Review of bg-2020-285:

Comparing modified substrate induced respiration with selective inhibition 2 (SIRIN) and N_2O isotope approaches to estimate fungal contribution to 3 denitrification in three arable soils under anoxic conditions

Fungal denitrification can make a significant contribution to N_2O production in soils, however emissions are poorly constrained. This study uses a variety of approaches to attempt to quantify the proportion of N_2O produced by fungal denitrification under anaerobic conditions. The methods are carefully applied however the complex treatment design is challenging to follow and a better overview is needed. The interpretation and statistical analysis is careful but somewhat basic and empirical – each of the methods is considered separately, and results from one are often used in another (eg. product ratios) which makes reasoning circular, and assumptions and uncertainties hard to follow. This type of multipronged approach would hugely benefit from a more complex statistical analysis, such as a Bayesian methodology whereby the results from all experiments as well as the uncertainties in many critical parameters from previous studies could all be brought together to gain a much clearer and more robust picture of the results and implications. It would be a great benefit to the paper if the authors would take the opportunity to use such methods to improve the results at this stage, although I suspect they may consider this beyond the scope of the paper and review. The use of English in the paper is not too bad, but would really improve following careful copyediting by a native speaker – it is often awkward and difficult to follow. Overall the paper is of a good scientific quality and worthy of publication, which I recommend once the comments in this review have been addressed.

- **Specific comments:**
  - L266: How did you calibrate N_2O isotopic values? Where values and/or precision dependent on N_2O concentration? Was interference from or dependency of isotope ratios on CO_2, H_2O or any other gas observed?
  - L290: I guess D is abiotic production, eg. chemodenitrification and similar. But if D is abiotic production and not any kind of artefact, why does it matter if D is lower than A, B and C for this calculation? And why is the denominator A-D? The equation then surely gives fungal production as a proportion of biotic denitrification production rather than as a proportion of total production, which would be more relevant?
  - L379: Why would production rates change with time throughout the incubation? Why did you only use the 10 h time to compare?
  - Table 2 / Results S 3.1: Rates for D are clearly not negligible, in fact usually on the order of around half of the total N2O production. I don't see this as a big problem for Eq. 3, as I stated earlier, but it is a significant problem for the use of Eq. 4, which assumes mixing of only FD and BD endmembers.
  - L451: Yes, it sounds like they are a valid estimate of emitted N_2O ie. without reduction, however the IEM still suffers from the problem of unrepresented processes as evidenced by significant fluxes from D.
  - L458: This maybe suggests a problem with either the product ratio or the fractionation factor?
  - L474: If inhibition was not successful, there would be less N2O following inhibition than was really produced (eg. lower denominator of Eq. 6), and the calculated product ratio would be larger than it should be. This seems to be the case in most of Expt 2 and in Expt 4 but in Expt 1 and 3 the opposite is observed. Why would you observe this effect, which is really strong for Expt 1? An unaccounted for process in tracing? Or an additional impact of +C2H2 on N cycling that is not just due to reduction? Also, it seems like you don't have complete inhibition for 2 and 4 – maybe 10% not inhibited – how much may this affect results?

- S3.4: This suggests that inhibition may have downstream effects on N cycling, eg. through inhibited processing of N species that are important as substrates for other processes. This could be a really significant problem for all your experiments, which all rely to some extent on SIRIN, and warrants a great deal more discussion.
- S3.5: As a rule of thumb, I would have thought that the further the points are from the BD origin, the more FD would be calculated. This appears to be the case for 4 -C2H2 but for 3 -C2H2 the calcuted F_{FD} values are very low. Why is this? Also, most of your points are close to the origin of BD. Can you use uncertainties in isotope measurements and in endmember values to put uncertainty ranges on the F_{FD} estimates? And can you give a minimum F_{FD} that you would detect by this approach? I think given the uncertainties in every term you would need a relatively strong contribution, eg. 20%, for it to be visible.
- S3.6: These are much lower than the endmember you used for FD. How does this impact your other results? If the fungal endmember was lower than you assumed, the FFD from both IEM and mapping approaches would have been underestimated. Indeed following half your calculated endmembers (4 of 8 are negative) FD and BD could be indistinguishable isotopically. Why do you think your endmembers are so low? Could this relate to underexpression when substrates are limiting, or some other effect?
- L723: Well, except that the FD endmembers you found were much lower than expected…?
- L768: I don't think you do show this, because you had really large variability in your FD map values, and no clear quantitative answer for f_{FD} because you had no clear endmember for soil water.

- **Minor comments:**
  - L60: This description of denitrification should be the first sentence in the paragraph.
  - L149-156: This discussion of whether fungal soil and pure culture values agree seems logically to fit before the more detailed introduction to IEM and mixing line approaches. Overall the introduction is a little hard to follow – it would be good to really think about the logical flow of the concepts from least to most complex and structure the intro accordingly.
  - L216-236: A table summarising the treatments and abbreviations used throughout would be very useful here. It is very confusing at the moment and needs to laid out much more clearly.
  - L239: The word "Experiment" here is confusing since it is really four different soils, right? It would be better to call the different soils "Soil 1" and so on. Also, why does Soil 4 get more fertiliser added?
  - L300: *f* rather than *F* would be a more common abbreviation for fraction. Also, this assumes no abiotic denitrification.
  - S2.5.1 is very hard to follow because of the treatment designations. Again, a table earlier in the methods is needed, much more clearly linking each specific treatment combination to a clear abbreviation code.
  - L322: *Product ratio* is much too long to be used repeatedly as a variable, maybe just f_{red} or *P* similar?
  - S3.2.2: -C2H2 is basically a control compared to +C2H2 and it seems like logically it should be discussed first.
  - L645: Partial pressure effects would potentially also be expected to affect N2O production, but you saw an increase in N2O production with time?
  - L4.2: Also potentially abiotic production.