

***Interactive comment on “Application of the
¹⁵N-Gas Flux method for measuring in situ N₂ and
N₂ fluxes
due to denitrification in natural and semi-natural
terrestrial ecosystems
and comparison with the acetylene inhibition
technique” by F. Sgouridis et al.***

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We are very grateful to the two reviewers for their comprehensive comments and suggestions for the improvement of the manuscript. We have attempted to accommodate all the suggestions and amended the manuscript accordingly where possible. Due to overlap of the comments between the two reviewers, we are presenting a joint response

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for all received comments.

Each reviewer comment is followed by a response:

Major comments

1)[Reviewer #2: The long enclosure (up to 20h) was used for the first time in field studies to my knowledge (previously up to 2 h, see details). Linearity check with 1, 2, 20h was not adequate due to the long interval between 2 and 20h. Previous studies (e.g. Tauchnitz et al, 2014) checked linearity by short intervals of 20 minutes. Linearity was only evaluated on the total data set, i.e. data from all sites from one system were pooled. But this check must be done for each site and sampling event. Physically linearity is extremely improbable, since concentration gradients decrease over time (e.g. Healy et al 1996). Moreover, the modelling by Healy et al. predicts that diffusion to subsoil increase with extended enclosure. This has been shown for denitrification studies (with the AIT) by Mahmood 1997. Although tests of this subsoil diffusion bias have never been published for the ^{15}N gas flux method to my knowledge, it is evident that this bias must be very significant for enclosure periods of almost 1 day. Note that Morse et al 2013 incubated in closed vessels when accumulating > 20h. I assume subsoil diffusion is the major reason why ^{15}N concentration did not increase significantly in many of the measurements. Request: - Evaluate linearity / non-linearity of N_2 and N_2O fluxes at each site and sampling date and discuss possible bias from subsoil diffusion during extended enclosure]

Response

In response to the reviewer's comment, we have carried out additional checks for the linearity of the evolved N_2 and N_2O gases per sampling plot and sampling event, which are presented in the Supplementary Information (SI) submitted with the revised manuscript (Supplementary Tables 4&5). This additional information is described in the results section (lines: 433-444) with reference to the SI. Despite, the reviewer's expectation for significant bias of the reported fluxes due to the extended enclosure

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period, this was not shown by the additional analysis, except for two cases, which are subsequently reported in the results, and discussed in lines (559-595). We suspect that subsoil diffusion may have not significantly affected our flux rates due to the relatively high water filled pore space (WFPS) of our field sites (mean WFPS data per site reported in discussion: lines 573-576) which may have limited the downwards diffusion of gases back into the soil despite the absence of a bottom barrier in our chambers. Jury et al. (1982) have shown that the wetter the soil the longer it takes for steady state gas diffusion to be established and this may take several hours from the start of gas production. The underestimation of flux rates due to a decreasing diffusion gradient between the soil surface and the chamber headspace (as modelled by Healy et al. 1996) does not constitute an issue for the N₂ gas, which is not a trace gas and is abundant in the atmosphere (78%). This was the main reason why we selected an extended incubation period to be able to detect a reliable ¹⁵N-N₂ signal in the N₂ rich chamber headspace. A decreasing gas diffusion gradient is more likely to be observed in the case of N₂O, but only where there is significant N₂O production, such as in fertilised grasslands for example (see R-IG in Supplementary Table 2). However, the majority of our field sites showed a very low N₂O production rate and it is unlikely that these have been affected by the gas diffusion gradient. It would have been desirable to perform the linearity checks at more frequent intervals, as suggested by the reviewer, but unfortunately this was not possible in the present study, where we focused more on constraining the spatial variability of the denitrification fluxes, at the expense of a more detailed temporal investigation (which was also the case in Tauchnitz et al. 2015). In subsequent applications of our methodology we will assess the temporal variability of N₂ and N₂O gas fluxes during varying incubation periods, as there seems to be a lack of conclusive results particularly for field applications of the ¹⁵N Gas-Flux method.

2)[Reviewer #1: The new method seems promising and the results here are certainly worthy of publication, but there needs to be a more thorough treatment of possible fertilization and water addition effects in the new method. The authors worked hard to minimize the amount of nitrate and water added to the field chambers but there needs

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to be a more clear statement of just how much the inorganic N pools and soil moisture content were increased by the additions. And once the extent of the increases is clarified, there should be some comparison with the literature to see if these increases have affected rates in previous studies.]

