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Interactive comment on "Continuous measurements of nitrous oxide isotopomers during incubation experiments" *by* Malte Winther et al.

Anonymous Referee #1

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The study by Winther et al. presents continuous measurements of nitrous oxide isotopomers at concentration levels close to ambient and intends to determine isotope effects for two different bacterial organisms. Especially the endeavour to bring such measurements towards ambient concentration levels is valuable for the scientific community. In this context, this paper is of interest for the broad audience Biogeosciences attracts. There are some flaws, such as 1. The initial NO3- isotopic composition had to be estimated. 2. There is no nitrate balance provided, which would be helpful with regard to constraining f. 3. Concentration of other products, such as NO have not been accounted for. However, the manuscript provides some interesting calculation approaches, and the experiments involving P. chlororaphis are straightforward. All in

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Discussion paper

all, I recommend acceptance for publication after addressing the below comments.

See some more detailed comments below. Title ok Highlights Not given Abstract P1, L10: Please specify the sentence “the continuous analysis of . . .”. The meaning is unclear without knowing the manuscript. Please change reveal to reveals.

Introduction P2,L19: please change to positions, instead of position The objectives and the added value of another experiment on fractionation factors could be more to the point. Materials and Methods P4, L12 and following: I suggest changing “before” to “upstream of”. In addition, the text does not comply with Figure1. From Figure 1, it seems like a Nafion and a Magnesium perchlorate / Ascarite trap was used. The Nafion reduces the water vapor to a certain dewpoint (please specity) and the magnesium perchlorate Mg(ClO4)2! removes the remaining water chemically. The Ascarite (this is not Mg(ClO4)2, but sodium hydroxide coated silica!!) removes the CO2!. This section needs to be corrected. P6,L1: the subsection head number is 2.4.1, but there is no 2.4.2, which does not make sense. I suggest numbering the subsection head to 2.5 P7,L20: please change results to result L7,P19/20: with regard to the reference to the supplementary, I suggest changing “unreacted” to “reacted” in P4,L14 of the supplementary (“. . .can therefore be calculated as the sum of the immediate product calculated for all reacted fractions of the substrate”), as the accumulated product is the result of the reacted fractions of the substrate. P8,(8): The numerator terms are quite clear, the denominator term is not required to my understanding. Please clarify. P8,L26: Please change to net production. P8,L30: This assumption is not in agreement with your expectations given on P3,L16-20. There it is assumed that d15Nalpha becomes enriched (I agree with this assumption). In general, all N2O isotopic species should become enriched in a situation in which only reduction occurs, as N2O is the substrate in this case, and a normal isotope effect occurs. However, this is not the case in Figures 6A/B. Please comment. P9,L5: the fractionation factor during reduction is varied between 1 and 2, this means only not-normal isotope effects are allowed for the reduction of N2O. A recent review on isotope effects in the N cycle, Denk et al. 2017

in Soil Biol. Biochem. (The nitrogen cycle: A review of isotope effects and isotope modeling approaches), shows that the literature has reported that N2O reduction is associated with a normal isotope effect for d15Nbulk. Please comment why this limitation was necessary. Results

Discussion P11,L2: The statement that the production rate is 10 times higher for P. Chlororaphis is ambiguous, since a net rate is compared to a “gross” rate (assuming direct conversion of NO3- to N2O). Please add this to your interpretation that reaction rate cannot account for the difference in isotopic fractionation alone. P11,L15-17: This is pertinent information. Thank you. I only suggest to change the numbering from 1) and 2) for the results of the DNA comparisons to i) and ii). I was a little confused with the (1)-(4) numbering.

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