Review response on "Isotopic fractionation of N2O to quantify N2O reduction to N2 – validation with Helium incubation and 15N gas flux methods" *by* Dominika Lewicka-Szczebak et al.

Anonymous Referee #1

Referee comments in red,
authors response in black.

Thank you very much for reviewing our manuscript and for your valuable suggestions, which allow us to improve the quality of the paper. We are very happy about your positive consideration of our manuscript. Below please find the response to your specific comments.

2. Specific comments

P9, L13 and eq. 5: It is not clear whether the N2 originating from non-labelled pools are considered similarly in the case of N2 because evidence of such N2 is described in section 3.2.2.

Unfortunately not. The method applied do not allow this because of the background unlabelled N2 present in the sample that cannot be distinguished from the N2 originating from unlabeled pool.

P9, eq. 6: In P8, the authors describe that three separated gas species (N2, N2+N2O, and N2O) were measured. Then why is fp_N2O not used in this equation? Or did they confirm mass balance like fp_N2+N2O = fp_N2 + fp_N2O?

The described calculation approach appeared to be more precise, because the quantification of fp_N2+N2O and fp_N2 base on the same method (similar peaks in the measurement procedure) and fp_N2O is determined from a different peak. Details are described in Lewicka-Szczebak et al., 2013, RCM. The balance was confirmed. This explanation will be added in the manuscript.

P11, L4: "average 15N abundance in nitrate" What was averaged? Initial and final values?

Yes. This will be clarified.

P18, section 3.2.2: Although the calculation procedure for r_N2O is explained in detail in section 2.5.2, results of r_N2O from the 15N treatment are not shown. Does this mean the 15N gas flux method failed to give r_N2O value?

These results are shown in the Fig. S2 together with the isotopomer results. We showed them there to allow for direct comparison with isotope data. But now we realise that this was a bad idea, and this should be shown in the graphs concerning 15N experiment (S3). This will be changed. This results are used all over the manuscript as a reference data for residual fraction for Exp.2.

P23, L6: "r_N2O values are always higher for Sc2" This is consistent with Figure 8, but I found the opposite statement in P16, L19. Please check the text and the figure.

True is: Sc 1 show lower rN2O, hence more N2O was reduced. P16, L19 must be corrected.

P23, L23: “for both soils in the anoxic treatment the cumulative non-labelled N2O flux is higher than the initial NH4+ pool plus the NH4+ possibly added” I could not follow. When I compare the 8th, 12nd, and 13th columns in Table S1, this is the case only for Min Soil with anoxic, 15N treatment.

This statement is true, but we poorly presented the units. The fluxes in Table S1 are given as rate for 24h, the unit should be described as [mg N kg^{-1} d^{-1}]

P24, L6: “it represents, respectively, 2 and 3 % of the nitrification rate (Table 1)” I cannot understand how I find this in Table 1. What does “respectively” means?

There is a mistake at the beginning of this paragraph – should be ‘oxic treatments’ instead of ‘anoxic’, which will be corrected. ‘Respectively’ means for Min and Org soil. It was calculated as: 0.01/0.3 = 0.033 and 0.07/1.93 = 0.036 – slight difference occurs due to rounding of the original values.

P24, L8: “observed increase in NO3-“ In Table S1, C_NO3t is always lower than C_NO30, so there seems to be no “increase in NO3-“. P27, L8: “15N-pool derived N2O characterized by higher d15Nsp values” In section 2.5, the authors did not mention that they measured d15Nsp of N2O in 15N gas flux method. Did they measure it?

This is based on the 15N dilution method. The concentration of nitrate decreases due to its consumption but based on 15N dilution we calculate how much nitrate was added to the nitrate pool. This will be clarified in the manuscript.

P32, L27: It seems that 15N gas flux method is useful to detect the processes such as producing hybrid N2O or N2, but I’m not convinced that it is really necessary to determine r_N2O (see above comment on section 3.2.2).

This was very useful and used in the manuscript as the main reference method for quantification of rN2O. We will add this data on the respective graphs (S3), and pay attention to make this clear in the manuscript. This information is very important and must be emphasised, we really regret that this was not sufficiently described.

