This manuscript presents the new Expanded Rayleigh model applicable for N2O synthesis for proper calculation of isotope effects regarding 2 different positions of N. This is very important development for enzymatic studies on N2O production in various pathways. It is very surprising that the differences while using classical Rayleigh model and the new developed Expanded Rayleigh model are so significant. It shows that it is highly necessary to apply this new calculation approach in such kind of studies.

The manuscript is clearly written and appropriate for publication in BG after minor edition.

Response: Thank you for your time and careful reading of our manuscript. We are happy to know that you find the manuscript clearly written and appropriate for publication in *Biogeochemistry*.

I have only a few minor comments.

L 46 s in a particular chemical compound – which chemical compound when you write about N2O, you mean any other? – info on compound-specific stable isotope analysis can be omitted in your introduction, since you do not need this afterwards

Response: As suggested, we have deleted "compound-specific" and the corresponding definition from the introduction.

Manuscript changes (*new text italicized*): "*One useful technique for N$_2$O source apportionment is stable isotope analysis.*"

L116 – please delete "*1000" from the equation, it shouldn't be part of the δ definition, is just the way of presenting in permil notation, see eg.
https://analyticalsciencejournals.onlinelibrary.wiley.com/doi/pdf/10.1002/rcm.8890

Response: The expression for δ can be written to define either the unitless form (without multiplying by 1000) or the per mil form (defined by multiplying the unitless form by 1000). Indeed, the original definition of δ was written using the per mil notation (Mckinney et al., 1950), and this definition has also been used in a more recent review (Casciotti et al., 2011). As δ values are commonly expressed in per mil, we have written many other equations in this manuscript to accommodate per mil δ notation (*e.g.*, Eq. 1 and Eqs. 27-30). Thus, to make it clear which form of δ we use throughout the manuscript, we included the multiplication by a factor of 1000 in our definition of δ (Eq. 6). We have also changed the manuscript text to make it clear that the per mil δ notation is used.

Casciotti, K. L., Buchwald, C., Santoro, A. E., and Frame, C.: Assessment of nitrogen and oxygen isotopic fractionation during nitrification and its expression in the marine environment, Methods in enzymology, 486, 253-280, 10.1016/b978-0-12-381294-0.00011-0, 2011.

McKinney, C. R., McCrea, J. M., Epstein, S., Allen, H. A., and Urey, H. C.: Improvements in mass spectrometers for the measurement of small differences in isotope abundance ratios, Rev Sci Instrum, 21, 724-730, 10.1063/1.1745698, 1950.

Manuscript changes: "Because there is naturally little variation in ^{15}N, $R_{sample}/R_{standard}$ values are typically close to 1, and isotopic ratios are usually converted to the δ notation, *which is commonly expressed in the per mil form (‰) by multiplying the unitless form of δ by 1000*:

$$\delta = \left(\left(\frac{R_{sample}}{R_{standard}} \right) - 1 \right) * 1000 \tag{6}$$

where R_{sample} is the isotopic ratio of the sample, and $R_{standard}$ is the isotopic ratio of the analytical standard (Mariotti et al., 1981). *The per mil form of δ is used throughout this manuscript.*"

Mariotti, A., Germon, J. C., Hubert, P., Kaiser, P., Letolle, R., Tardieux, A., and Tardieux, P.: Experimental determination of nitrogen kinetic isotope fractionation: Some principles; illustration for the denitrification and nitrification processes, Plant Soil, 62, 413-430, 10.1007/BF02374138, 1981.

On a related note, while reading the paper the reviewer referenced above (Skrzypek and Dunn, 2020), we realized that it would be more accurate to use 0.0036765 as the value of $R_{standard}$ instead of 0.003663 (atom percent for ^{15}N) (Mariotti et al., 1981). As the two values are very similar, using 0.0036765 had essentially no effect on the outcome of our calculations. We have, however, updated the manuscript text and corresponding calculations to be as accurate as possible.

Manuscript change (Section 2.1): "*(Atom percent of ^{15}N in atmospheric N_2 (air) is 0.3663 ± 0.0004%, so R = 0.0036765) (Junk and Svec, 1958; Mariotti et al., 1981; Skrzypek and Dunn, 2020).*"

Junk, G. A. and Svec, H. J.: Nitrogen isotope abundance measurements, US atomic energy commission. Office of technical information., ISC 1138, 1958.

Mariotti, A., Germon, J. C., Hubert, P., Kaiser, P., Letolle, R., Tardieux, A., and Tardieux, P.: Experimental determination of nitrogen kinetic isotope fractionation: Some principles; illustration for the denitrification and nitrification processes, Plant Soil, 62, 413-430, 10.1007/BF02374138, 1981.

Skrzypek, G. and Dunn, P. J. H.: Absolute isotope ratios defining isotope scales used in isotope ratio mass spectrometers and optical isotope instruments, Rapid Communications in Mass Spectrometry, 34, e8890, https://doi.org/10.1002/rcm.8890, 2020.

L121 – the equation is mathematically not true, you define Nbulk as total number of atoms and then this is equal mol N2O?! When mol represents 6.022*10²³ atoms! You mix here up different units. This is not equal. -The same wrong statement you have later in L 225

Response: The main goal of equation 7 was to express the relationship between N^{bulk} and N^{α} and N^{β} (*i.e.*, $0.5*N^{bulk} = N^{\alpha} = N^{\beta}$). This relationship remains the same whether N is quantified in terms of atoms or moles/equivalents. However, as the reviewer correctly points out, because N^{bulk} is formally defined as $0.5*mol\ N_2O$ in Eq. 7, we should avoid using "atoms" and "moles" interchangeably. Thus, we have changed the text to match Eq. 7 by defining N^{bulk} as the total number of moles of N at the α and β positions. Note that adopting this definition means the text from L225 does not need to be changed.

