

*Author's response on*

**“N<sub>2</sub>O isotope approaches for source partitioning of N<sub>2</sub>O production and estimation of N<sub>2</sub>O reduction – validation with <sup>15</sup>N gas-flux method in laboratory and field studies”  
by Dominika Lewicka-Szczebak et al.**

*Review response for Anonymous referee #1*

- (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.

We will take into account all the comments and especially clarify the endmembers isotope values corrections we applied. Here we provide detailed responses for the raised points, minor corrections have been accepted and will be included into reviewed manuscript.

*L70 I am not sure if I understand correctly. The observed isotope effect for N<sub>2</sub>O reduction is quite stable and doesn't depend on r N<sub>2</sub>O. Do you mean “change in N<sub>2</sub>O isotopic composition due to N<sub>2</sub>O reduction depends largely on y n<sub>2o</sub>”?*

Isotope fractionation during N<sub>2</sub>O reduction is independent of rN<sub>2</sub>O (mostly). But with the isotope effect we meant the change in N<sub>2</sub>O isotopic signature, as you suggest. To avoid confusion this will be changed in the manuscript to: **'the increase in  $\delta^{18}\text{O}$ ,  $\delta^{15}\text{N}$  and  $\delta^{15}\text{N}^{\text{SP}}$  of the residual N<sub>2</sub>O due to N<sub>2</sub>O reduction, is related to  $r_{\text{N}_2\text{O}}$ '**

*L77-78: I don't understand “N<sub>2</sub>O mixing ratio between two N<sub>2</sub>O production pathways and rN<sub>2</sub>O”.*

This will be changed to: **'N<sub>2</sub>O mixing proportions of two N<sub>2</sub>O production pathways and  $r_{\text{N}_2\text{O}}$ '**

*Section 2.1: has this experiment been conducted in 2015/2016. Please confirm if this is the procedure that has been applied in the past and state clearly that this paper reports on past campaigns and current incubations of the same soil.*

Yes, the field campaigns were conducted in 2015 and 2016, and the incubations in 2018. This information will be added: **'The soil for incubations, upper 30cm layer, was collected on the**

18.01.2018 from the experimental plot used previously for field campaigns and the incubation was conducted from 19.02.2018 to 05.03.2018.'

#### *Materials and Methods*

*L192 cf: On the one hand, water was added to obtain a certain water content in the soil. On the other hand, columns were continuously flushed with He/O<sub>2</sub>/N<sub>2</sub>. These gases are dry (i.e., very low dew points), so that they will take up significant amounts of water from the soil, considering a flush time of 5 days. As a consequence, the soil columns will dry out under this treatment. How did the authors account for this drying effect when referring to the WFPS values? Are the WFPS values an average or the initially values that was aimed at?*

The WFPS was analysed with mineral nitrogen analyses and do not show significant decrease. The given WFPS values are the actual measured values, not the theoretical target values. The results are presented in the supplement (Fig.S1). Water was also added in the middle of the experiment therefore the drying effect was not significant. The reference to the WFPS results shown in supplement will be added in this section: 'The WFPS values were controlled during the experiment (Fig. S1).'

*L210: I suggest mentioning that fluxes were calculated based on the dynamic chamber principle. Correction for the inlet concentration is omitted since the gas mixture used for flushing is N<sub>2</sub>O-free. Do I get this right?*

Yes, thank you, this will be added.

*L310: Please give a rationale for expecting higher fungal denitrification than Ni*

This is rather high soil moisture (>60% WFPS) and low ammonium content (Table 1). This explanation will be added in the text.

*L319: I suggest explaining what the cases are: Case 1 assumes reduction of N<sub>2</sub>O produced through bacterial denitrification and subsequent mixing, whereas case 2 assumes mixing and subsequent reduction of the mixed pool by denitrifiers. : : : if I am getting you right.*

Yes, this is right, but this is just explained a few lines above. I would like to rather avoid repetitions.

*L320: please explain c, and explain what happens if the condition is met.*

Sorry, this was a mistake in properly displaying of this formula, it should be:

$$L_{i+1}/L_i \geq \alpha,$$

'c' was a typo.

The condition of  $L_{i+1}/L_i \geq \alpha$  is the main concept of standard Metropolis algorithm. The newly generated state is accepted as a valid configuration if the likelihood function ratio  $L_{i+1}/L_i$  is greater or equal than a random variable  $\alpha$ . If the state is accepted it becomes a new

reference (appearing in the denominator) in the next iteration. If it is not accepted the next iteration is performed with unchanged value in the denominator in likelihood ratios.

