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Interactive comment on "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" *by* Lena Rohe et al.

Anonymous Referee #2

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The manuscript submitted here presents an interesting combination of approaches for assessing the contribution of fungal denitrification to the N2O. By using some SIRIN and two isotopic techniques (endmember mixing) and SP/delta18O mapping, they conclude that the fungal contribution to N2O fluxes under anaerobic conditions in the three investigated soils is modest. In general, the manuscript is well written, and the methods are well elaborated. I however miss a clear rationale for the study. As a consequence, the reader is not guided through the work, so that it is hard to get the main conclusions

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of the work, and how the different pieces of the work fit together (i.e. how the methods compare to each other). Below, you can find some critical parts which should be revisited, with regard to objectives, experimental design and methods and discussion of the results. At the end, some more specific comments.

I think the manuscript would benefit from a more straightforward formulation of the objectives. As it is now, the three objectives (L155-162) are hard to differentiate from each other (ie, using three approaches to determine the fungal contribution to N2O efflux; compare the fungal contribution obtained by the three, and evaluate the use of SPN2O values); even more importantly, in the abstract I don′t see a connection with such objectives. You can think on hypotheses -e.g. methods (do not) perform equal-, and ways to test them.

It is also not clear to me the reasoning for the selection of the three soils and how this relates to the objectives, so it is hard to evaluate the suitability of the approach. Is it just to get an idea of variability? In L165 you stated that the soils differ in texture to provide different conditions for denitrification, so that might be the reason behind. The experiments take place under anoxic conditions, so texture might not be that relevant, and one may argue that, for example, different C sources for denitrification maybe more important, or different proportion of fungal vs. bacterial microbial biomass. However, the variability across soils, or the potential role of texture on the results is not discussed at all. Furthermore, one of the soils was sampled twice. Why? What is the difference between experiment 1 and 4? Is it about seasonality? Which kind of information did you want to obtain, and which kind of lessons you learned in hand of the results? I am missing this information in the discussion.

As you said, the microbial inhibitors did not have the expected effects. This is evident not only for N2O, but also for CO2. I understand this kind of results are disappointing when investing large efforts in conducting the analysis. But this is a key issue which deserves more attention, since it has important implications for the relevance of the whole study. For example, L609-610 read: “the SIRIN results [. . .] were rather

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unsatisfactory and led to unsolved questions" (a similar statement at the end of the discussion, L827-830). This is quite a statement which, to be honest, it is not reflected in the abstract, which describes that, for the one soil where it was possible to quantify the fungal contribution, this was 28%, higher than what obtained by the other methods. Thus, as a reader I would infer that SIRIN might overestimate the fungal contribution to the N2O fluxes, which is quite a different conclusion compared to "SIRIN results were rather unsatisfactory". On the contrary, the conclusions have a totally different approach, focusing almost only in the caveats of the SIRIN approach. By the way, you said you tested the concentrations of inhibitors applied; thus, were the preliminary tests performing better than the "real runs"? How did you test the optimal concentration? In general, when presenting the results of the different methods and discussing them, I missed a profound analysis on which method should be applied, what the cons and pros are and whether methods provide complementary information, which would support the simultaneous use. As it is now, they are presented almost separately, thus failing in in the objective which can be derived from the tile: "comparing . . .approaches to estimate fungal contribution to denitrification . . ."). And I honestly consider this is a serial issue, especially because you are making use of some of the outputs of selected methods as input for the rest of the methods, making all of them dependent to each other. It is only a suggestion, but consider including some table or graph with the main features of each methods and the key info, so that a reader can get an overview at first glance

Specific comments:

L48: What do you mean by "under conditions ensuring larger fungal N2O fluxes"?

L165-169: A more detailed description of the soil sampled will help. In general, the use of experiment/soil/treatment and variety is confusing, e.g. the same soil is used in two "experiments" (see above) and variety might refer to the use of c2h2 or 15N tracer.