Manuscript changes: "*N^{α} and N^{β} are defined as the number of moles of N at the α (central) and β (outer) positions of N_2O, respectively.*

$$0.5 * N^{bulk} = N^{\alpha} = N^{\beta} = mol\ N_2O \qquad (7)$$

Thus, $^{15}N^{bulk}$ is defined as the total number of moles of ^{15}N at both the α and β positions:

$$^{15}N^{bulk} = {}^{15}N^{\alpha} + {}^{15}N^{\beta} \qquad (8)$$

Similarly, $^{14}N^{bulk}$ is defined as the total number of moles of ^{14}N at the α and β positions of N_2O:

$$^{14}N^{bulk} = {}^{14}N^{\alpha} + {}^{14}N^{\beta} \qquad (9)$$"

Fig 4 and 5 it would be interesting to see the comparison of values calculated with normal and expanded Rayleigh model

Response: With the exception of the $\delta^{15}N^{s0}$ values shown in Fig. 5, all of the δ values shown in Figs. 4 and 5 were experimentally measured and are the same regardless of which model is applied. The $\delta^{15}N^{s0}$ values from Fig. 5 were back-calculated from $\delta^{15}N^{bulk}$ values using the standard Rayleigh model (Eq. 2), which can be accurately applied to relate $\delta^{15}N^{bulk}$ to $\delta^{15}N^{s0}$. Note that the Expanded Rayleigh model also uses Eq. 2 for bulk $\delta^{15}N$ values, so these $\delta^{15}N^{s0}$ values are the same regardless of which model is used. Tables 5-6 present comparisons of values calculated by applying the standard and Expanded Rayleigh models to the data from Figs. 4-5, and the differences between the two models are described in section 3.3, "Application of the Expanded Rayleigh model to previously published isotopic data."

L 410-418 You present here the extreme cases with strong differences in fractionation in alfa and beta position, of course, then the differences between both calculation models are large. However, I wonder if these cases are realistic in any way? Is there any possible process which shows that different isotope discrimination in both positions?

Response: In addition to the simulated datasets, we evaluated two experimental datasets with fairly large differences in isotope discrimination between the α and β positions. The differences between position-specific KIE values calculated for the standard and Expanded Rayleigh models are similar for the simulated datasets and the experimental datasets. (Absolute relative differences between standard KIE $^{15}N^{\alpha}$ or KIE $^{15}N^{\beta}$ values and Expanded Rayleigh model values range from 0.01-0.04 for simulated datasets and from 0.02-0.04 for the experimental datasets.) As with the simulated data, the differences between the values calculated for the two models are

large enough to be of practical significance for the experimental datasets: Some of the experimental standard Rayleigh KIE values for N^α and N^β are qualitatively inaccurate, causing incorrect determination of the type of isotope effect at either the α position (Table 6) or β position (Table 5). In contrast, the Expanded Rayleigh KIE values correctly identify the type of position-specific isotope effect for both positions (as determined by qualitative comparison of $\delta^{15}N^{s0}$ with $\delta^{15}N^\alpha$ and $\delta^{15}N^\beta$).

L432 – the sentence from "To address this issue, we have developed…" is a repetition of what you already said in methods section, not needed here. Please pay attention to omit unnecessary repetitions

Response: The sentence in question briefly summarizes how the Expanded Rayleigh model differs from the original Rayleigh model (*i.e.*, by the addition of new parameters, ρ and τ). Because the remainder of the paragraph discusses the results for ρ and τ calculations, we feel it is appropriate to include this statement to remind the reader what these newly defined terms mean. Nevertheless, to address the reviewer's concern, we shortened this sentence to avoid unnecessary repetition.

Manuscript change: "*To address this issue, we developed the Expanded Rayleigh model that introduces two new parameters, ρ and τ, to define how $^{15}N^{bulk}$ and $^{14}N^{bulk}$, respectively, are apportioned between N^α and N^β.*"

Table 5 – what is Expanded 1 and 2? Please explain in Fig caption or footnotes

Response: We have modified footnote e to highlight the difference between Expanded Rayleigh models 1 and 2. Please see the new sentence at the end of the footnote below. We have also changed the model descriptions in the first column of Tables 5 and 6 to clarify which model is being used (*i.e.*, "Standard Rayleigh" or "Expanded Rayleigh").

Manuscript changes: Table 5 footnote e: "For the Expanded Rayleigh model, bulk values ($\alpha_{N\text{-bulk}}$, $\varepsilon_{N\text{-bulk}}$, and KIE $^{15}N^{bulk}$) were determined with the standard Rayleigh approach, ρ was determined via nonlinear regression, and τ was determined by averaging $^{14}N^\alpha/^{14}N^{bulk}$ for every step of the reaction. Then $\alpha_{N\text{-}\alpha}$ and $\alpha_{N\text{-}\beta}$ were calculated with Eq. (21) or Eq. (22) and converted to KIE values using Eq. (10). *The only difference between Expanded Rayleigh models 1 (Eq. (29)) and 2 (Eq. (30)) is which δ value ($\delta^{15}N^\alpha$ or $\delta^{15}N^\beta$) is substituted with a ρ-containing expression in the nonlinear model.*"