SOIL Discuss.,
https://doi.org/10.5194/soil-2018-7-RC1, 2018

Interactive comment on "Microbial community responses determine how soil-atmosphere exchange of carbonyl sulfide, carbon monoxide and nitric oxide respond to soil moisture" *by* Thomas Behrendt et al.

Anonymous Referee #1

Received and published: 14 June 2018

In "Microbial community responses determine how soil-atmosphere exchange of carbonyl sulfide, carbon monoxide and nitric oxide respond to soil moisture, " Behrendt and co-authors combine new and previously published gas flux measurements with quantification of soil thiocyanate, microbial phylogenetic rRNA profiles, and qPCR analysis of specific marker genes. The authors find some nice trends in OCS fluxes as a function of soil moisture with biome and land use, and report the surprising result that production rates of OCS are inversely related to soil thiocyanate concentrations. Using RNA-based community profiling methods, the authors report significant differences

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not in the bacterial and archaeal populations, but instead the fungal populations as a function of OCS concentration. Finally, the authors present also CO and NO trace gas measurements using sweep air devoid of those gases, which gives an unresolved balance between their production and consumption in soils as a function of soil moisture. The gene copy number of ammonia oxidizer and rubisco genes are assessed in soils using quantitative PCR alongside OCS fluxes, and results are reported as a function of OCS concentration, though more commonly associated genes such as carbonic anhydrase and thiocyanate hydrolase are not assessed. While the authors present some interesting results, they are not convincingly connected and the study feels as if disparate measurements were forced together. In more than one case, literature is misinterpreted and unsupported conclusions are drawn from results. I believe there are some useful findings and ideas in this paper, but significant work needs to be done to tip the scales away from the weaker aspects.

General comments:

1) Generally, the introduction and conclusions should be more focused to pertain to the main methods and approach of the study. Specific comments on the introduction and conclusions are likely mute at this point given the amount revisions required in the results and discussion section. The methods are wordy and could be written much more concisely. In general, material should be presented in a more organized fashionâĂŤsome paragraphs contain multiple, unrelated concepts. Check that both () are given

2) Details regarding replicates and the timing of different parts of the experiment (measurements from wet to dry, and time of sub sampling) should be made abundantly clear.

3) Because CO- and NO-free air were used in the experiments, it is difficult to evaluate the relative role of consumption and production of those trace gases. This needs to be accounted for in the discussion and conclusion sections. Furthermore, this makes the

data collected here incomparable to that published by Sun et al., 2017 and Section 4.3 and all related matter should be completely cut.

4) There is currently no known link between higher availability of OCS and higher CA levels in soils and/or selection for CA-expressing organisms. It is not known whether organisms expressing CA in soils do so in response to OCS availability, or instead to utilize CA for more well known functions (e.g., pH regulation, C concentration). Therefore, interpreting changes in microbial community structure in response to OCS concentration may need to be more carefully discussed

5) The qPCR results are difficult to interpret with regards to OCS concentration treatment because only one moisture level is available for comparison, and initial differences were not quantified.

6) Limitations of assuming that rRNA reflects microbial activity should be acknowledged. A reference for this can be found here: http://fiererlab.org/2017/12/20/is-rna-a-useful-measure-of-microbial-activity/

7) That said, the differences you observe are interesting. Could you better describe the conditions of the differential OCS treatment. Were there any differences in the amount of time soils were stored, wetted before measurement, or the duration of the measurement that could also contribute to differences in RNA patterns?

8) It could be quite interesting to compare the abundance of taxonomic groups at various ranks other than phylum. If you look at lower levels, do you see more or fewer differences? Given that CA and other genes are not necessarily conserved at the phylum level, you could find that summarizing at the phylum level washes out trends.

9) However, I am extremely concerned that the ITS region is not suitable for this type of rRNA analysis. ITS regions are not preserved in the ribosomal RNA maturation process, instead they are excised. I have not seen previous work showing that their RNA abundance is a proxy for eukaryotic activity. If this is a suitable technique, please

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provide ample references, and I apologize for my ignorance. If not, you will have to re-evaluate your interpretation of the ITS data and any results and conclusions.

Specific comments:

Make sure the term ‘red-like’ is defined.

L195: elemental

L196-200: check that chemical names are accurate

L191 vs L210: difficult to tell if how samples were treated and whether they were homogenized and then again subsampled. What does technical replicate indicate here? If it refers to ‘runs’, that is actually not defined until L213. Concepts should be defined when first mentioned.

