Response to Anonymous Referee #2
The authors' answer is in red fond.

1. General comments
This paper embodies an attempt to answer crucial questions how oxygen isotope ratio in N2O is determined during its formation in soil microbial processes and how it can be used to differentiate various production pathways of this greenhouse and ozone depleting trace gas. While nitrogen isotope ratio including position specific 15N/14N in NNO molecule has been successfully used to trace production or consumption pathways of N2O like nitrification and denitrification, 18O/16O information is often difficult to interpret because it is controlled by more factors compared to 15N/14N. The authors applied both 18O/16O and 17O/16O analyses to N2O emitted from anaerobically incubated soils under several conditions to elucidate the effects of O isotope ratios in nitrate and water, soil type, temperature, and oxygen stress on O isotope ratio in N_2O produced mainly by denitrification. The title of this paper should be specified to show that they studied denitrification "in soils" because there are many publications with respect to isotope fractionation during N2O production in soils, waters, pure culture of microbes. The experiments are well designed and data are almost comprehensively presented, although Figure 3 is difficult to understand (see below). The most significant outcome of this work is that the extent of oxygen exchange between water and intermediates of N2O production was precisely determined by two independent methods using materials with natural isotope abundance and that robust 18O/16O fractionation for the O-exchange (epsilon value) is obtained for soil denitrification. These findings would help and stimulate isotopic studies of N2O production/consumption processes considerably. However, I found an error in their model-based discussion on the branching isotope effect, and consider the error might be critical as shown below. In summary, I consider that this paper might be acceptable for publication in Biogeoscience after correction for the error and improvement of some minor points below.
Thank you very much for your positive opinion on the manuscript and for critical comments, very helpful in improving our manuscript.
As suggested, we will specify the title to soil denitrification: 'Oxygen isotope fractionation during N_2O production by soil denitrification'. The data shown in Figure 3 will be better clarified.
The 'error' you found in model-based discussion, is not really an error, but an assumption that can be well justified. We explain this below in more detail, also more discussion on this issue will be added in the manuscript.

2. Specific comments
P17017, L17 "The incubation vessels were cooled to 2C . . ." Although the authors describes there was no temperature effect on the O-exchange, I wonder whether the manipulation of temperature before the incubation might affect the activity of microbes because they discuss the possibility of activation of different microorganism groups due to the initial gas treatment in Exp. 2.
It is theoretically possible but we do not know any studies indicating activation of specific microbial groups due to low temperature. Therefore, we suppose that the oxic conditions in the initial gas treatment are much more crucial than temperature, since it has been shown that fungal species may be activated by oxic atmosphere (Zhou et al., 2001).

P17017, L22 "During the incubation the headspace was constantly flushed . . ." This means water was constantly evaporating from the soil. How WFPS was maintained? Wasn't there any isotope fractionation of H2O during the incubation?
Yes, water was constantly evaporating but due to quite short duration of the experiments water losses were not large, up to 3% WFPS for sandy soil, whereas organic soil showed no measureable water loss at all. The change in $\delta^{18}O$ of soil water was within the analytical precision of 0.5‰.

P17018, L17
The authors used "Delta" series mass spec, for which I think linearity problem has been previously reported for NO+ fragment analysis. I suggest to add correction procedure/method if they applied.
The non-linear correction has been applied. For correction of non-linear effect due to variable gas amount five different standard gas mole fractions (0.3, 1, 5, 10, 20 µmol mol^{-1}) were analyzed in each sample run. Samples with similar N_2O mole fractions were run together with at least two standard gases with similar mole fractions. This information will be added in the manuscript.

P17026, L23 "19.1+-0.5 (Table 1)" Does this mean average and 1sd of 12 data presented in Table 1?

Yes, this will be clarified in the manuscript.

P17026, L26 "It can be noted . . ." I cannot follow this because Figure 3 is complicated. It seems this figure shows more data than those presented in Table 1. For example, I thought Exp. 1.1a was conducted with nitrate with high d18O from Table 1, but blue open triangle in Figure 3 suggests this experiment was also carried out with low-d18O nitrate.
Yes, Exp1.1 was also carried out with synthetic nitrate of low-d18O. But the O-exchange was not measured for these samples therefore they weren't shown in Table1. To be consistent, we will also delete the samples with synthetic nitrate in Exp1.2 from Table 1, since the O-exchange could not be precisely determined there and they are not further used for modelling. We will better explain the selection of different treatments for tables and graphs in the manuscript. Moreover, we will add an appendix with a summary of all the treatments and way of their presentation in tables and figures. These changes allow to present equivalent results in Table 1, Fig.3 and Table 4.

