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

Anonymous Referee #2

Received and published: 8 August 2016

The study by Winther et al. presents continuous measurements of nitrous oxide isotopomers to determine isotope effects for two different bacterial organisms. N2O isotopomers have been identified as a promising tool for the identification of the different processes generating N2O. For this reason, such measurements are valuable for the scientific community. Especially spectroscopic measurements have been shown to be very well suited for the determination of the isotopomers (Mohn et al., 2014, Rapid Comm. Mass Spec.), owing to the selectivity of this method. In this context, this paper is also from a methodologic perspective of interest for the broad audience Biogeosciences attracts. However, I want to raise some general points:

1. The paper unfortunately stops short of providing technical details on the instrument
used and the performance during the incubation experiments. For potential users, information on the stability of the measurements over a deployment period of weeks to months would be interesting. Further, the stability of the concentration dependence and the calibration coefficients is of interest for readers interested in spectroscopic methods. In my opinion the authors should elaborate on the named points a little more than stating “P4, L29: Over the course of the experiments, no further instrumental drift was observed”. For example, what is the duration of the course of the experiments?

2. The headspace was flushed with pure N2 and, thus, the composition of N2 and O2 was not constant. At the same time, the calibration gases were provided in synthetic air. The composition of the analysed gas is crucial for adequate calibration, and the authors need to show the influence of changing N2/O2 ratio.

3. The determination of isotope effects for the bacterial organisms is based on the Rayleigh approach. However, for P. fluorescens the determination of the isotope effect during production was considered. In this consideration, a parameter, gamma, is introduced reflecting the ratio between reduction rate and production rate. In the next section, the authors state that they used an iterative procedure to fit their experimental data to the derived Rayleigh model, but the sensitivity of the resulting isotope effect is not mentioned at all throughout the manuscript. Also the authors state that it is assume that the ratio between consumption and production is constant. In my opinion, a sensitivity analysis of the model towards non-constant gamma should be provided.

4. It is surprising that for SP, the measurements seem to differ quite a bit from published values, though the isotope effect associated with SP, and especially for N2O reduction, is in general considered to be the most reproducible. I suggest discussing this in more detail.

5. The manuscript uses informal language at many points, not always provides adequate references (e.g., section 2.5) and also the figure captions sometimes lack basic information such as which bacterial organism is the subject of the figure (see below). The introduction is currently rather a sequence of statements, so that I suggest rearranging it to a concise introduction. See some more detailed comments below.
Title
ok
Abstract
P1, L1: “feed-back loop”: global warming is not necessarily enforcing global N2O emissions per se. Please explain the feed-back loop mentioned.
P1, L3/4 sounds odd. I suggest “A rare 15N atom can substitute the abundant 14N atom either at the central or terminal position in the linear N=N=O molecule.”
P1, L6: Please also define site preference
Introduction
The introduction draws an arch from isotopomers to climate change. This needs to be specified as currently the capabilities of quantitative source partitioning still need to be proven.
P2,L5: point (1) sounds odd. I suggest “(1) enhanced radiative forcing with N2O being the third most important GHG”. Further, e.g. is followed by a comma. Please change all e.g. to e.g.,
P2,L9: Please make clear that there was a positive correlation.
P2,L13/14: Please elaborate more in how isotopomers can help understanding climate change. The radiative forcing is almost exclusively due to 14N14N16O and, as said before, atmospheric N2O concentration is correlated.
P2,L24/25: I suggest starting the d-value description form the generic d15Nsample: “The isotopic composition of a sample is usually reported as d-value which represents the deviation of the elemental isotope ratio R in the sample from a reference material. Delta values can be calculated for bulk N2O as well as for d15Na and d15N beta.” Please refer to AIR-N2 as standard material.
P2,L27: Park et al is not suited for the definition of SP. It was introduced in 1999 by Brenninkmeijer and Röckmann (as indicated in the text) and by Toyoda and Yoshida, Annal. Chem 71, 4711-4718. I think these two publications deserve the credit for SP.
P3, L2: It sounds like denitrifying bacteria exclusively produces N2, which is not the case. Please rephrase.
P3, L14-17: Is it a method application or investigation of different bacteria? The latter is not really new.