3. Technical corrections

All the required corrections will be included in the manuscript. Thank you very much for your careful check.

Anonymous Referee #3

Referee comments in red,
authors response in black.

Thank you very much for reviewing our manuscript and for your valuable suggestions, which allow us to improve the quality of the paper. We are happy to hear you appreciate the scientific value of the paper and will do our best to improve the presentation of the material according to your suggestions.

The sentences were often long and awkwardly written.

We will rewrite the awkward sentences and improve the language quality of the paper.

Additionally, many of the terms were not well defined and described early in the manuscript. Then when the results were presented it was difficult to understand what each variable meant, and why they were important.

We will pay attention to precisely define each term in the manuscript.

The figures were also cluttered with too much information. I would have liked to seen there be more things distilled down for the reader, rather than showing all the data and every experiment. I wonder if all the experiments or data should be presented in one paper or if some of this information would be best split into multiple manuscripts.

We will try to simplify the figures, but we would not like to remove any data from the manuscript. We had been also thinking about it before to split the paper, but we decided, this makes not much sense, since the data are all connected and needed to check the performance of the reduction fractionation and mapping approaches (*i.e.,* isotope data to calculate SP and $d^{18}O$ of produced N_2O and its uncertainty from Exp1 and Exp2, ^{15}N tracing data from parallel experiments – Exp2 - for independent estimates of N_2O reduction as well as formation of hybrid N_2 and N_2O to check for pathways other than bacterial denitrification, N_2 fluxes from He incubations – Exp1 - as the most precise independent measure of N_2O reduction).

We believe it would be even more difficult for the reader if the data were split into separate papers. We wanted to present possibly all the basic data, because the methods we are describing are still under development. But all this very detailed information is placed in the supplement, and only readers especially interested in some particular points will need this. The paper should be understandable without this additional detailed information placed in the supplement. We will check the manuscript carefully, if this is the case.

Specific Comments:

P1 L1- I would suggest editing the title to make it catchier and less awkward.

Our new idea for the title is: Quantifying N2O reduction to N2 based on N2O isotopocules – validation with independent methods (Helium incubation and 15N gas flux method)

P1 L10- Rephrase, “the main unknown magnitude”

It will be rephrased to ‘a missing quantity’.

P1 L11- Define in the abstract what the residual fraction is.

The definition will be added – remaining unreduced N2O.

P2 Introduction- Add more description on the importance of being able to determine N2O reduction. Also, give a better background on all the important terms to be later used in the manuscript and why they are important. A figure or table might be helpful for showing previous work and how the terms fit into the overall picture.

This information will be added: N2O reduction to N2 is the key quantity of N cycle that is poorly quantified, cause loss of fertilizer N and lowering of N leaching, and is the least well understood N flux. N2O reduction is crucial to know in order to close the nitrogen budget. We actually gave a background of the important terms discussed later, but we will try to better explain their importance.

P2 L4-6, P3 L1-3, P3 L26-28- Long and awkward sentences, consider rewriting.

They will be corrected as follows:

P2 L4-6 Commonly applied analytical techniques enable us to quantitatively analyse only the intermediate product of this process, N_2O, but not the final product, N2. This is due to the high atmospheric N2 background precluding direct measurements of N_2 emissions.

P3 L1-3 Its advantage over the ^{15}N gas flux method lies in its easier and non-invasive application, no need of additional fertilization, and much lower costs. This expands the application potential of the isotopic fractionation method and enables its more widespread use.

P3 L26-28 There are still some open questions: (i) whether the isotopic fractionation factors for denitrification processes determined in laboratory experiments are transferable to field conditions, (ii) how robustly the N_2O residual fraction can be determined and (iii) whether the quantification of the entire nitrogen loss due to denitrification is possible.

P4 L19- The heading title has “experiment 1 and Exp 1” I would only write it once in the title.

This will be corrected, we wanted to introduce the abbreviation by the first mention of the particular experiment, and this is in this subtitle. But we will introduce this in the main text.