Table 1: inorganic N is expressed in mg/L, which is fine for solutions, but not for de-

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scribing soils. The usual unit for me is mg N /kg soil. Please check consistence of these numbers. See my comments below, these values can have high temporal variability, with consequences on your analysis. Further to the table: what is “natural soil NO3-“? In general, how stable are these numbers for the Braunschweig soils, and for the rest of the sites? They are arable soils, probably subject to fertilization.

L243: What is the rationale of having target soil densities? Do they correspond to the field bulk density?

L248/L378/Figure 1: How did you calculate the N2O (and CO2) fluxes? Since you flushed with N2, I presume that, for t=0, you used a background concentration of 0 for both N2O and CO2 and then calculated the rate of change after 6, 8 or 10 hours (or 2, 4 and 8 in experiment 4). However, you mentioned average production rates (L378, L390); thus, where does the average come from? As you show in Figure 1, rates vary in some cases by more than 100% depending on the incubation length. So, what is your view on this and, more importantly, what is your suggestion for future experiments?

L254: How can you have a fixed measurement precision across different incubation lengths? How was this calculated? The precision is some orders of magnitude lower than the calculated fluxes, but there are some large variations within the same treatment. Is this solely due to spatial variability? Or are you presenting only the analytical precision and leaving out some other sources of uncertainty?

L289: I am not sure I understood this section. I suspect you used two approaches, but they are presented in a mixed way. With the IEM, one calculates the fungal contribution to N2O by solving the equation 4 using the SP of the N2O produced in the acetylene treatment (variety A) and the assumed SP for fungal and bacteria (33.6 for fungi, and -7.5 to +3.7 per mil for bacteria). The N2O from the acetylene is used to eliminate the distortion coming from N2O reduction in the non-acetylene treatment. But according to L304-307, you solve the equation for SP of fungi using FFD. Please clarify this, since it is highly misleading as it is now.

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L352: Where did you get the amount of N in unfertilized soils from? Table 1?

L445: How did you assess the success of the acetylene blockage? A rough look to table 2 suggests that is experiment 2 which did not work. In general, this is a fundamental problem, since you don't know the n2o reduction rate a priori (it is precisely the info you want to obtain), unless e.g. application of 15N labelled substrate is combined with N2 isotopic analyses (what you did in section 3.2.3, but I don't see results for the acetylene treatment, or for N2).

L450: Significance level was established at 0.1 (L366) so p = 0.037 is significant

L461: What are the implications for exp. 4? Was there a significant amount of NO3- available in the soil which may compromise your results? Interestingly, many replicates were not analysed; why? For those values coming from two replicates, why didn't you include the standard deviation (as you did in Table 2)?

L479: you probably mean eq. 8

Table 4 and 5: Why did you set the negative values to 0 in Table 5, but not in Table 4?

Further to table 5: How are the ranges calculated? Are they coming from the different replicates, or from different SP and delta18O, or both?

Section 4.1 and 4.2 should be better streamlined. Actually, section 4.1 refers almost completely to inhibitors (which is section 4.2)

L642: It is not clear to me whether high partial pressure induces physiological changes or rather provokes methodological artifacts (or both). In the former case, respiratory effects might also influence denitrification activity. In the latter case, do you expect an effect on diffusion?

L653: The role of abiotic processes should be briefly discussed here.

L674-678: Experiments 1 and 4 were performed on the same soils, but you got completely different results. And this applies for the mismatch between tracers and acety-

lene method (only in treatment 1, as you say), but also for the tracers results per se (70-80% N2O production ratios in exp. 1, 50-60% in exp. 4). How do you explain this?

L701: Include the papers you refer to.

L702-706: The explanation is right, but I suspect you have many uncertainties in the application of inhibitors. Take into account that CO2 release was not affected by the inhibitors, and N2O not as high as expected, so you may have a significant contribution of non-inhibitable organisms, so that the substrate effect on N2O reduction rates may not be that important

L724-727: The whole sentence is contradictory. Is the SP not an isotopic approach? Please, clarify

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