Materials and Methods
P3, L22: Please change spectrometry to spectroscopy. The paper reports on a spectroscopic method.

P5, L30: The incubation chamber’s headspace was flushed with pure N2 to create anaerobic conditions. However, the calibration gases are N2O in synthetic air. For spectroscopy, the gas matrix is of importance as it affects the line shape of the investigated gas. Please show the influence of increasing N2 content on determined isotope ratios at constant N2O concentration. It is fundamental to address gas matrix in such an experiment.

P6, L7-8: Though this statement addresses the concern raised above, but actually it is not only O2 spectral lines that affect the measurements, but also the collision partner itself. For this reason, the authors need to show empirical evidence here that the effect of a changed gas matrix did not change the determined isotope ratio (as described above).

P6, L12: Equation 3 is given with Rs and Rs,0, whereas L12 refers to “reactant”. The “s” in Rs,0 stands for substrate. In my opinion, it is easier to follow if the abbreviation would be the same as the word used. Thus, I suggest using “substrate instead of reactant”

P6, L22: The term isotope enrichment for the bulk is a little confusing here. For normal isotope effects, the accumulated product is depleted compared to the initial substrate. In this context, the section head 2.5 “Analysis of isotope enrichment” is also not ideal. As far as I can see the whole section aims at the determination of the isotope effect. I suggest using “Determination of isotope effects” as section head and introduce epsilon as isotope effect, which is from my point of view the agreed term (this also refers to line 15/16).

P7, L2: in equation 8, the correction term is not specified, however the isotope ratio is. I assume, the correction term needs a subscript identifier as well, e.g., a or b. In
addition, the section beginning P6, L 25 needs references.
P7, L3-10: Also this section needs references and at least the terms in eq. 9 and 10 should be explained properly. Rp, r is most likely the isotope ratio of remaining N2O after reduction? The manuscript is cumbersome to read if this (basic) information needs to be deduced by the reader.
P7, L17: In my opinion it's odd to have a quite extensive section 2.5 followed by a sole subsection 2.5.1. This type of structure does not make sense to me and I suggest the following structure: 2.5 Determination of the isotope effects, 2.5.1. Modifications to the Rayleigh model, 2.5.2 Fitting procedure.
P7, L 30: I am not aware that CDC has been introduced so far. What does it mean?
Whole 2.5.1: The second section is somewhat repetitive as the R2 value is mentioned twice as optimization criterion and, thus is too verbose. Please make this section more concise and provide information on what “is iteratively found” means exactly (P7, L23).
Results
P8, L7ff. The evolution of N2O concentration (please add the word concentration in line 7) during the experiments is described for both organisms, though there are subsections for the respective organisms following. From this perspective, the introduction in section 3 is odd. Though it may be controversial, I suggest starting the subsection 3.1 directly following section 3 and moving the description of N2O concentration to the respective subsection.
P8, L14: The reference to Fig 4a and 4B is ok, but the caption of Fig. 4 does not indicate which organism the figure refers to. Please add this information.
P8, L15: Please avoid terms like “in fig . . . we plot . . . . “. This occurs throughout the manuscript, sounds informal and could easily be replaced by Figure xxx shows . . . .
P8, L17-18: The match was relatively high to what? The correlation coefficient is R, the coefficient of determination is R2. Please consider.
P8, L24: please also here add the organism to caption of Fig. 5 and remove “the” in front of d15Na. The results section does not contain any information on the parameter gamma, representing the share of reduction and production. Please give details.
Discussion
The discussion lacks information on the share of reduction and production of N2O during the onset of the experiment involving P. fluorescens. What is the uncertainty of this parameter on derived isotope effects?