Response to Anonymous Referee #4
The authors' answer is in red fond.

Summary
The authors present an analysis of oxygen isotope dynamics and fractionation during biological N2O production in soils. Using a variety of approaches, they aim to discern the location and magnitude of fractionation mechanisms including 1) equilibration of N-oxyanion intermediates with water, 2) kinetic isotope effects during each step of denitrification, as well as 3) branching isotope effects during abstraction of oxygen atoms at each of these steps. The authors incorporate both a 17O approach and 18O labeled water experiments as well as acetylene inhibition in the context of both batch and flow-through incubations. In particular, I feel that this manuscript does a good job of laying out the oxygen isotope dynamics involving both 'intra-' and 'inter-molecular' fractionation mechanisms and clarifying the meaning behind 'branching' isotope effects in the context of denitrification. In general, I think the discussion about intra- and intermolecular isotope effects is among the most valuable parts of this manuscript. The idea of fungi contributing to N2O production is also intriguing, especially if there is the possibility of teasing their role apart from bacteria. Overall, I think the paper could benefit from some clarifications in many areas – as I have noted below. [Although the presentation of the results and interpretation is generally clear – there are sections of the text (especially, the discussion) that would benefit from editing by a native-English speaker.]
Thank you very much for your positive opinion on the manuscript and for critical comments, which will be very helpful to improve the manuscript.

General Comments
Based on my understanding (and also the reading of Casciotti et al., (2007)), the exchange of O atoms between nitrite and water occurs as the result of the chemical dissociation of nitrous acid and is enhanced depending on their respective equilibrium concentrations. With a pKa value of _3.4, a lower pH accelerates the exchange of oxygen atoms because the pool size of nitrous acid increases, increasing the rates of forward/reverse equilibrium reactions between nitrite and nitrous acid (during which O atoms are lost/gained). This pH influence is well-known in other oxy-anion systems as well (sulfate, carbonate and phosphate, etc.). Given this important control on oxygen isotope equilibrium dynamics, I am surprised that the authors have not reported pH values for their soil incubations and solutions. Can more consideration be included about the pH of these soils and the porewaters – and their possible role in the oxygen isotope dynamics?
Information about soil pH values will be added in the manuscript: loamy sand: 5.7, silt loam: 7.4, organic soil: 5.9, sandy soil: 5.3. In Exp.1 oxygen exchange for loamy sand and silt loam is not significantly different, while the difference in soil pH is pronounced. Also for Exp.2 no relation between pH and isotope exchange could be found.

The authors report traditional 'delta' and 'epsilon' values in units of 'permil,' yet in all of the equations the authors use 'un-normalized' delta and epsilon values. Perhaps this is simply style issue – but I feel that it can lead to confusion. For example – in the text when the branching isotope effect is estimated as '17‰´-- this is not the numerical value that is used in the equations throughout. At a minimum, some clarity might be provided by stating how the values should be converted (e.g., not multiplied by 1000). For example, on P 18, L 16 – here the epsilon values which were reported on P 15, L2 as "18.2‰´' and "17.1‰´' are being reported as equal to 0.0181 and 0.0172. While I understand the desire to somewhat simplify the equations – there appears to be some inconsistency – which I think would be very confusing to the casual reader.
It will be clarified in the methods section. Permil values are just the fraction values multiplied by 1000 ‰.
On P 18, L 16 - values from Fig.2 - this is a mistake! Should be 0.0182 and 0.0171, sorry, this will be corrected.

P 4 L5-10: In general I think it would be good to be clearer about how epsilon-n here is calculated (e.g., I think it would be useful to see this mathematically expressed).
It is just a sum of both intra and intermolecular effect. I do not think introducing an extra equation in introduction would be needed, as it would be just epsilon-n = intramolecular effect + intermolecular effect, associated with each reduction step. We will clarify this better in the text.

