

Review of Ibraim et al

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The authors present an innovative and thorough assessment of the microbial origins of N<sub>2</sub>O emissions from a grassland soil based on spectroscopic measurements. This data set is clearly an advance for the field as extensive time-series data sets for the isotopic composition of N<sub>2</sub>O are rare but very important to constrain the dynamic nature of N<sub>2</sub>O production from soils. While I appreciate the thoroughness of the data set and interpretations I have a few central concerns regarding calibration and with the assessment of microbial origins of N<sub>2</sub>O described in the paper.

Characterization of sample isotope values based on two isotopically characterized standards is certainly the minimum necessary. Critical is also that the range of isotope values of the samples be encompassed by the range in isotope values of the standards. The range in isotope values for  $\delta^{15}\text{N}^{\alpha}$  and  $\delta^{15}\text{N}^{\beta}$  is quite good (although I don't know what fraction of the samples lie outside these ranges) the range in  $\delta^{18}\text{O}$  values of the standards is small and does not well encompass the range in sample values. The issue with this is not a just with the precision (which can be determined) but with the accuracy; values outside the range of the standards cannot be considered accurate even if precise. My personal opinion is to not publish isotope values outside the range of standards however, I appreciate that there may be a statistical approach for providing greater confidence in this situation.

While the authors present an extensive data set on the concentration and isotopic composition of N<sub>2</sub>O in air much of the data set is derived from periods of low flux when the concentration of N<sub>2</sub>O is only slightly above atmospheric. The calculation of soil-derived N<sub>2</sub>O is obtained from a simple isotope mixing model (soil-derived mixing with atmospheric N<sub>2</sub>O). In this model, there is considerable error associated with the soil-derived isotope values at low N<sub>2</sub>O concentration (the error increases as the concentration of N<sub>2</sub>O declines). Further, the error around each calculated soil-derived isotope value increases as the difference in the isotopic composition between soil and atmospheric N<sub>2</sub>O decreases. For this reason, in our laboratory, we generally don't publish data on samples in which the N<sub>2</sub>O concentration is less than 30% greater than atmospheric (although this is an arbitrary value). The Keeling-plot approach does provide greater accuracy and precision but it is critical that the authors (1) report the uncertainty obtained for all soil-derived isotope values, (2) provide a clear discussion of this issue, and (3) consider not reporting isotope values for which the error is very large and a reasonable explanation for what the cut-off error should be.

There are two important assumptions in the graphical approach to tracing the microbial origins of N<sub>2</sub>O that is used by the authors: (1) that  $\delta^{18}\text{O}$  is a conservative tracer of origins and (2) that the kinetic isotope effects associated with production of N<sub>2</sub>O as well as reduction of N<sub>2</sub>O are constants. As I discuss below I question the validity of both of these assumptions. I will acknowledge, however, that this is a common approach in the literature but ask that the authors, at least, discuss

these issues and how variation in the kinetic isotope effects might impact their model outcomes.

Page 1, Line 17: Was data corrected based on measured values for the Target gas?

Page 5, Line 26: Was the concentration correction performed daily? Did the range in concentration standards encompass the range in sample concentrations observed?

Page 5, line 34: Did the Keeling plot approach include calculation of the uncertainty surrounding the isotope values for the soil derived N<sub>2</sub>O? Was this evaluated using the Monte Carlo model as described? At concentrations only slightly above ambient this error can be very large. We generally discard data with a N<sub>2</sub>O concentration < 30% above atmospheric levels although the decision to discard data should probably be made on the basis of the magnitude of the uncertainty.

Page 5, Line 36: Why was a correlation between  $\delta^{15}\text{N}^{\alpha}$  and  $\delta^{15}\text{N}^{\beta}$  considered a criterion for a valid measurement? While unpublished, our data indicates that most of the variation in SP during N<sub>2</sub>O production from the cNOR enzyme is driven by  $\delta^{15}\text{N}^{\beta}$ ; which suggests that a lack of correlation could be a normal feature of production from denitrification.

Page 6, Line 5: It is not really necessary to specify the “hard disk” failure as the cause of missing data. Simply stating equipment failure is fine. This is also mentioned in the Figure 4 legend.

Page 7, line 26: Delete “during”: word not needed.

Page 8, Line 22: On what basis was an  $r^2$  of 0.2 similarly used to exclude data? This seems like a very poor degree of correlation.

Page 8, Line 23: Perhaps “calculated” is a better word than “extracted”.

Page 8, line 24: What are “relatively large uncertainties”? It would be better to state the uncertainty in the text as well as in the figures. Perhaps it would be best to exclude data with large uncertainties; as long as some reasonable criteria can be established to exclude data beyond a certain uncertainty (perhaps based on the range in isotope values between the sources).

Page 8, line 30: Yes, the poorer data quality for  $\delta^{18}\text{O}$  is likely a consequence of the fact that the standards do not encompass the data. Generally, standards must encompass the range in data to assure accuracy in any calibration relationship. The issue is that this does not only result in poorer precision but, more importantly, results in poorer accuracy. The authors are presenting data for which the accuracy

is questionable. If the  $\delta^{18}\text{O}$  values obtained are substantially outside the range of the standards the authors should give considerable thought as to whether they should be excluded.

Page 9, line 19: I am not fond of the graphical approach used by Koba et al to apportion sources of  $\text{N}_2\text{O}$  largely because  $\delta^{18}\text{O}$  is a poor tracer of microbial origins. It is well known that oxygen exchanges with water during the microbial production of  $\text{N}_2\text{O}$  which can alter  $\delta^{18}\text{O}$  values (e.g. Kool references). Further, this approach relies on limited data sets for  $\delta^{18}\text{O}$  generated by cultures of fungal denitrification, bacterial denitrification and nitrification. Given this, I suggest that the authors, at least, acknowledge the limitations of the graphical approach in assessment of the origins of  $\text{N}_2\text{O}$ .

For  $\delta^{15}\text{N}$ , this approach also depends not only on the isotopic composition of the nutrient source but also the kinetic isotope effect (KIE) associated with  $\text{N}_2\text{O}$  production. The KIE is more accurately described as a net isotope effect and can be highly variable depending upon conditions in the soil. The authors need to consider and discuss the effect of variation in the KIE on their model outcomes.

Page 10, Line 5: Here again a single value is chosen for the KIE associated with  $\text{N}_2\text{O}$  reduction whereas there are many papers that show variation. In Jinuntuya-Nortman et al (2010) we demonstrate that the SP net isotope effect associated with  $\text{N}_2\text{O}$  reduction can approach zero per mil and is inversely correlated with water filled pore space. The authors demonstrate that water filled pore space varies. What is the affect of a varying NIE on the model outcomes? Indeed their inference that low SP values occur at high WFPS is entirely consistent with the role of WFPS in masking expression of fractionation during  $\text{N}_2\text{O}$  reduction and may not reflect variation in the proportion of  $\text{N}_2\text{O}$  derived from nitrification and denitrification. The estimates of the rates of  $\text{N}_2\text{O}$  reduction may not be meaningful if the net isotope effect for reduction varies (as it likely does).

Page 12, line 36: Given the issues raised above I am not convinced by the author's statement that natural abundance isotope studies are "an effective way to disentangle  $\text{N}_2\text{O}$  production pathways". A frank assessment of the strengths and weaknesses of the approach is needed.