

General comments:

The manuscript of Malte Winther and co-authors with the title “Continuous measurements of nitrous oxide isotopomers during incubation experiments” presents measurements of N₂O isotopic composition ($\delta^{15}\text{N}^\alpha$ and $\delta^{15}\text{N}^\beta$) with a prototype Picarro CRDS analyser.

Research on GHG isotopologues is very active and the manuscript is therefore timely and of high interest for readers of Biogeosciences and potential future users of this technique. The wording is colloquial and should be strongly improved. I have a number of suggestions for technical corrections the authors have to consider for improving the consistency and readability of the manuscript.

The manuscript gives details on “prototype” applications of the novel technique on N₂O produced by two bacterial strains. I have strong concerns, regarding the interpretation of results using a simplified modelling approach, which in the end leads to results for isotope enrichment factors (ϵ^{SP}), which are in contrast to existing literature! I would strongly recommend to either reassess the data analysis + interpretation or focus more on performance tests of the developed analyser and mention the limitations of the applied approach.

Moreover, the manuscript gives the impression that this is the first time that N₂O isotopomers were analysed continuously by mid-infrared spectroscopy. The author ignore previously published using a similar CRDS analyser (D. V. Eler et al., *Limnol. Oceanogr. Methods* 13, 391–401 (2015)) and several years earlier with mid-infrared absorption spectroscopy QCLAS (see specific comments below related to page 2 Line 20). The respective citations have to be included in the manuscript before publication!

In summary, I suggest publication in Biogeosciences after careful revisions.

Specific comments:

Page 1 Line 3 -5: I would suggest to rephrase this sentence possibly to: “In the linear N=N=O molecule ¹⁵N substitution is possible in two distinct positions, central and terminal. The respective molecules, ¹⁴N¹⁵NO and ¹⁵N¹⁴NO, are called isotopomers.”

Page 1 Line 5: The sentence is colloquial and should be changed to something like: “ ... that N₂O produced by nitrifying or denitrifying microbes exhibits a different relative abundance of both isotopomers.”

Page 1 Line 6: Please define the term “site preference”.

Page 1 Line 7: What is the meaning of the term “in the order of days” – would it not be feasible to perform analysis for weeks or even months – please comment?

Page 1 Line 10: The term “position dependent measurement” might be changed to “analysis of N₂O isotopomers” or similar.

Page 1 Line 16: The limitations of the applied data analysis, in particular to disentangle N₂O production and N₂O reduction should be mentioned. In addition, the discrepancy of the enrichment factor ϵ^{SP} for N₂O reduction with existing literature should be mentioned or the number deleted from the abstract.

Page 2 Line 17: The positions in the N₂O molecule are named α and β but not the isotopomers, please correct.

Page 2 Line 20: “ ... spectral regions ...”

Page 2 Line 20: Mid-infrared spectroscopy and exactly the same spectral region (around 2188 cm⁻¹) was already applied earlier for continuous analysis of N₂O isotopologues. The authors have to cite the respective publications: H. Wächter et al. *Optics Express* 16 (12), 9239-9244 (2008), J. Heil et al. *Geochimica et Cosmochimica Acta* 139, 72–82 (2014), J. R. Köster et al. *Rapid Commun. Mass Spectrom.* 27, 216–222 (2013), J. Mohn et al. *Atmos. Meas. Tech.*, 5, 1601–1609 (2012).

Page 2 Line 25: The international isotope ratio scale is AIR-N₂ and the standard is atmospheric nitrogen.

Page 3 Lines 0 – 17: Please add a few sentences on the effect of N₂O reduction on the N₂O isotopic composition.

Page 3 Line 20: “determined”

Page 3 Line 25: Please rephrase the term “¹⁴N absorption feature”.

Page 3 Line 26 – 28: Please give information for which “time interval” the precision values are given.

Page 4 Line 0 ff: Was there any provision to avoid under- or overpressure in the setup?

Page 4 Line 7: Flushing the system with N₂ most probably has changed the O₂/N₂ ratio in the setup, which in turn would have affected the analysis of N₂O isotopologues – please comment? The statement given on page 6 Line 7 – 8 is not sufficient as the gas matrix (O₂/N₂ ratio), i.e. differences in pressure broadening, is known to affect the of the analysed spectral lines (e.g. D. V. Erler et al., *Limnol. Oceanogr. Methods* 13, 391–401 (2015)).

Page 5 Line 1: If the two diluted gases are new standard gases they might be named different than the original ones.

Page 5 Line 3 – 14: The two statements “measured according to an international standard reference” and “relative to atmospheric air” is contradictory as the primary anchor of the international scale is atmospheric N₂ and not N₂O – please correct. If measurements were anchored to atmospheric N₂O please give the values which were adopted for $\delta^{15}\text{N}^\alpha$ and $\delta^{15}\text{N}^\beta$ of atmospheric N₂O by different laboratories.

