Author's response on

"N_2O isotope approaches for source partitioning of N_2O production and estimation of N_2O reduction – validation with ^{15}N gas-flux method in laboratory and field studies"
by Dominika Lewicka-Szczebak et al.

Review response for Anonymous referee #2

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

Thank you very much for your positive evaluation of the manuscript and your critical comments which helped us to improve our work.

Line 61: It would be helpful to provide the actual value of sensitivity increase here so that a direct comparison can be made.

This is about 80-fold increase in sensitivity. This information will be added in the text.

Line 68: 'budget' is a more appropriate word here rather than emission. It is probably much easier to measure N2O fluxes directly if emission is intended.

Thank you, this will be changed.

Line 76: Should diffusion of N2O be taken into account as one of the processes determining the final N2O isotopes? Either here or in discussion, why the fractionation factor of diffusion is not considered in this study should be briefly mentioned.

We consider rather enzymatic processes than diffusion to be rate-limiting since enzymatic isotope fractionation is rather determining the apparent isotope effect. This has been more deeply discussed in our previous publications (Lewicka-Szczebak et al., 2014, 2015) and we will add this information here.

Line 98: What was the non-identical treatment here? Suggest briefly describe to give the readers some idea on the treatment differences which should be avoided

Different fertilizer application procedures: needle injection of fertilizer solution for ^{15}N treatments and surface distribution of fertilizer in NA treatments, different sizes of ^{15}N and NA microplots and chambers). This information will be added.

Line 117: Suggest the authors indicate their reference method here

This is ^{15}N gas-flux method. This information will be added.

Line 159: Should also briefly mention what is the same treatment strategy employed in this study.

This is: identical fertilizer application procedure as fertilizer solution applied with needle injection technique, identical water and fertilizer addition and identical plots and chamber sizes. This information will be added.

Line 178: Why 20 mg N/kg of soil in lab incubation compared to 10 mg N/kg soil in field fertilisation?

This was wrongly described and will be corrected for: in both lab and field study total fertilization was 20 mg N per kg soil added as $NaNO_3$ (10 mg N) and NH_4Cl (10 mg N)).

Line 141: This is confusing. Why the dates for 'next field campaigns' do not correspond to what is written earlier?

These are dates when the cylinders were reinstalled, this was done at least one month before the next filed campaign. This will be clarified.

Line 217: How was N2O converted to N2? In-line conversion?

Yes, in-line reduction, this information will be added.

Line 298: nD is not included here. Why? Understand that the isotopic ranges are not very different between bD and nD but authors should briefly mention why this is not included here to avoid confusion. Also to show that the authors have considered the nD pathway as well.

nD cannot be really separated with this approach from bD. It will be clarified that the bD fraction here can possibly include nD as well.

Line 310: Why higher fungal DN compared to nitrification in this instance? The data in Table S2 can be used to briefly justify this?

We deal with rather high soil moisture, mostly over 65% WFPS, and also ammonium content was low, which rather favours fD than Ni. This explanation will be added.

Line 379: Some of the N2 fluxes are above the detection limit but the values written here are below the detection limit mentioned in line 373.

Sorry, this was a mistake, it is from 23 to 304 g N-N2. This will be corrected. Thank you for careful reading!

Line 421 - 423: Don't quite get what you mean here. Consider rephrasing. You mean corrected precursor ranges based on different fractionation factors?

It will be clarified. In this sentence one word was incorrectly used – precursors instead of endmembers. Sorry for this mistake.

Line 434: The minimum reduction line is not described in Fig. 1. The dotted lines and the mixing should be clearly described in the legend/caption.

This explanation will be added to the Fig.1 caption: The soild lines (bD-fD mixing and mean reduction line) are main assumptions used in the calculation procedures for SP/O Map. The grey dashed line shows the alternative bD-Ni mixing line (calculations with this alternative scenario are also presented in the supplement Table S2). The red dashed line shows the minimum reduction line – for the case of minimal delta values of the bD endmember. And for Fig.3 caption: The dashed line shows the linear fit for all the points with its equation and statistics above.

Line 461: There was relatively large discrepancy between lab and field NO3- and NH4+ values. In fact, the d15N-NH4+ is very heavy and the possible factors driving these values should be discussed.

We comment this in the discussion, L680: This indicates that the ammonium pool was highly fractionated and nearly exhausted. This is most probably due to adsorption processes. But this is just a speculation so far. The discussion on this issue will be extended in the follow up paper, where we also include the 15N-NH4 treatment which was not presented here. This information will be added in the manuscript.

Line 490: The authors mentioned that the high d15N-NH4+ has shifted the location of the nD and Ni in the end member mixing plot. What is the author comparing the shift to?

Ccompared to cases when similar $\delta^{15}N_{NH4}$ and $\delta^{15}N_{NO3}$ values are determined or assumed – this will be clarified in the text.