[Reviewer #2: The amount of label added: it was variable and pretty low, but this is not well justified, since no mineral N data of sites were shown. It is thus not possible to see to which extent denitrification was potentially enhanced by increasing nitrate. In nitrate-free soils, 1 kg NO₃-N/ha would clearly enhance denitrification. Request: -Show mineral N and 15N label amendment for each site (in an appendix) and discuss based on that the possible dilution and consumption of the label]

Response

In response to the above comments by both reviewers we have added ambient soil nitrate data as well as the estimated soil nitrate pool enrichment for each land use type in Table 2 at the end of the manuscript. Moreover, in the Supplementary Information we have added Supplementary Table 2 that details the 15N label amendment per field site for the present study and compares with the annual average soil nitrate pool enrichment for the period April 2013 to October 2014. Based on these data, the range of soil nitrate enrichment was quite variable (range: 2- 40 %) and above our annual average and this was attributed to discrepancies between the soil nitrate content on the day of the measurement and the data used for calculating the required tracer concentration (data from previous campaign). Our aim was to enrich the total soil nitrate pool by no more than 10% with 15N-NO₃⁻, but clearly this was not always possible unless we were able to measure the ambient soil nitrate pool on the day of the 15N amendment, which was logistically impossible. To our knowledge only Kulkarni et al. (2014) have applied the 15N Gas-Flux method in the field with soil nitrate enrichment levels lower than in our study, but in their case this resulted in poorly detected 15N-N₂ fluxes. Even at slightly higher soil nitrate enrichment levels that we originally aimed for, our tracer application rates corresponded to daily N atmospheric deposition rates in the case of the organic

soils and daily fertilizer application rates for the improved grasslands. Therefore, we believe that our field denitrification rates using the 15N Gas-Flux method reflect as close as possible 'true' in situ rates. (Manuscript changes: Lines: 411-416 and 597-621)

The range of the augmented water content was between 3 and 5 %. Detailed data from each sampling plot are presented in Supplementary Table 1. The manuscript has been amended in lines 265-269.

3)[Reviewer #2: The 15N distribution was not well explained since the grid distance of injection was not given. It is thus not possible to judge potential non-homogeneity of labeling. For this, the volume of each injection and the distance must be reported. You might compare your pattern to Wu et al 2011 who optimized injection volume to achieve homogeneity.]

Response

The information on number of injections, volume per injection and the distances of the grid have been added to the methods section (Lines: 254-257). Wu et al. (2011) have optimised the number of injections and the volume of tracer needed to achieve homogeneous labelling of a soil core (diameter 15 cm; height 20 cm) and reported that 38 injections of 4 mL volume each were necessary. We have used only 10 injections of 5- 20 mL volume (depending on the soil water content of each land use type) to minimise the disturbance of the soil pore water:air matrix, particularly in highly porous media such as peatland soils, and this may have affected the homogeneous distribution of the tracer. This comparison has been added to the Discussion (Lines: 633-639).

4)[Reviewer #2: Another artefact from long enclosures is the decrease in $N_2O/(N_2+N_2O)$ ratio due to increasing N_2O reduction as N_2O concentration increases during accumulation. This is straightforward and has been repeatedly shown (unfortunately I have no reference at hand). This effect is not addressed at all in this paper and might in part explain why ratios were mostly very small. Request: -Evaluate the

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change in product ratio during 1, 2, 20h sampling for each site and discuss the bias of the 20h values]

Response

The change of the denitrification product ratio with incubation time was evaluated in each sampling plot where both N₂ and N₂O 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 ratio was observed after 2 hours of incubation. This was indeed an indication of some further reduction of the denitrification derived N₂O to N₂ during the extended closure period of up to 20 hours, even though the N₂O increased linearly during 20 hours incubation (apart from the R-IG), as discussed in the response for the major comment 1. This observation has been included in the Results section (Lines: 460-466). We refer to this observation in the discussion as well where we make the recommendation 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 N₂O/ N₂ + N₂O ratio. A longer incubation is warranted under conditions of low flux seasons (winter) or low flux sites such as the organic soils (Lines: 681-691).

5)[Reviewer #2: AIT was used as a reference but the major bias from this method was not discussed i.e. catalytic NO decomposition (Bollman & Conrad 1997, Nadeem et al 2013). Hence this method is today considered to be inadequate for field quantification (e.g. Felber et al, 2013). Moreover, the C₂H₂ treated cores were sealed from the bottom thus avoiding subsoil diffusion. If the ¹⁵N labeled cores had been sealed from the bottom, discrepancies between the methods would certainly have been even larger than reported. -Discuss all factors of bias of the AIT and take into account the absence of subsoil diffusion.]

