Re-review of bg-2020-285:

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

This manuscript has benefitted greatly from the first review round, and now it is close to ready for publication, following the comments listed below. The premise and experimental work is good, but the manuscript is often confusingly written and not concise enough.

- **Specific comments:**
  - Abstract: The abstract is confusing to read due to the complex set up and somewhat inconclusive results. There seems to be too much focus on SIRIN, with less focus on the other approaches. I would suggest to rewrite the abstract to (briefly!) answer the two main questions of this study: i) comparing the three approaches, first in method application and then in results, and ii) quantify importance of fungal denit. The current abstract is also perhaps too long; most of the first paragraph is intro and most of the last is conclusions – both should be shortened to just one concise sentence in the abstract.
  - L72: This is confusing – did L&S 2002 think fungal or co denitrification contributed 92%? Did they distinguish the two? The link here to codenitrification is not properly explained. Even if they often co-occur, surely that doesn't mean codenitrification is an indicator fungal denitrification – co-denit can occur under many conditions without FD also.
  - Introduction: The introduction is quite hard to follow and still somewhat unclear. I recommend to restructure to something very easy to follow (considering the complexity of the topic), eg.:
    - an introduction to the topic as a whole,
    - followed by a single paragraph about each of the three methods (how the method is applied, examples from prev studies, strengths, risks and weaknesses),
    - followed by a synthesis of comparability of the methods as well as things like acetylene inhibition (it is currently very unclear how this relates to the three methods), and
    - finally a summary of what you hope to achieve, questions, hypotheses.

    The exact structure is of course up to you but at the moment it is very hard to follow, and for a complex topic such as this a clear intro is very important.
  - L165: This naming is super confusing. The soil sampled twice should be called 1.1 and 1.2, not 1 and 4, to clarify throughout that these are "more similar" that soils 2 and 3.
  - Figure 1: This table is an improvement but still extremely confusing. Why is acetylene only mentioned at the very right hand side? The formatting in the second-from-right boxes is really odd. The use of acronyms (esp in the second-from-right boxes) means the table is not a good overview for a reader who has not yet finished reading the paper. The linkages eg. between expt varieties is odd. This figure still needs a lot of work – it should function as an overview to guide a reader into the experiments and clarify exactly what was applied to which soils in which order. Using colour would probably help to separate different aspects.
  - L201-216: These results should be shown in a figure in the SI.
  - L216: Is this a valid assumption, considering these are pretty different processes? What are the implications if conditions are not optimal for denitrification?
  - L250: Why more fertiliser for soil 4? And different sample timing (L264)?
  - L307: I understand from your response to the initial review that A-D is conventionally used as the denominator in this equation, ie. you determine FD

relative to FD + inhibitable BD, rather than as a proportion of total denitrification. All the same, I find that using just A as a denominator and calculating FD as a proportion of total denit would be more appropriate in this case, as it is more comparable to the isotope approaches, which are essentially finding FD as a proportion of total denit. I think it would be good to calculate f-FD with A as a denominator in addition to the more traditional A-D. It is well within the scope of this paper to try to improve these methods rather than just apply them as previously done.

- S2.5.1: I think you could consider an addition to this section, which would allow you to also use the non-acetylene experiments with the IEM approach. FD, nitrification, and reduction all act to increase SP. Only denitrification (bacterial, codenit, nitrifier denit…) produce low SP N_2O. Therefore, you can calculate a "minimum" denitrification contribution, and thus a "maximum" FD contribution. If you measure, for example, SP = 2 permil, with endmembers of 0 and 33 permil for BD and FD/nitrif (this is just a quick example, of course you want to consider uncertainties!), using Eq. 4 you get a minimum denitrification contribution of (2-0)/(33-0)=94% and thus a maximum FD of 6%. This approach gives a relatively strong constraint on fungal denitrification when you measure a low SP (can't have much FD) and very little or no constraint on FD when you measure a high SP.
- In S2.5.1 I'm also unsure why you completely discount nitrification. I know you have wettish soils and flush with N_2 but potentially there is still aerobic microsites, oxygen production in the soils from other processes, … Do you have an estimate or previous study to show nitrification is truly negligible?
- L386: Why 0.1? This is a high significance level. And at L405, why do you sometimes report the P (eg. 0.002, 0.008, 0.027) and sometimes just the comparison to the sig level ($P < 0.010$)? It would be better to always report the P, unless it is very small, eg. $P<0.001$.
- Table 4: I would think that the errors causing negative values would also apply to positive values, eg. this simply reflects a high uncertainty of the mapping approach. Simply rejecting negative values will therefore bias any interpretations or aggregations towards an overestimation of fungal denitrification using this technique.
    - This is also reflective of the fact that the mapping approach is extremely uncertain as your points are mostly very close together, providing little constraint on the gradient. The mapping approach would presumably be better suited only to samples with larger ranges (a couple of yours have more range but most not).
- L605-619: SIRIN could be an overestimation simply by definition, as it does not include non-inhibitable in the definition, and FD-MAP could be an overestimation because of the rejection of negative values – so I would say the endmember approach gives the highest confidence.
- S4.4: You have very little evidence at all relating to codenitrification and I think it could be more appropriate to remove this section, or at the very least reduce it to a short paragraph at the end of a different section.
- Discussion: The discussion is much improved from the previous version, and provides a good overview now. The authors could still reread the discussion and ensure (like the introduction) that it is a clear and concise coverage of the results in relation to the research questions and hypotheses.

- **Minor comments:**
    - L99: change to *…interferes with quantification of FD based on SP values…* or something similar.
    - L480: Change thanof to than of
    - S4.2: Should the title be *with* and not *without*?

- L744: I think it would be more appropriate to change “revealed” to “were consistent with”. Also, this sentence is long and a bit repetitive and could be improved.