Line 551: Amplitude for 3D1 model, case 1 is not always lower than the reference – at the start and towards the end of sampling, the amplitude is higher than the reference method. Any explanation on why this is the case?

I meant lower amplitude of the temporal changes, this will be clarified in the text. The uncertainty of each method mostly depend on the standard deviation of 4 repetitions of which each time sample consists.

Line 670: I agree with the authors that recalculation of the literature mixing endmember values is important but my question is what fractionation factors should be considered when correcting these values and how to evaluate that these corrected ranges are justified?

We can take the literature ranges for fractionation factors based on pure culture studies (we have presented the summarised values in Table S1, they are also summarised in the supplement to new perspective paper Yu et al., 2020 (https://onlinelibrary.wiley.com/doi/abs/10.1002/rcm.8858)) . These values can be also determined experimentally for the particular soil under study, at least for denitrification, but this is complex and time consuming. But importantly the literature fractionation factors for particular processes must be corrected with the substrate isotopic signatures, which should be determined for each soil study. The procedure of this correction is presented in Table S1. We have also extended the description of this correction and will move this whole paragraph to the methods section.

For the graphical presentation of dual isotope plots for sampling points always $\delta^{18}O$ and $\delta^{15}N$ values of emitted N_2O are plotted ($\delta^{18}O_{N2O}$, $\delta^{15}N_{N2O}$). But the precursors isotopic signatures ($\delta^{18}O_{H2O}$, $\delta^{15}N_{NO3-}$, $\delta^{15}N_{NH4+}$) are taken into account by respective correction of mixing endmembers isotopic ranges (see Table S1). The literature endmember ranges are given as isotope effects (ε) expressed in relation to particular precursor relevant for particular pathway, e.g. for $\delta^{18}O$ of bD the $\varepsilon_{N2O/H2O}$ is calculated by subtracting the precursor isotopic signature (δ_{H2O}) from the measured δ_{N2O} values:

$$\boldsymbol{\varepsilon}_{N2O/precursor} = \delta_{N2O} - \delta_{precursor} \quad (11)$$

e.g. for $\delta^{18}O$ of bD: $\delta_{N2O} = 10$, $\delta_{H2O} = -9$; $\boldsymbol{\varepsilon}_{N2O/H2O} = 19$

Afterwards, the literature isotope effects are corrected with the actually measured precursor values determined for the particular study ($\delta_{actual\ precursor}$) to determine the characteristic isotopic signature of N_2O emitted from the particular mixing endmember for this particular study conditions ($\delta_{N2O,\ endmember}$):

$$\delta_{N2O\_endmember} = \boldsymbol{\varepsilon}_{N2O/precursor} + \delta_{actual\ precursor} \quad (12)$$

e.g. $\delta^{18}O$ of bD: $\boldsymbol{\varepsilon}_{N2O/H2O} = 19$, $\delta_{actual\ H2O} = -6.4$, $\delta_{N2O\_bD} = 12.6$.

Hence, the endmember ranges represent the expected isotopic signatures of N_2O originating from each mixing endmember for the particular case study characterised by specific precursor isotopic signatures. Such approach allows for presenting all data in the common isotopic scales without presumption on the dominating pathway and dominating precursor. Hence, this new approach presented here is actually a further development of Maps, since this allows for correcting both Ni and bD, fD and nD endmembers with relevant distinct precursors, in contrast to only correcting measured values with one common assumed precursor isotopic signature. In previous papers, where $\delta^{18}O$ and $\delta^{15}N$ related to precursors ($\delta^{18}O_{N2O/H2O}$, $\delta^{15}N_{N2O/NO3}$) were plotted (Ibraim et al., 2019; Lewicka-Szczebak et al., 2017; Lewicka-Szczebak et al., 2016) it was assumed that denitrification must be the dominating N_2O production pathway.

We will also move the Table 1 into the main manuscript, since it contains important information for these corrections.

Line 686: Be specific of what shift is meant here? Temporal?

Yes, temporal shift, this will be added.

Line 820: This sentence is rather subjective. Is it possible to provide a more definitive range here? Can the authors make use of a sensitivity analysis to show the extent of substrate isotopic variations effects on the accuracy of the mapping approach?

This is quite a complex analysis – it has been done for SP/O Map (Wu et al., 2019 https://www.sciencedirect.com/science/article/abs/pii/S0013935119306036) but not yet for isotope Maps applying d15N. This is definitely the topic for the further work and it is planned to be done soon. Without a precise analysis it is not possible to provide a precise numbers here.

Line 825: Can the author suggest the lowest N2O fluxes without compromising the precision of isotope maps and the 2DI model? This will be helpful as a guideline for future studies wanting to use these approaches.

Based on our F1 and F2 field case studies we can say that where N_2O flux was mostly below 10 gN-N_2O $ha^{-1}d^{-1}$ the pathways partitioning was biased. This information will be added in the text.