L213: Field capacity is not the same as 100% water filled pore space in most cases because some pores do not retain water. How was field capacity and WFPS determined?

L217: Explain the point of the quotations around zero. Why is ‘CO2 ambient” trailing, and what does it mean?

Table 1 should be referenced (in full, not abbreviated), especially before referring to abbreviated sample names ∼L214.

L220: Why give this vague reference to how fluxes are calculated here? Seems out of the blue. Co-locate with Equation 2

L227: Citation refers to a paper that shows this method for NO, not OCS, and the sentence should be worded to reflect this. The following paper has applied this method to OCS and should be cited: Kaisermann et al., 2018, https://www.atmos-chem-phys-discuss.net/acp-2017-1229/

L236: We can’t see Bourtsoukidis et al., submitted and it is not included in your refer-

ences, so this procedure should be suitably described here. What is ts?

L242: How long did saturated soils sit before air flow was initiated?

L243: How do the second and first parts of this paragraph relate? Given an overview that describes the rational for WHY the particular set of experiments were performed with the particular treatments. Why weren't treatments applied uniformly to all soils? How is the analysis here different from those using the same data (Bunk et al., submitted; maybe the other Behrendt et al., 2014 paper though not clear how that data relate to Table 1)?

L247: "the gas fluxes represented active microbial genes" This statement is vague. Please be more specific, or simply say that they were subsampled for molecular analysis and expand upon that procedure later in the methods. Clarify and perhaps more concisely explain the subsampling approach. I'm confused whether these all refer to samples for molecular analysis.

L259, L395: Is 'fumigated' the right term for inlet air with sub-ambient OCS concentrations?

L274: State what the accuracy and precision is. You should state that you are assuming it is similar, but have not measured it in the analyzer used if that is the case.

Equation 2: define Msoil

L280: How long was each soil dried out. Please list the duration in Table 1 for each soil.

L292-297: The justification for this sampling procedure needs to be clarified significantly. What is the objective? Explain why it was desirable to "to minimize OCS consumption compared to OCS production" and likewise why only one subsample is needed to look at OCS consumption. It should be noted that maximal OCS consumption rates in soils is not only a consequence of high numbers or activity of OCS consuming organisms, but is significantly impacted by the control of soil moisture on

trace gas diffusion due to purely abiotic processes. Citation is needed for statement in 296-297.

L362: List also the agricultural soils that did emit OCS.

L364: Flipped implies overturning, when here it is just a shift in balance between production and consumption. I would use ‘switched’ or ‘changed’

L367: Again, it’s really important to state how long these measurements proceeded from the first to last data point to fully appreciate the relationship with soil moisture and time.

L368: Spell out ‘less than’ instead of <.

L372: Spell out agricultural instead of A (hasn’t been defined as an abbreviation and is awkward)

L375: Was soil texture determined, or is sandy a qualitative statement?

L379: The justification for measuring should be given in intro and appropriately cited. Could be repeated here as a question, which would be more suitable, but as a statement it needs a citation. Why is the reference for the method given again (Environment Agency, 2011)? Please include only information relevant to the results section here and keep it concise.

L382: Could you give a statistical justification for removing A2 as an outlier? Were there more roots in that soil? The justification should be given in the results section rather than in Figure 2 caption.

L386: Indicate direction you are moving on x-axis – below 10%.

L390: Stay in past tense.

L395: A topic sentence to reorient the reader would be appreciated. Would be useful to remind reader that 16S reflects bacterial and archaeal populations.

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L400: I would not say this ‘indicates’ their importance, but could suggest it.

L406: give significance of trend.

L409: The title of this section focuses on CO, but the first part of the results focus only on the sensitivity of OCS fluxes to [OCS]. I would suggest renaming section to be more broad and add topic sentence to orient readers.

L415: How can you be sure that consumption changed instead of production? There is likely both CO production and consumption in those soils, but the experiment does not test the sensitivity to consumption of incoming CO (you used CO and NO-free air) so there is no constraint on whether production or consumption changed. Please state what the standard deviation represents and how many soil replicates were used per treatment. It might be worth noting that there is a lot of variability making it difficult to assess differences between the two treatments.

L428: Cite Figure here.

L432: But should state whether those trends are significant given variability.

L434: Were there replicates on the OCS at 1000 ppt cbbL qPCR measurement? Is the variability very low? If there were fewer reps, explain why.

L441: “seems to affect NO release rates and thereby nitrification.”Wouldn’t it be the other way around?