### Results

*L421-425: Actually, I can't follow this short statement entirely. I understand that the authors aim at getting rid of the implicit assumption of DELTAdelta maps that the dominant source of N2O is denitrification. I thank the authors for this advancement.*

There was one mistake in this paragraph: wrong word 'precursor' was used instead of 'endmember' (L422), which probably made the understanding of this section difficult. We will correct this and further clarify the points below.

*Some unclarities remain however:*

*1. For a 18O/SP map, the endmember values for bD, nD and fD are changed by the same amount, -6.4 per mil of water. Correcting for 18O of water makes sense, if nitrate exchanges O with water completely. However, when looking in the literature cited, it seems like the endmember values in the literature have been corrected for 18O already. For this reason, and I want to discuss this with the authors, I suggest correcting for the difference in d18O water between the presented study and the body of literature. Please clarify.*

Since the O-exchange is usually high correcting with water makes more sense than with nitrate. Of course, if we knew the exact O-exchange this could be done more precisely, but usually it is not known. These effects and associated uncertainties are discussed in Section 4.1, L641-655. The cited literature values (Table S1) are the isotope effects – so, the isotope shift between the water applied and the emitted N2O – to avoid confusion we will denote this with  $\epsilon$ :

$$\epsilon_{\text{N}_2\text{O}/\text{H}_2\text{O}} = \delta_{\text{N}_2\text{O}} - \delta_{\text{H}_2\text{O}}, \text{ e.g. } \delta_{\text{N}_2\text{O}} = 10, \delta_{\text{H}_2\text{O}} = -9 \Rightarrow \epsilon_{\text{N}_2\text{O}/\text{H}_2\text{O}} = 19$$

We aim to calculate the expected N2O  $\delta$  values emitted from the particular pathways for our case studies, with  $\delta_{\text{H}_2\text{O}} = -6.4$  so need to calculate:

$$\delta_{\text{N}_2\text{O}} = \epsilon_{\text{N}_2\text{O}/\text{H}_2\text{O}} + \delta_{\text{H}_2\text{O}} = 12.6$$

All these values are presented in Table S1 in the supplement. But now I realise these are very important data for the study so this table will be moved to the main manuscript text and placed below the calculation explanations.

This more exact explanation will be added in the manuscript as well.

*2. Again, for a 18O/SP map, this correction is equivalent with a correction of the measured N2O 18O signature with water 18O, as correcting all endmembers for the same value is mathematically the same as correcting the measured values.*

We are not correcting all the endmembers with water, because  $\delta^{18}\text{O}$  for Ni depends on atmospheric oxygen (since this is quite stable we do not assume a stable value for this source). You are right – for the case of bD and fD mixing for SP/O Map it doesn't make a difference but it does for bD-Ni mixing and for all results of the 3DI model.

3. For  $^{15}\text{N}/\text{SP}$  maps, this is actually a further development, since this allows correcting both  $\text{Ni}$  and  $\text{bD}$ ,  $\text{fD}$  and  $\text{nD}$  endmembers, in contrast to only correcting measured values with one assumed precursor composition.

I am asking the authors to comment on this and make this clear in the text if they agree.

Please also explain the sign of your correction. It interferes with the definition of the apparent isotope effect in your reference 4 of the supplementary material (Sutka 2006).

Thank you, this statement will be added in the manuscript. But this works for both  $\text{SP}/\text{N}$  and  $\text{SP}/\text{O}$  Map - because  $\delta^{18}\text{O}$  for  $\text{Ni}$  in this approach is not corrected with water but can be corrected to the atmospheric oxygen (if different than mean value, which may be the case in e.g. aquatic studies).

We will add the definition of  $\epsilon$  to make the signs and recalculation procedures clear (the literature data is recalculated according to our definition). But I couldn't find the definition of the apparent isotope effect in (Sutka et al., 2006) and any supplementary material to this paper.

