## *Review response for Anonymous referee #1 on*

# $^{15}$N gas-flux method to determine $N_2$ emission and $N_2O$ pathways: a comparison of different tracer addition approaches

by Dominika Lewicka-Szczebak and Reinhard Well

(1) *comments from referees*
(2) authors response
(3) authors changes in manuscript

*This is an informative and relevant study, the experiments are well planned and conclusions are sound. Prior to publication, a few clarifications are needed. The paper Gould also benefit from language editing (e.g. past and present tense are mixed).*

Thank you. We will make the clarifications needed and the professional language editing will be performed after the needed corrections and their acceptance.

*Both, the introduction and discussion could benefit from including references that support your statements. There are quite a few statements, which are unsupported by references and/or your results. Although this might be the first paper on the effect of 15N tracer approach on the N gas source partitioning, some other papers have investigated the effects of tracer addition on the soil N cycle (Davidson et al., 1991; Gütlein et al., 2016; Kaur et al., 2010). It might be worth looking at those (you do not need to cite those necessarily, but they might contribute to your discussion).*

Thank you for the very adequate citation suggestions. These and further references will be included in the manuscript introduction and discussion:

Introduction: line 32 (Davidson et al., 1991), line 33(Gütlein et al., 2016; Kaur et al., 2010), line 36 (Davidson et al., 1991),
Discussion: line 163 (Davidson et al., 1991), line 118: (Kaur et al., 2010).

*The tracer addition (with a 15N fraction of 73 %), resulted in an initial 15N fraction of soil NO3- of 42.5 % (line 51). This means that soil NO3- content was more than doubled, which is much above common recommendations of tracer addition (10 – 25 % of native soil N). What was the motivation for such a high addition of tracer and what are the consequences for your results? I would like to see a discussion on this.*

The reason for high N addition was the limited sensitivity of 15N gas flux method. The N2 gas flux is only detectable for the high 15N content. The common recommendations for low N additions are important for the studies where we want to trace the natural N transformation for this soil and the fertilization effect must be as minimal as possible. Here our aim was to compare the effects of the method of tracer addition, i.e. homogenisation vs injection, so it was important to obtain a well detectable N2 flux and it was not intended to draw conclusions on the denitrification activity for the particular study site. If we compare the different addition strategies by addition of even more N than usual, the potential experimental artefacts should be even enhanced, which would be a positive consequence for our study objectives. This discussion will be added to the manuscript at the beginning of 3.2 section, line 110 :

In this study the addition of N to the soil was quite high resulting in more than doubled NO3- content. This was much above the common recommendations of tracer addition of 10-25% of native soil N (Davidson et al., 1991). These recommendations are motivated by the need of minimizing the fertilization effect to trace the naturally occurring N transformation processes. But, in this study we only aimed at comparison of tracer addition strategies and not intended to draw conclusions for this particular study site. Establishing a high $^{15}$N enrichment of the $NO_3^-$ by high addition of $^{15}$N-labelled $NO_3^-$ enhanced the sensitivity of $N_2$ fluxes detection, which is a prerequisite for reliably identifying potential experimental artefacts, which we aimed to evaluated in this study.

We will also add this information in the introduction:

To determine soil gross N transformation rates, enrichment in $^{15}$N of a few percent (e.g. 10 at% $^{15}$N) is sufficient (Müller et al., 2004). However, in applications where $N_2$ fluxes are analysed ($^{15}$N gas-flux method) the labelled N pool (e.g. $NO_3^-$) should ideally be enriched by approximately 50 at% $^{15}$N to achieve precise results (Stevens et al., 1993).

*Your comparison of the 15N fraction of NO3- (a_NO3) with the calculated a_p values (line 127) makes only sense if NO3- was the sole source of N2O and n2, i.e. all gases were produced via denitrification. What supports this assumption? You speculate yourself later about the possibility for hybrid N2 (line 148). And N2O production from nitrification is also possible.*

Quite a high soil moisture favours denitrification. We only labelled the nitrate pool so when calculating aP this refers to labelled pool, nitrate. Other gas sources, originating from unlabelled pools, like eg. nitrification, are obtained from the isotope ratios of emitted N2O (data not shown).
If hybrid gases are present the aP values are lower than nitrate a15N. That’s why we speculate either about heterogenity or hybrid gas production.

*Specific comments*

All the specific comments have been taken into account and the relevant changes will be incorporated into the manuscript

*Line 11: please be more specific what kind of results.*

We aimed at comparing the N2 flux determined by the gas flux method

*Line 13: “wider range” is unclear, be more specific.*

It will be changed to: larger variability

*Line 51: what is "initial condition"? Is this prior to trace addition or immediately after? Please clarify.*

This will be clarified:
measured in the subsamples of the homogenized soil immediately after tracer addition and mixing

*Line 66: the ap values, are those calculated or measured? I think this part would benefit from showing all equations rather than referring solely to other papers.*

The equations will be added:

Based on these measurements the following values are calculated according to the respective equations (after Spott et al. (2006)):

- $^{15}$N abundance of $^{15}$N-labelled pool ($a_\mathrm{P}$) from which $N_2$ ($a_\mathrm{P\_N2}$) or $N_2O$ ($a_\mathrm{P\_N2O}$) originate:

$$a_\mathrm{P} = \frac{^{30}x_\mathrm{M} - a_\mathrm{M} \cdot a_\mathrm{bgd}}{a_\mathrm{M} - a_\mathrm{bgd}} \tag{1}$$