Page 5 Line 3 – 14: The spread of results observed by different laboratories for the same calibration gas is considerable. This should be mentioned in the text with reference to a recent inter-laboratory campaign, which showed similar results (Mohn et al. *Rapid Commun. Mass Spectrom.* 28, 1995–2007 (2014)). In addition it should be mentioned that Tokyo Institute of Technology is supposed to be the only laboratory in the group to anchor their measurements to the AIR-N₂ scale through NH₄NO₃ thermal decomposition.

Page 6 Section 2.5: All acronyms should be defined in the text.

Page 6 Line 14: Rs is supposed to be the isotope ratio of the substrate at time t.

Page 6 Line 15: For “ ϵ ” the wording “enrichment factor” is usually applied (e.g. Well et al. JOURNAL OF GEOPHYSICAL RESEARCH, VOL. 114, G02020 (2009)) – please correct throughout the text.

Page 6 Line 16: “We did not measure ...”

Page 6 Line 17: The statement “has to be identical” might be too strong and should be replaced by “can be used to estimate”.

Page 6 Line 19: “ ... the bulk $^{15}\text{N}/^{14}\text{N}$ isotope ratio ...”

Page 6 Line 20: “ ... for the accumulated product $R_{p, acc}^{bulk}$ is:”

Page & Formula 4: It should be “ $R_{s,0}$ ” instead of “ R_0 ”.

Page 7 Formula 8: Some of the acronyms are not defined and for “ ϕ ” “ ϕ^α ” might be correct.

Page 7 Formula 9 + 10: It is hard to follow the argumentation as some of the acronyms are not explained in the text. Please add the definition and give more details on the derivation of the formula and the involved literature.

Page 7 Line 7: The assumption that the ratio of reduction and production rate is constant is highly questionably based on past experimental evidence (e.g. Lewicka et al., Rapid Commun. Mass Spectrom. 29, 269–282 (2015)) – please comment?

Page 7 Line 21 – 22: This sentence might be wrong, as the “net production rate” is negative after the point of maximum N_2O concentration (Figure 3) – please clarify?

Page 7 Line 30: The term “CDC” is given here for the first time, please define.

Page 8 Line 4: “ γ ” was defined as ratio between reduction and production rate (page 7 Line 6 – 7) and is named reduction correction parameter here – please unify.

Page 8 Line 27: The statement, that N_2O production is absent when both $\delta^{15}\text{N}^\alpha$ and $\delta^{15}\text{N}^\beta$ decrease is without proof – please comment?

Page 8 Line 24: Please delete “the”.

Page 8 Line 27: The wording “the bulk” is colloquial, please correct.

Page 9 Section 4 Discussion: Some statements in the discussion are in contrast to existing literature. Therefore, the authors should carefully check the interpretation of their results in relation to existing literature – details are given below.

Page 9 Line 13: To clarify “nutrient” might be exchanged by “substrate”.

Page 9 Line 18 – 19: The wording “the isotopomers are depleted” is colloquial as the term isotopomer relates to the molecules $^{15}\text{N}^{14}\text{NO}$ and $^{14}\text{N}^{15}\text{NO}$.

Page 9 Line 19: The wording “the Rayleigh” is colloquial please change.

Page 9 Line 21: What are “measurements of *P. cholororaphis*”?

Page 9 Line 23: The statement “that the conclusion applies to all denitrifying bacteria” is too strong based on the presented measurements and might be deleted. Please also discuss

results in relation to relevant work by other authors: e.g. Sakae Toyoda et al. *Soil Biology & Biochemistry* 37 1535–1545 (2005).

Page 9 Line 24: The observed difference in the enrichment factor ϵ^{bulk} could also be explained by just differences in the reaction rate as already demonstrated by A. Mariotti et al. *Can. J. Soil Sci.* 62: 221-241 (1982).

Page 10 Line 9: R. Well et al. *Rapid Commun. Mass Spectrom.* 2008; 22: 2621–2628 published ϵ^{SP} values for diffusion, which are in contrast to the authors speculation, please add the reference and comment?

Page 10 Line 24 – 25: The authors state that “for N₂O reduction their results (ϵ^{bulk} and ϵ^{SP}) are in line with earlier studies”. However, all previous studies consistently show negative ϵ^{SP} values for N₂O reduction, e.g. D. Lewicka *Geochimica et Cosmochimica Acta* 134 55–73 (2014); D. Lewicka et al. *Rapid Commun. Mass Spectrom.* 29, 269–282 (2015), R. Well et al. *Rapid Commun. Mass Spectrom.* 23: 2996–3002 (2009); J. R. Köster et al. *Rapid Commun. Mass Spectrom.*, 27, 2363–2373 (2013). Please add references and comment!

Figure 3: I assume the “N₂O production” is “net N₂O production” – please clarify and change here and elsewhere in the text?

Figure 4A, 4B, 5A and 5B: The wording in the legends is “poor” and should be rephrase to something like “ $\delta^{15}\text{N}^{\alpha}$ as a function of N₂O concentration ... The blue curve is the instantaneous signal of the CRDS analyser, the black curve the five minutes running average. The blue arrow indicates ...”. Please add information on the bacterial strains involved in Figure 4 and 5.

Table 3: The enrichment factors given for N₂O reduction are in contrast to all existing literature. Therefore the limitations of the applied data analysis should be mentioned.