After addition of clarifications this paragraph will expand and will not fit anymore to results section. It will be moved to Methods section 2.5, as:

For the graphical presentation of dual isotope plots for sampling points always  $\delta^{18}\text{O}$  and  $\delta^{15}\text{N}$  values of emitted  $\text{N}_2\text{O}$  are plotted ( $\delta^{18}\text{O}_{\text{N}_2\text{O}}$ ,  $\delta^{15}\text{N}_{\text{N}_2\text{O}}$ ). But the precursors isotopic signatures ( $\delta^{18}\text{O}_{\text{H}_2\text{O}}$ ,  $\delta^{15}\text{N}_{\text{NO}_3^-}$ ,  $\delta^{15}\text{N}_{\text{NH}_4^+}$ ) are taken into account by respective correction of mixing endmembers isotopic ranges (see Table S1). The literature endmember ranges are given as isotope effects ( $\epsilon$ ) expressed in relation to particular precursor relevant for particular pathway, e.g. for  $\delta^{18}\text{O}$  of  $\text{bD}$  the  $\epsilon_{\text{N}_2\text{O}/\text{H}_2\text{O}}$  is calculated by subtracting the precursor isotopic signature ( $\delta_{\text{H}_2\text{O}}$ ) from the measured  $\delta_{\text{N}_2\text{O}}$  values:

$$\epsilon_{\text{N}_2\text{O}/\text{precursor}} = \delta_{\text{N}_2\text{O}} - \delta_{\text{precursor}} \quad (11)$$

e.g. for  $\delta^{18}\text{O}$  of  $\text{bD}$ :  $\delta_{\text{N}_2\text{O}} = 10$ ,  $\delta_{\text{H}_2\text{O}} = -9$ ;  $\epsilon_{\text{N}_2\text{O}/\text{H}_2\text{O}} = 19$

Afterwards, the literature isotope effects are corrected with the actually measured precursor values determined for the particular study ( $\delta_{\text{actual precursor}}$ ) to determine the characteristic isotopic signature of  $\text{N}_2\text{O}$  emitted from the particular mixing endmember for this particular study conditions ( $\delta_{\text{N}_2\text{O, endmember}}$ ):

$$\delta_{\text{N}_2\text{O, endmember}} = \epsilon_{\text{N}_2\text{O}/\text{precursor}} + \delta_{\text{actual precursor}} \quad (12)$$

e.g.  $\delta^{18}\text{O}$  of  $\text{bD}$ :  $\epsilon_{\text{N}_2\text{O}/\text{H}_2\text{O}} = 19$ ,  $\delta_{\text{actual H}_2\text{O}} = -6.4$ ,  $\delta_{\text{N}_2\text{O, bD}} = 12.6$ .

Hence, the endmember ranges represent the expected isotopic signatures of  $\text{N}_2\text{O}$  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  $\delta^{18}\text{O}$  and  $\delta^{15}\text{N}$  related to precursors ( $\delta^{18}\text{O}_{\text{N}_2\text{O}/\text{H}_2\text{O}}$ ,  $\delta^{15}\text{N}_{\text{N}_2\text{O}/\text{NO}_3}$ ) 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  $\text{N}_2\text{O}$  production pathway.

*L434: Please define minimum reduction line. I guess it is the dashed red line, but it hasn't been defined in the figure caption.*

This definition will be added, it is indeed the dashed line. Sorry for the missing information.

*L461: there is a large discrepancy of field and laboratory  $\text{NH}_4$  delta values. This observation should also be taken up in the discussion again.*

This is most probably due to differences in fertilizer addition techniques. In field studies the fertilizer solution was injected into the soil intact columns and in laboratory studies it was mixed and afterwards packed into the vessels. In both studies we observe a very fast decrease in ammonium content which is most probably due to its adsorption. The more detailed interpretation of  $^{15}\text{N}$  experimental results including Ntrace model is ongoing, but probably during mixing this adsorption process is more enhanced when compared to injection technique. 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  $^{15}\text{N}$ - $\text{NH}_4$  treatment which was not presented here. This information will be added in the manuscript.

*L495: In my opinion, the pie charts are i) not well described and ii) consume more space than necessary. If the authors stick to the pie charts, I ask them to indicate that the share of  $\text{N}_2$  produced during  $\text{N}_2\text{O}$  reduction is given as  $\text{N}_2$  in percent. Hatched superimposed patterns show the source processes for  $\text{N}_2\text{O}$  that has been reduced to  $\text{N}_2$ . All in all, my suggestion is showing 2 bar plots (case 1 and case 2) with categories bD (bd $\text{N}_2\text{O}$ , bd $\text{N}_2$ ), nD, fD, Ni,  $\text{N}_2$ .*

Thank you for the nice idea with bar plots. We hoped to nicely present the comparison between cases with the pie plots, but apparently did not succeed with this idea, we fully agree that bar plots are better for this aim. This will be changed and such new Figures will be included in the manuscript:

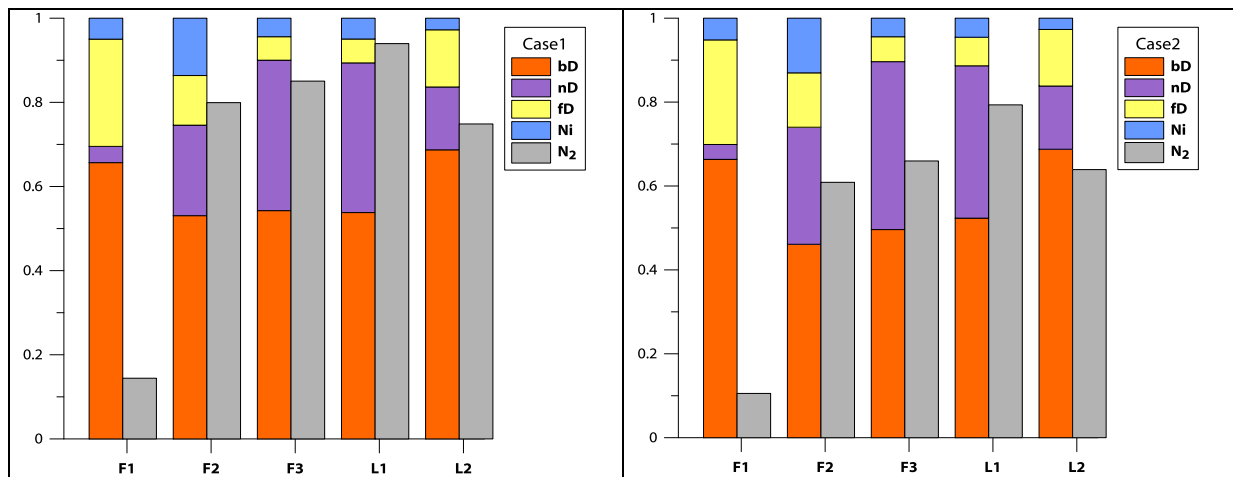


Figure 5: figure 5 gives everything but an overview of the results. I suggest showing only one case for Laboratory or field and instead have the numbers intelligible, and annotate scales. Please only show significant correlations, and don't correlate font size with correlation strength.

Ok, I see your points. I think we can fully move this Figure to the supplement. Since the number of points (individual iterations) is very high – ca. 1000 points – most correlations are significant, even showing low R values. These are also standard graphs for the isotope mixing models introduced by the trophic nets research and we wanted to keep the idea similar. Showing 2 graphs only will not be informative, and actually the needed information is referred in the text and all the graphs will be presented in the supplement.

L569: why is  $p < 0.1$  considered as significant? I suggest rephrasing the sentence, proved sounds odd in terms of statistical inference. The  $p$  agreement between reference method and SP/O Map ( $p < 0.1??$ ) as well as Case 2 of 3DI model ( $p < 0.05$ ) was statistically significant.

$p < 0.1$  may be assumed as statistically significant, we have changed this in the statistical methods, Section 2.7. Although only  $p < 0.05$  is usually accepted as significant, here we think that the values obtained for SP/O Map are also important indication and should be accepted, with a clear statement that this statistical significance is weak ( $p$  values are shown). The sentence will be corrected.

Discussion L718-720: could the authors please explain how the uncertainty was actually transferred in the model. In line 331-332, the authors state that “: : uncertainties of the source's data ifs fed into the model through the variance in the calculation of unnormalized likelihood”. However, I don't understand then the following sentence, that the prior distribution was assumed uninformative. Does this mean that for each endmember isotopic composition, the whole range of values was allowed? Please clarify how this actually works.

The sentence ‘the prior distribution was assumed uninformative’ is misleading. It refers to starting values for the model – we do not assume any preference for any pathway, that's why flat Dirichlet distribution is applied. We will modify this sentence to avoid confusion.

We have applied the sources data with their uncertainty into the model, as defined in Tab S1.

### *Conclusion*

*L863-864: I think this needs to be rephrased. The authors haven't presented a triple isotope plot, but rather present a likelihood function that allows for including three isotopic quantities and 4 associated isotopic endmembers.*

This sentence will be corrected to:

- Here we present for the first time the idea of applying a model based on three N<sub>2</sub>O isotopic signatures. We are convinced that this is a powerful step forward in development of N<sub>2</sub>O isotopocule methods to quantify especially  $r_{\text{N}_2\text{O}}$ , but also estimate some mixing proportions of the four N<sub>2</sub>O pathways included in the model.