P17028, eqs. (7) and (8)
It seems the authors assume that epsilons for NIR- and NOR-mediated O exchange processes are identical. But I think it is not trivial because chemical species that exchange O atom with water are different between the two processes. Rationale or speculation should be added.
Yes, we assumed a common epsilon for O exchange by NIR and NOR. This value has only been measured for the exchange water-nitrite and water-nitrate and is not known for the potential NO-H_2O exchange. But this study and also previous studies show that the exchange associated with NIR enzyme is most probably dominant. Previous studies applied the same assumption (Rohe et al., 2014; Snider et al. 2011). We will better discuss this uncertainty in the manuscript.

P17029 , L1 "We have neglected the possible fractionation associated with the NAR reduction, . . ." I disagree with this statement. The authors write this was investigated in Rohe et al. (2014a), but I could not find any experimental evidence in the cited paper. I found a quotation from Casciotti et al. (2007) in the caption of Table 4 in Rohe et al. But Casciotti et al. (2007) describes that "branching isotope effect between nitrate and nitrite is 25-30 permil". Please explain why the authors considered the branching isotope effect is significant in nitrite-NO reduction step, not the nitrate-nitrite step. If nitrate nitrite step is more important regarding the branching isotope effect as Casciotti et al. showed, delta-n in equation (11) should be d18O of nitrite, not nitrate, and the authors' model calculation results presented in Table 4 would change especially for Exp. 2.
The reason why we neglected the Nar fractionation is the compensation of two opposite isotope effects: intermolecular and intramolecular effect. This was also stated in Rohe et al.:
"we assumed no branching effect during the nitrate-to-nitrite reduction step, since this branching isotope effect due to the intramolecular $^{18}O/^{16}O$ fractionation (positive ε) is compensated by the intermolecular isotope effect resulting in preferential reduction of ^{18}O-depleted NO_3 (negative ε)."
Rohe et al. investigated fungal pure cultures in two treatments: with nitrite and nitrate as electron acceptor, where for both the branching effects were modelled. When we compare the results of both treatments we see that for nitrate treatments we obtain in some cases lower total branching as in nitrite treatment, and never higher. This shows that there is no additional branching effect associated with this step. The difference to the study of Casciotti et al. (2007) is that there the nitrate was completely consumed and they observe only the intramolecular effect associated with removal of oxygen atom which is about 30‰. In contrast, in our experiments and in those of Rohe et al. only a small fraction of nitrate is consumed and we also deal with intermolecular effect, i.e. the preferential reduction of ^{16}O-nitrate. This effect can attain values as negative as -37 ‰ (Lewicka-Szczebak et al. (2014)), and hence compensate the intramolecular effect.
This compensation should be most pronounced for the NAR step, since the residual nitrate pool is big, whereas the next intermediates are less stable and primarily reduced more quickly, which may minimise the compensation by the intermolecular effect.
This statement will be also better clarified in the manuscript.

P17030, L7 "Since the mean value of 0 was assumed for . . ." From eq (10), epsilon-NOR does not necessarily equal to zero when epsilon-n is zero.
We assumed that $\varepsilon_{NIR} = \varepsilon_{NOR}$. We are aware they can differ, but we are not able to differentiate between them and determine them individually. Therefore in our model we rather look at total ε_N which is a summary effect of both ε_{NIR} and ε_{NOR} as defined in Eq.10. For calculation purposes we have equally distributed the total ε_N between ε_{NIR} and ε_{NOR}. Any assumption can be made here, and this is the easiest one. But we do not draw any conclusions from that, and a different assumption will not significantly change the general results of total ε_N.

Since we assume $\varepsilon_{NIR} = \varepsilon_{NOR}$ - in case ε_N is 0, eNOR must be also equal 0. Theoretically it could be different, but unfortunately our data do not allow us to differentiate this, because the intermediates were not measured.

3. Technical corrections
P17032, L25 and 27 "intra-molecular effect". This should be "inter-molecular effect"?
P17042, second column of Table 2. The unit of production rate should be consistent with those appear in Table 1 and text: microgram/kg/h.
P17044, caption of Table 4. Number or position of bracket(s) are awkward in the first sentence.
P17045, Figure 1 "epsilon-n"s are better noted as "epsilon-NAR, -Nir, -NOR" to be consistent with text.
Thank you, all these mistakes will be corrected in the manuscript.