Response

Our intention was to use the AIT as an alternative field method to compare against

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and in essence ‘fool-proof’ our measurements with the adapted 15N Gas-Flux method that was applied for the first time in the field. The good agreement we got between the two methods gives an additional indication that our adapted method gives reasonable estimates of in situ denitrification. However we agree with the comment here and are aware of the several drawbacks of the AIT as a field quantification method and the fact that subsoil diffusion was not possible with the AIT, which also did not receive any nitrate amendment, preclude the direct comparison of the two methods. In response to the reviewer’s comments we have adapted the respective section of the discussion 4.3 (Lines: 697-737) to reflect several possible sources of uncertainty that may be responsible for the discrepancies observed between the two field methods.

Minor comments

Reviewer #2:

1)[P 12654 (54), L 15 : check reported precision, do you mean 0.5% of 0.367 at%? This would be d15N of 5 per mil, i.e. one to two orders worth than previous methods. L 18 give units of volume/surface ratio L18 20h accumulation time far too long, see above L 24 C2H2 bias not fully addressed (see above)]

Response

The coefficient of variation (CV) of 0.5 % refers to the R29 and R30 precision reported in Table 1. The units for the chamber volume/surface are cm³:cm².

2)[P55 L9 but not only with respect to EXCESS nitrogen]

Response

The word ‘excess’ is deleted

3)[P56 L 18-20 AIT not adequately discussed (see above, check Bollman & Conrad, 1997 and Nadeem et al. 2013)]

Response

The effect of acetylene on the catalytic decomposition of NO has been added as a significant drawback of the AIT for quantifying in situ denitrification rates with reference to Nadeem et al. 2013 (Lines: 105-109).

4)[P57 L1 This statement is incorrect since the 15N gas flux method is inadequate for saturated soils (see Tauchnitz et al 2014 and references therein) where only the push-pull method is suitable for quantification. L7 refer also to Tauchnitz et al 2014]

Response

The statement on the suitability of the 15N Gas-Flux method for saturated soils has been deleted. The reference to the study by Tauchnitz et al (2015) in restored peatland soils has been added to the literature review in Lines 119-122.

5)[P59 L18-21; P60 L11: Not clear what per mil means here L12 not clear what 3 mL * 100 mL means]

Response

The per mil units refer to the standard deviation of $\delta^{15}\text{N}$. The clarification has been added to the section 2.1. In L12 the mistake is a typo. It reads now three 100 mL flasks.

6)[P61 L2 Small insertion depth of 10 cm further enhances subsoil diffusion (see Healy et al, 1996) L10 the purpose of a vent is incorrectly addressed here. It is needed to allow pressure pumping, and this is independent of cover volume. Exclusion of pressure pumping affects fluxes, please discuss. L12 did you check temperature during 20h closure? If so, please report data L15 report number of injections and grid dimensions L25 since water content is among the main drivers: more detail is needed here: what was the range of augmented water content and discuss potential effects. An increase of 5% (g/g) is quite a lot.]

Response

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According to Healy et al. (1996), inserting the chamber walls into the soil up to the depth of gas production could minimise the error due to the distortion of the gas concentration gradient by increasing vertical (upward) diffusion and minimising any radial diffusion. The collars were inserted at approximately 10 cm depth, which was also the depth of the tracer injection. Therefore, the top 10 cm of soil was considered our gas production depth and this was surrounded by the collar walls, thus minimising radial diffusion. Deeper insertion of the collars would not have affected subsoil diffusion downward, as the reviewer suggests, but it would rather minimise any further radial diffusion (see Healy et al, 1996).

We did not use a vent tube (as suggested by Hutchinson and Mosier, 1981) in our chamber design, which could have diluted the chamber headspace with atmospheric N₂, as part of our effort to increase the probability of a detectable 15N-N₂ signal in the chamber headspace. The build-up of positive pressure within the chamber's headspace, particularly during the extended 20 hours incubation, may have potentially led to underestimations of the N₂ and N₂O fluxes and thus we amended the manuscript to recognize this underestimation. (See manuscript amendments: Lines 248-252 and 567-570).

The soil temperature was not recorded inside the soil enclosure during the incubation, since we wanted to minimise any further disturbance of the soil matrix but measured within the m² plots assuming similar temperature inside and outside of the chamber. To avoid any over-heating of the enclosed soils, we covered our chambers with reflective foil

The number of injections and grid dimensions are reported in line 255.

The range of the augmented water content was between 3 and 5 %. Detailed data from each sampling plot are presented in Supplementary Table 1. The manuscript has been amended in lines 265-269.