The calculation of $a_\mathrm{P}$ is based on the non-random distribution of $N_2$ and $N_2O$ isotopologues (Spott et al., 2006) where $^{30}x_\mathrm{M}$ is the fraction of $^{30}N_2$ in the total gas mixture:

$$^{30}x_\mathrm{M} = \frac{^{30}R}{1 + {}^{29}R + {}^{30}R} \tag{2}$$

$a_\mathrm{M}$ is $^{15}$N abundance in total gas mixture

$$a_\mathrm{M} = \frac{^{29}R + 2\,{}^{30}R}{2(1 + {}^{29}R + {}^{30}R)} \tag{3}$$

$a_\mathrm{bgd}$ is $^{15}$N abundance of non-labelled pool (atmospheric background or experimental matrix)

- the fraction originating from the $^{15}$N-labelled pool ($f_\mathrm{P}$) for $N_2$ ($f_\mathrm{P\_N2}$), $N_2$+$N_2O$ ($f_\mathrm{P\_N2+N2O}$ )and $N_2O$ ($f_\mathrm{P\_N2O}$) within the sample:

- $$f_\mathrm{P} = \frac{a_\mathrm{M} - a_\mathrm{bgd}}{a_\mathrm{P} - a_\mathrm{bgd}} \tag{4}$$

- $N_2O$ residual fraction ($r_\mathrm{N2O}$) representing the unreduced $N_2O$ mole fraction of pool-derived gross $N_2O$ production (Lewicka-Szczebak et al., 2017).:

- $$r_\mathrm{N2O} = \frac{y_\mathrm{N2O}}{y_\mathrm{N2} + y_\mathrm{N2O}} = \frac{f_\mathrm{P\_N2+N2O} - f_\mathrm{P\_N2}}{f_\mathrm{P\_N2+N2O}} \tag{5}$$

- where $y$ represents the mole fractions.

*Line 102: This sentence needs rephrasing; "we may deal with" is unclear.*

This will be rephrased to: we probably observe

*Line 110 (&114): The phrase "column heterogeneity" is unclear and might be confusing. As I understand you mean the heterogeneity between different columns, but it sounds like the within column heterogeneity. The latter, you actually cannot conclude about.*

This is heterogenity within one column, determined at the end of experiment by destructive sampling of multiple samples within one column. This will be clarified, by using 'heterogeneity within columns'

*Line 117: For me it is unclear why the initial NO3- content should differ between the treatments. After all, it is the same soil. Alternatively, it might be due to stimulated nitrification in the mixed soil (see e.g. Kaur et al., 2010).*

This is due to storage, sieving and homogenisation - same as indicated by Kaur et al, 2010. Thank you for information on this paper! Explanation and citation will be added:

Storing of mixed soil or sieving and homogenization procedures probably intensified N mineralization and formation of additional nitrate through intensified nitrification, which has been also observed in previous studies (Kaur et al., 2010).

*Line 119-123: This sounds somewhat unlikely to me. If less 15N was injected, you certainly should have noted that during the injections.*

This could have not been noted during the injections. For all columns 3L of solution were prepared, this included 400mL reserve above the calculated needed amount (needed e.g. for flushing the needles before injection). I didn't measured exactly the amount lost during injection and left after injection, hence I also wasn't able to assess the unplanned losses during the injection.

*Line 129 (& 136): Suggest moving the text in parentheses (after colon) to the Methods.*

This will be moved to the methods section 2.4

In Table 3 for the comparison of particular $a_{NO3}$ and $a_{\mathrm{P}}$ values we applied following calculated parameters:

- cumulative relative difference (cum diff) calculated as a sum of differences in $^{15}$N enrichment of different pools for all 24 samples: cum diff = $\boldsymbol{\sum_{i=1}^{n}(a_1 - a_2)_i}$
- absolute mean difference (mean abs diff) calculated as a mean of modulus of differences in $^{15}$N enrichment of different pools: mean abs diff =$\boldsymbol{(\sum_{i=1}^{n}|(a_1 - a_2)_i|)/n}$

In the above equations $a_1$ and $a_2$ represent the $^{15}$N enrichment of two compared pools ($a_{NO3}$ or $a_{\mathrm{P\_N2}}$ or $a_{\mathrm{P\_N2O}}$).

*Line 131 & 144: The "differences" you refer to, is this the cumulative or mean?*

First cumulative and later mean. This will be added in the text.

*Line 172: here you use for the first time "content" of inorganic N, while otherwise you use concentration. In fact, content is the correct term.*

This will be corrected for content in the whole manuscript.

*Table 2: Unclear what is compared statistically, withintreatment of between? Also, what is the "mean" referring to, mean of what? The "Injection point", is this for both layers?*

This caption will be modified

Table 2: Soil analyses at the end of the experiment: mixed samples, and separately from the top and bottom layer and for injected columns also from injection points (including both top and bottom layer). Statistically significant differences are indicated with uppercase letters (**p<0.01, ***p<0.001). For individual values, the differences within treatment and for mean values the differences between treatments were tested.

*Table 3: Suggest moving the equations (with additional explanations) to the method section.*

They will be moved to the section 2.4.