P 5 L 11-13: This sentence seems out of place.
I think the use of the word "Dynamic" incubations seems a little misleading – perhaps consider using 'steady-state' or 'open-system' or 'flow-through' experiments instead? I think of 'dynamic' as indicating an important changing parameter – whereas here conditions are held constant (with the exception of the temperature and perhaps soil moisture).

Good idea, thanks for this suggestion, this will be changed to 'flow-through' experiments.

Does one need to account for the non-random 17O in the calculations of Site Preference?
Or does the low abundance of 17O not impact the accuracy?
The ^{17}O correction changed Site Preference values of up to 0.4 permil. It has been applied for Exp2 data, where the measured $\Delta^{17}O$ in N_2O is pronounced - up to 5.3 permil. For Exp1 the measured $\Delta^{17}O$ in N_2O is very low, hence the correction was not needed.

P 20 L 11: Here the authors conceptualize oxygen isotope exchange as occurring 'later than' the branching isotope effect. However, in the equations – they are mathematically occurring simultaneously. Indeed, would it not be probable that the O abstraction and exchange with water are occurring as the result of the same enzymatic process – and that the 'intra-' and 'inter-molecular' isotope effects are related (while not necessarily their fractional contributions). At least for a single organisms or class of enzymes – I would think this would be the case.
Of course, in one step the both effects may occur simultaneously, but in this sentence we compare the exchange at NIR and branching occurring afterwards at NOR, as is assumed in scenario 1. Branching associated with NOR must occur later, after formation of NO.

P 20, L 25: "Out of plausible range of values" – please include reference to your line of reasoning here (e.g., based on what?).
The references providing plausible range of values (Casciotti et al., 2007; Rohe et al., 2014a) will be added.

P21 L 10-15: Something is not clear about these statements. I understand how the effect observed by Casciotti (2007) represents only the 'intra-molecular' effect – (e.g., the O abstraction) – and that Casciotti (2007) refers to this as the branching effect. Here the authors then refer to this as being the 'maximal possible branching effect' – which also makes sense. Then, referring to the work by Rohe et al (2014), since the NO3- pool is not completely consumed – both the 'inter-' and 'intra-molecular effects' should be observed. But I fail to understand the next statement about the values of e-NIR of 10‰ and eNAR assumed to be 0‰ – and how this supports their observations. Please clarify.
These are values indicated by the model applied in that study. The lower values obtained indicate that the maximal branching effect was partially compensated by intermolecular effect, which results in lower values for net branching effect. This will be better clarified in the revised manuscript.

Regarding the source of the positive _17O values others have observed in atmospheric N2O, this would imply denitrification of specifically atmospherically derived NO3-. Is this a reasonable assumption? Their data indeed show that a large degree of the original NO3- oxygen isotope composition is erased during equilibration with water – which has important implications for the transfer (or not) of this signal to N2O. So – to the degree that this signal is not erased, it would be possible to retain some amount of this _17O signal. However, does the size of the _17O signal in N2O represent a reasonable fraction of N2O derived from NO3- of atmospheric origin? I think these are open questions that may not be easily addressed with their data.
Yes, we included a short discussion on this issue in Section 4.6, but our results show actually wide range of possible oxygen exchange, i.e. of possible loss of ^{17}O signal. Therefore a precise calculation of yielded anomalous N_2O is difficult. Moreover, we would need to assume that there is no turnover of nitrate in soil prior to emission as nitrous oxide and be able to assess how much of atmospheric nitrate is emitted as N_2O. There are too many unknowns to calculate this.

Specific Comments
The title could be a little more specific (e.g., referring to soils).
Title will be corrected: 'Oxygen isotope fractionation during N_2O production by soil denitrification'
P 17 L 11: 'Oxygen fractionation' = not clear whether you are referring here to molecular O2, O in water or O in N-bearing species.
It is not exactly defined in this stage, we leave this question still open, we know there is a mix of O precursor from soil nitrate and soil water, but this is clarified in the next section with the model.
We will clarify in the text that this fractionation is related to the entire process of investigated N_2O formation.
P21 L 17 and L19: I think this should be 'inter-molecular effects.'
Yes, this will be corrected.