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Interactive comment on "The mechanism of oxygen isotope fractionation during N_2O production by denitrification" *by* D. Lewicka-Szczebak et al.

Anonymous Referee #2

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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 in-

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cubated 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 N2O 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.

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.

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?

P17018, L17

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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.

P17026, L23 "19.1+-0.5 (Table 1)"

Does this mean average and 1sd of 12 data presented in Table 1?

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.

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.

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.

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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.

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.

Interactive comment on Biogeosciences Discuss., 12, 17009, 2015.