

***Interactive comment on “Application of the  
<sup>15</sup>N-Gas Flux method for measuring in situ N<sub>2</sub> and  
N<sub>2</sub> fluxes  
due to denitrification in natural and semi-natural  
terrestrial ecosystems  
and comparison with the acetylene inhibition  
technique” by F. Sgouridis et al.***

**R. Well (Referee)**

reinhard.well@ti.bund.de

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General comments

The study reports a modified analytical approach to measure N<sub>2</sub> and N<sub>2</sub>O evolved from <sup>15</sup>N-labelled nitrate in soil. The aim was to adapt the method to natural and semi-

C5309

natural systems and apply it in a large number of sites, since there are few <sup>15</sup>N flux data from these systems. Modifications include the increase in incubation time (up to 20 h compared to 1 or 2 hours in previous studies) and minimizing of added <sup>15</sup>N-label to minimize system disturbance. Field <sup>15</sup>N flux data with closed chambers were compared to the acetylene inhibition soil core method.

The topic is of high relevance in view of scarce field data and the prominent role of denitrification in the N cycle. Due to the difficulty to measure denitrification, especially in the field there are few robust field studies until now. The presented methodical work and the large data-set would thus be a valuable contribution to make progress in this field.

However there are several problems and uncertainties associated with the methods used that are not sufficiently addressed and discussed and might have caused severe bias in the reported fluxes. These issues must be addressed and probably data need to be re-analysed and/or presented more detailed to show uncertainties. Overall, I think the data are far more uncertain than reported. Nevertheless they are probably worth to be published in Biogeosciences and I recommend resubmission after major review.

The following aspects lead to uncertainty of the data:

-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 modeling 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

C5310

been published for the  $^{15}\text{N}$  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  $^{15}\text{N}$  concentration did not increase significantly in many of the measurements.

-Another artefact from long enclosures is the decrease in  $\text{N}_2\text{O}/(\text{N}_2+\text{N}_2\text{O})$  ratio due to increasing  $\text{N}_2\text{O}$  reduction as  $\text{N}_2\text{O}$  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 ratio were mostly very small.

-AIT was used as a reference but the major bias from this method was not discussed i.e. catalytic  $\text{NO}$  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  $\text{C}_2\text{H}_2$  treated cores were sealed from the bottom thus avoiding subsoil diffusion. If the  $^{15}\text{N}$  labeled cores had been sealed from the bottom, discrepancies between the methods would certainly have been even larger than reported. -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,  $1\text{kg NO}_3\text{-N/ha}$  would clearly enhance denitrification.

The  $^{15}\text{N}$  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.

A revised version of the manuscripts should thus include the following:

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

C5311

-Evaluate linearity / non-linearity of  $\text{N}_2$  and  $\text{N}_2\text{O}$  fluxes at each site and sampling date and discuss possible bias from subsoil diffusion during extended enclosure.

-Discuss all factors of bias of the AIT and take into account the absence of subsoil diffusion.

-Show mineral N and  $^{15}\text{N}$  label amendment for each site (in an appendix) and discuss based on that the possible dilution and consumption of the label

-Report the uncertainty of reported fluxes based on all factors including those mentioned above and take into account when discussing results and need for future improvements.

Further aspects are addressed in the details below. I did not give detailed technical corrections in view of the substantial changes needed.

☞ Specific comments

P 12654 (54), L 15 : check reported precision, do you mean 0.5% of 0.367 at%? This would be  $\delta^{15}\text{N}$  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  $\text{C}_2\text{H}_2$  bias not fully addressed (see above)

P55 L9 but not only with respect to EXCESS nitrogen

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

P57 L1f This statement is incorrect since the  $^{15}\text{N}$  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

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

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P61 L2 Small insertion depth of 10 cm further enhances subsoil diffusion (see Healy et al, 1996) L10 the purpose of a vent in 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.

P62 L 10 capping the bottom precludes comparison with 15N gas fluxes since the soil was not capped at the bottom in the 15N treated microplots (see comments on subsoil diffusion)

P63 suggest to give also an equation for evolved N<sub>2</sub>O

P64 L1-5 linearity is not expected for 20h closure. Please address time course data and linearity for each site and sampling (see above) L22 15XN of N<sub>2</sub> and N<sub>2</sub>O can be very different due to inhomogeneity of labeling and formation of hybrid N<sub>2</sub> or N<sub>2</sub>O (Spott et al 2007). Please discuss uncertainty from assuming equal 15XN of N<sub>2</sub> and N<sub>2</sub>O. Did you get useful 15XN of N<sub>2</sub> in high flux plots? If so how 15XN of N<sub>2</sub> and N<sub>2</sub>O agreed in those cases. (data of individual sites should be given in an appendix)

In section 2.3: please explain how you calculate N<sub>2</sub>O flux from other sources.

P67 L 1 this analysis is not 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.

P69 I 1-3: the lower NO<sub>x</sub> 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 N<sub>2</sub> and/or

C5313

N<sub>2</sub>O L 16 but note that your precision was not better than older data, eg Well et al 1998.

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

P71 L 6-24 in this discussion please also address that you did not measure 15XN of N<sub>2</sub>

L27 the arguing for hybrid N fluxes should be better explained. You can only check this precisely if you have good estimates for the enrichment of NO<sub>3</sub> (15a\_NO<sub>3</sub>). If 15XN < 15a\_NO<sub>3</sub> 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-NO<sub>3</sub> 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\_NO<sub>3</sub>, should be shown in appendix) and discuss more in detail. P72 L4 not clear to me. I agree that nitrification might dilute the 15N in NO<sub>3</sub> causing a decrease in 15XN. But N<sub>2</sub>O 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. 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.

P73 L 5 This does not apply to all organic soils, i.e. to bogs, but not to fens L9 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. L22-26 since the AIT is not quantitative this arguing is not suitable (see above)

C5314

P74 L12-15 this is a weak argument since N<sub>2</sub>O flux is by no means equal to denitrification.

L22-26 see comments on AIT above

Fig.1: the meaning of N<sub>2</sub> and N<sub>2</sub>O in the Fig. is not clear. NO is not removed in the furnace but reduced to N<sub>2</sub>

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

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C5315

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Interactive comment on *Biogeosciences Discuss.*, 12, 12653, 2015.

C5316