7)[P62 L 10 capping the bottom precludes comparison with 15N gas fluxes since the

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soil was not capped at the bottom in the 15N treated microplots (see comments on subsoil diffusion)]

Response

The intact soil cores used in the AIT technique were capped at both ends to make sure that cored soil (up to 10 cm depth) is retained during incubation within the tube without falling out to avoid any overdose of soil with C₂H₂ and to maintain similar soil pore and headspace C₂H₂ across the sites A significant effect of subsoil diffusion was not demonstrated for the 15N Gas-Flux method in the majority of the sampling plots (as shown in Supplementary Tables 4 &5) and this mismatch of the two methods in terms of sealing is discussed in the major comments above. Therefore, we believe that a comparison of denitrification rates between the two field methods cannot be precluded on the basis of the subsoil diffusion effect

8)[P63 suggest to give also an equation for evolved N₂O]

Response

The equation for calculating the evolved N₂O is exactly the same with equation (5), where N₂ concentration is replaced by the total N₂O concentration. This is described in the manuscript in lines 346-350, and therefore we believe that repeating the same equation for a second time would be redundant.

9)[In section 2.3: please explain how you calculate N₂O flux from other sources.]

Response

We did not partition the sources of N₂O in this study, but rather measured total N₂O flux (from all possible sources) to be used in equation (5) for estimating the evolved N₂O due to denitrification. This is explained in the methods section in lines 346-350.

10)[P64 L1-5 linearity is not expected for 20h closure. Please address time course data and linearity for each site and sampling (see above) P67 L 1 this analysis is not

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adequate. Each site and date must be checked individually (see above, data might be shown in appendix). Please check also which values were significantly different from background air. Data not significantly different must be excluded from linearity checks.]

Response

Please see response for major comment 1 above.

11)[P64 L22 15XN of N₂ and N₂O can be very different due to inhomogeneity of labeling and formation of hybrid N₂ or N₂O (Spott et al 2007). Please discuss uncertainty from assuming equal 15XN of N₂ and N₂O. Did you get useful 15XN of N₂ in high flux plots? If so how 15XN of N₂ and N₂O agreed in those cases. (data of individual sites should be given in an appendix)

[P71 L 6-24 in this discussion please also address that you did not measure 15XN of N₂]

[P71 L27 the arguing for hybrid N fluxes should better explained. You can only check this precisely if you have good estimates for the enrichment of NO₃ (15a_NO₃). If 15XN < 15a_NO₃ then you obtain positive values for hybrid N according to Spott et al 2007. But this might be also due to non-homogeneity. You did not measure 15a-NO₃ but have initial estimates which are lower than 15XN. So this indicates strong non-homogeneity. This is an important observation. Would be good to show the data (15XN and calculated 15a_NO₃, should be shown in appendix) and discuss more in detail.]

Response

We were able to calculate 15XN from the N₂ isotope ratio data mostly from the woodland and grassland plots. Data from all plots where the 15XN could be calculated from both the N₂ and the N₂O isotope ratio data are shown in Supplementary Table 3. When comparing the mean 15XN from the two data sources for each land use type, these were not significantly different, thus indicating negligible effect from hybrid N₂

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and N₂O fluxes. This comparison has been added in the results, lines: 427-431 and the discussion for further clarification, lines: 652-657.

12)[P69 | 1-3: the lower NO_x formation is probably due to the different geometry of the ion source of the IRMS and not due to injection volume. L10 note that true values are needed when using the equations by Spott et al 2007 to calculate hybrid N₂ and/or N₂O L 16 but note that your precision was not better than older data, eg Well et al 1998.]

Response

Clarifications were added in the discussion section 4.1 (Lines: 509-538) to address the above comments by the reviewer.

13)[P70 L14 note that Morse and Bernhard incubated in closed systems which did not allow subsoil diffusion. 20 h closure has never before been employed for ¹⁵N gas flux studies in the field, to my knowledge. L18 this is not adequately proven because it was only tested using averages of all sites of one system, but it needs to be shown on individual sites /dates (see above) L20 please show WFPS data]

Response

The difference between our approach and the one described in Morse and Bernhard (2013) has been made explicit in the Discussion (Lines: 564-567). The rest of this comment is addressed in our response above (comment 1). The mean WFPS data per field site are presented in the Discussion (Lines: 573-576).

14)[P72 L4 not clear to me. I agree that nitrification might dilute the ¹⁵N in NO₃ causing a decrease in ¹⁵XN. But N₂O from nitrification is another issue. You can calculate that based on the Bergsma (2001) equations and it would be a valuable extension of your data.]