P5 L2- Missing “a” in “application of a N2-free atmosphere”

The mistake will be corrected.

P5 L15- Fix section heading, as above.

This will be corrected.

P5 L21- Why is the nitrate treatment different in Exp2 than Exp1?

In Exp2 we applied more nitrate because this experiment lasted longer and the nitrate amendment was proportionally higher to ensure we have residual nitrate for analyses at the end of the experiment. We will add this explanation in the manuscript.

P6 L8- Define NA.

It was defined before, P5, L24.

Supplemental Figures S1 and S2- I suggest removing some of the variables from the figures and putting a simplified figure in the main text. I was also confused with the labeling and order of the figure S2, such that it went from 2.1 a) to 2.2 a) and then back to 2.1 b), could you combine panels onto 1 page and make them a b c and d?

The Figures will be improved and labelling will be corrected as suggested. We will delete the information about the residual N2O fraction (rN2O) from the graphs showing data from NA Experiments, since this information is gained from 15N Experiments and we realised it makes confusion. This will be shown in graphs for 15N experiments.
But we would not like to include this graphs in the paper main text, because this is lot of basic data, and they are all shown in other graphs in the paper, but not as time series. We believe the detailed time series are not the most important to show in the paper. This will make the reading rather more difficult.

P14 L24- In the "mapping approach" how much will the answer change if you use different end member values? The boxes for possible values are large and suspect it could be large.

Yes, this is a very important issue, but also quite complex, it will be addressed in the following paper, to be submitted very soon to Biogeosciences: *Buchen, C., Lewicka-Szczebak, D., Giesemann, A., Well, R., in preparation. Estimating N2O processes during grassland renovation and grassland conversion to arable land using N2O isotopocules*. In that manuscript we present the possible range of results taking into account the wide ranges of endmember values and also fractionation factors due to reduction. We will add the respective citation in this manuscript.

P15 L2-7: What is the value are you referring to in this paragraph?

To the values given above: from 17.4 to 21.4 ‰. We will clarify this.

P16 L1: I was surprised that the reduction isotope ratios were the same for oxic and anoxic incubations. Why is that?

The same process in both incubations is responsible for the N2O reduction. Oxic incubations were conducted in very humid conditions, hence, even for the oxic atmosphere many of soil microsites will maintain anoxic conditions.

P19 L3: What is N immobilization?

Transfer of mineral nitrogen into organic nitrogen forms. It has been defined earlier in the manuscript, in Section 2.6, Eq. (16).

P19 L17: What is hybrid N2O? Could you define it earlier in the manuscript?

It has been defined earlier in the manuscript, in Section 2.5.2, Eq. (10,11).

P20 L4: What correlations?

Correlations between N2O residual fraction r_{N2O} and measured δ_r values. It was explained in 2.7.1, but we will repeat this information in the discussion.

P21-22: What are the differences between Val 1 and Val 2? Can you state them more clearly before presenting the results?

This was explained in the method section in 2.7.2, P14, L1-9.

P22 L25: Could the data in Table 3 be put into a simplified graph in the main text? That might be helpful for the reader.

We do not really see the possibility to present this information on a simple graph, because the table lists the results of 13 functions from several experiments. The graphs are presented in the supplement, and we will add this link to the heading.

P25 L10-15: I'd suggest putting the historical data in a table with the current findings.

We just wanted to indicate some typical problems and compare to our results. We will try to make the description more clear and add a table presenting 15N contents in soil nitrate and the released N2O in our and previous studies.

P27 L22: The title "Calibration and Validation" is vague, calibration and validation of what?

"-of rN2O quantification" - will be added to this subheading

Table 1: I suggest putting the full names of the variables in the table header row.

It will be corrected as suggested

Figure 2 and 3: Why is the x-axis on the right hand side?

No reason, we will move the y-axis on the left hand side.

Figure 4, 6 and 8: Symbols are similar and hard to distinguish in the figure.

We will use larger symbols.

Figure 5: There is no legend.

The legend will be added.