L446-448: A more careful reading of Conrad, 1996 would have revealed that there is great uncertainty in the role of thiocyanate as written in this passage by R. Conrad: “However, the mechanism of OCS production in soils that are not treated with thiocyanate is still unknown”, as only upon artificial amendment of thiocyanate has a potential role been illustrated.

L459: Were there crusts on your desert soils? These should have been visible. If not, this is not a relevant discussion point for your results.

L465: Low concentrations of what?

L482: Suggest adding: “although some were net consumers of OCS.”

L484: The relationship of CO18O to the paper and discussion point needs to be given.

L495: Describe how these two processes represent related niches, especially if OCS production mechanisms are not known and CA are involved in additional processes besides CO2 fixation (e.g., pH regulation).

L501: In general, the term “RNA relative abundance” is a more common way to discuss your community profiling results than using the term “transcripts”, which was used earlier.

L502: I’m not sure why this is relevant: “Our results are supported by a study which found 503 that in agricultural soils, where the lignin content of organic matter is typically low, 504 Ascomycota are the key decomposers (Ma et al., 2013).”

L506: Where is this statement supported: “which might be more resistant to desiccation”? Conjecture is not appropriate.

L510: How is this statement supported by Ogawa et al., 2016?

L445-L476: Despite the results showing a decrease in OCS production with increasing thiocyanate concentrations, the discussion still gives the sense that the authors support a role for thiocyanate in the production of OCS and attempt to explain away the observed trends by bringing up other OCS precursors that might be involved in particular cases or that additional compounds (e.g., organic carbon compounds) are also needed to efficiently utilize thiocyanate. This section also mixes discussions of the drivers of OCS uptake and emissions. I would advise that the authors distill key discussion points, remove repeated results, and embrace their surprising result that thiocyanate concentrations exhibited the exact opposite trend as expected and suggest possible explanations.

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L508-519: This discussion paragraph contradicts itself. You both state that CA classes may differ in their kinetics, that they are distributed in a complex way, and that they should therefore behave in a uniform way. To my knowledge, it has not been shown that CA activity is uniform across its diversity in soils.

L522: I do think that this point about H2S is a good one. It could be useful to estimate the rate of H2S production from full OCS conversion and its potential ability to support sulfur oxidizing bacteria and/or its potential toxicity to soil prokaryotes and eukaryotes.

Table 1: Don't abbreviate Table. The temperature should be listed in the methods. The point that "Note that OCS fluxes for F3, F4, F5 and A1 are presented in a separate study including the compensation points (Bunk et al., submitted). " should be limited to the footnote. Neither are needed in the caption. If the ** designation is defined, it should be found in the table. The use of ' and || is confusing. What does + and - mean? How is A1 different from A2? Why is A5 found under a different line? Spell out countries or define abbreviations. pH units of [1] don't need to be listed. The full row of the second and third occurrence of A1 should be filled out or somehow made easier to understand. This table needs significant improvement to be helpful.

Figure 1: Spell out figure in caption. Define LM, MM, HM. Useful to point out in caption that scales are different on subfigures.

Figure 2: Please color or label all the points with the site name so trends with land use and biome can be discerned. Is the fit to the trend important or meaningful to give?

Figure 3: The source of the standard deviation should be better described in the methods. At which stage in the analysis were replicates considered, and what is represented here? Resolution on this figure should be improved. Why are some groups in []? Make sure color scheme is colorblind friendly (comment applies to all figures). Clean up formatting on labels (remove _, -, etc. . .). What is the difference between unidentified and other?

Figure 5: standard deviation on qPCR results should be offset so they can be seen on all points. Subplots should be designated with letters.

Section 4.3: This is essentially a new results section that is not consistent with the scope or methods presented in this paper. This section should be cut from the paper. The data set of Sun et al., 2017 is not comparable to the data in this paper as they measured fluxes at ambient [CO] concentrations, and therefore can observe net CO uptake, while here the soil were starved of incoming CO. This is essentially comparing one dataset with mostly production (likely abiotic, this study) to another with mostly uptake (likely CO-oxidizing microbes, Sun et al., 2017). Very unclear why CH4 is discussed extensively when it was not measured. For a consistent, self-contained study I advise cutting L526-L575.

L579-L600 is a reasonable discussion providing an interpretation of the data in this study.

L601-610: Your results were not significant, and it is unclear how this is related to OCS, the main topic of your study. I would cut this section. Sauze's reference needs to be given, and this is the only relevant sentence in the paragraph and it's another person's work, so I would just cut it.

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