Response

A clarification has been added to the Discussion (Lines: 658-660) to address the above

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comment. It now reads ‘...the slope of 15XN with time was negative suggesting dilution of the 15N-labelled soil NO₃- pool by the oxidation of the ambient ammonium (nitrification).’ The source partitioning of the N₂O is the subject of a separate publication and we do not think that adding this information here is within the scope of this methodological study.

15)[P72 L 9 to 20. This discussion is too simple as it only compares ranges of values without addressing denitrification controls. So if you want to keep this, compare soil types, mineral N level, organic C, moisture and so on, and discuss in which cases agreement or disagreement of data was expected.]

Response

In this part of the Discussion (Lines: 665-677, revised manuscript) we are comparing our measured in situ denitrification rates with the published literature, where similar methodological approaches were used. There is a general agreement of our rates with the rates reported for low 15N field applications, whereas our rates are significantly lower compared to fertiliser level applications of 15N. We believe that this part of the discussion is important as it shows that the denitrification rates measured with our adapted method generally agree with the literature and are not unreasonable. We do not expand our discussion to discuss the observed differences in denitrification rates between land use types and the effect of soil variables in controlling process rates, as this discussion would be beyond the scope of this methodological study, but instead we make reference to the separate publication that focuses on ‘The relative magnitude and controls of in situ N₂ and N₂O fluxes due to denitrification in natural and semi-natural terrestrial ecosystems using 15N tracers’ (Sgouridis and Ullah, accepted). In response to the reviewer’s comment we have removed the part of the discussion between P12672 L25 and P12673 L15 (Pages and lines refer to the pdf of the manuscript published in the Biogeosciences Discussion forum).

16)[P73 L 5 This does not apply to all organic soils, i.e. to bogs, but not to fens L9

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this needs clarification. Not adequate to leave BD values out, but include them as zero fluxes or 50 % of detection limit. Which option is advisable depends on the number of BD values. If you have only few, then 50% of detection limit would be adequate from my view.]

Response

The comment for P73 L5 does no longer apply as this part of the discussion has been removed (see previous comment). As for the comment for P73 L9, although this statement has also been removed from the discussion we would like to provide a clarification. By 'N₂ fluxes below the detection limit' we meant those samples that did not pass our minimum detectable concentration filter (MDC, described in the manuscript) and therefore they were not regarded as valid samples. As to the reason why these samples were not valid we cannot be certain as it may had to do with the sampling procedure, or simply that the 15N-N₂ signal even over 20 hour incubation may have been too low to be detected by our IRMS. Therefore, we chose to not use these samples as invalid, rather than assuming a potentially false 0 flux, which would have seriously underestimated the mean flux rate calculation.

17)[P73 L22-26 since the AIT is not quantitative this arguing is not suitable (see above)]

Response

This argument has been removed from the discussion. See also response to major comment 5.

18)[P74 L12-15 this is a weak argument since N₂O flux is by no means equal to denitrification.

And also reviewer #1: The authors correctly point out that “adding nitrate to the C₂H₂ amended cores would have been desirable for evaluating directly the priming effect of the added substrate on denitrification rates”, yet they did not do this. As a result, they cannot really conclude that the AIT rates were lower due to incomplete blockage

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of N₂O reduction from the data you have. The idea that “if the ¹⁵N tracer addition in the static chambers, even at such low rate (< 1 kg N/ha), were to stimulate the denitrification activity, this might have been reflected through high bulk N₂O flux from the chamber compared to the intact cores” is not really valid, as the vast majority of the denitrification flux went to N₂. So it would be hard to see a fertilization effect in the bulk N₂O flux.]

Response

We agree with the comments of both reviewers and in response we amended the respective section of the discussion as such: ‘Adding nitrate to the C₂H₂ amended cores would have been desirable for evaluating directly the priming effect of the added substrate on denitrification rates. Even though the ¹⁵N 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, 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 ¹⁵N Gas-Flux methods.’ Discussion Lines: 702-714.

19)[Fig.1: the meaning of N₂ and N₂O in the Fig. is not clear. NO is not removed in the furnace but reduced to N₂]

Response

Figure 1 was adapted to clarify the above comment. N₂O is removed in the liquid nitrogen trap. NO is not removed but reduced to N₂ in the furnace and finally N₂ is directed to the IRMS.

20)[Fig 2: Units: $\mu\text{g N/m}^2/\text{h}$?]

Response

The units in Figure 2 are $\mu\text{g N}$ as the evolved N₂ and N₂O refer to amounts of gas accumulated in the chamber headspace at the different incubation times.

Please also note the supplement to this comment:

<http://www.biogeosciences-discuss.net/12/C7335/2015/bgd-12-C7335-2015-supplement.pdf>

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