

## ***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.***

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Anonymous Referee #2 This is an ambitious study that attempts to understand the microbial controls CO, COS and NO soil-atmosphere gas exchange at different soil moisture contents. In addition the experiment benefits from investigating how the net carbonyl sulfide (COS) exchange varied between a range of soils from different land uses and biomes including two desert, two tropical forest and a set of agricultural sites that had different pH and S contents. They also included in the study an experiment to look at changes in the microbial community composition of an agricultural soil when

exposed to different COS concentrations (50 and 1000ppt) and furthermore used a qpcr approach to look at some enzyme genes linked to C and N cycling. As stated above reconciling these types of data to arrive at clear insights on microbial function are a real challenge especially when one is trying to link interactions between a set of different gas species. The communication of this challenge was not helped by this paper as there was often a lack of clarity when explaining the conceptual logic linking the metabolic reactions of the different species in the introduction or later in the discussion. The content is not clearly presented and seems to jump from one idea to another and/or another gas species. This made the manuscript quite hard to read and sometimes I had to read paragraphs over and over to try to understand the link between ideas or statements.

We thank R#2 for the valuable comments. We addressed all points and improved the manuscript accordingly.

The paper would definitely benefit from a set of clear and informative conceptual drawings that explain how these gases interact and the enzymes implicated. For example re-reading the Conrad paper from 30 years ago! we see that these pathways can be presented very clearly. Furthermore the authors need to state clearly what new information this study brings beyond that of Conrad or indeed many of the more recent studies on COS soil-atmosphere exchange. If I ask myself what new information this study brings beyond that of Conrad or indeed many of the more recent studies on COS and soil moisture such as Van Diest & Kesselmeier, 2007 or Bunk et al., 2017. I think the new data is obviously the microbial community analysis with the main result in a bar chart showing the response of bacterial and fungal groups to COS fumigation concentration. However, it is not only the logic that is often hard to interpret but also the methodology. It is not clear how long this fumigation experiment takes place for, how long were the soils incubated at these COS concentrations and how long was the soil moisture experiment?

The incubation time for each experiment is now given in table 1.

It is also not clear what level of replication occurred in each experiment. The authors need to state clearly what replicates there are in each of the figures and show error bars on all plots. The study should also be more quantitative when presenting the gas exchange analysis for example they say the CO response is different at different COS concentrations across drying soils. Can they test this quantitatively with statistics?

See our reply to previous comments. We focus the discussion in the improved manuscript on the qPCR results at 50 ppt OCS.

It is also not really clear why the authors try to attribute microbial taxa trends from an agricultural soil to the soil moisture response of a Finnish forest that did not form part of the present gas exchange study nor was sampled for microbial community. I would remove this graph and cut that discussion from the paper.

We followed the suggestion from the reviewer and removed that point from the paper.

Finally it seemed that the authors want to demonstrate that other enzymes besides that of carbonic anhydrase are responsible for the uptake of COS. However, this experiment really was not designed to test this they did not partition the net fluxes of each gas species nor did they measure the gene expression of CA alongside their other candidate genes. They also never attempted to remove the influence of soil properties and CA from their dataset to look at what unexplained signal was left and how this correlated with other candidate genes and or trace gas fluxes. I think these steps would have all been necessary to test this hypothesis, however with the current study their results do not support any of these hypotheses.

We agree partially with the rationale of the reviewer. Knowing limitations of the study we down-toned our conclusions and highlight that a combination of stable isotope probing plus metagenomics might lead to more concise conclusions if beyond CA also further enzymes were involved.

Minor points Line 61 upland is a bit specific here I would change to oxalic

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Upland changed into oxic.

Line 65 I think Ogee et al. 2016 go quite far in explaining COS uptake but rather pointed out production was somewhat unclear.

We agree that Ogee et al., 2016 present a well elaborated model for OCS uptake. However, the author's stated "We recognize that Eq. (9) is an oversimplification of the reality in the sense that  $k_{cat}$  and  $K_m$  are not true kinetic parameters but rather volume-averaged parameters for the entire soil microbial community. Also Eq. 9 neglects the competition for CA by CO<sub>2</sub> molecules and the co-limitation of the uptake by diffusional constraints." There is evidence that different forms of CA are involved in OCS and CO<sub>2</sub> exchange (Meredith et al., 2018b) and even other enzymes might be involved (our study).

Line 71 I am not sure there is quantitative evidence to support this statement yet.

We agree and modified that statement and included Meredith et al., 2018a.

Line 76 I don't think Bunk et al 2017 showed this.

Modified in the improved manuscript.

Line 99 You say elevated CO<sub>2</sub> would inhibit rubisco but not CA however there are many studies that microbes grown in elevated CO<sub>2</sub> down regulate CA activity and in fact microbes with CA knocked out cannot survive in low CO<sub>2</sub> but can in high CO<sub>2</sub>. You should read and cite some of these studies.

We thank the reviewer for this comment. Under some environmental conditions autotrophs might contribute to OCS exchange (e.g. Sauze et al., 2016). We discuss our data more carefully.

Line 122 The Bunk experiments did not estimate or measure CA activity so at least point out that the role of CA was putative.

We agree and modified that in the improved manuscript.

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Line 220-224 this is really not clear

We use a + or – to indicate in table 1 if NO and CO exchange rates have been measured. While CO and NO in incoming air was scrubbed, 50, 500 or 1000 ppt OCS mixing ratio was used.

Line 236 remove this citation

Citation was removed.

Line 237 should this not be msoil(ti)?

Corrected.

Line 258 from how many soil replicates?

We performed technical replication which is explained in the method section.

Line 273-274 this is super vague and confusing

We included accuracy and precision in the improved manuscript.

Line 290-297 is repetition should be removed

Has been removed.

Line 363 A3 is still producing too

Corrected.

Line 382 don't you mean A2 here?

A3 is corrected to A2.

Line 389-392 repetition again

Removed.

Section 3.2 title not helpful with A2 not sure about the replication, timescale of exper-

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iments and why you expect to see a difference over such a short temporal and conc change? Also are the other basidiomycetes not also sig. diff?

Title was changed to “Fungal activity correlated with POCS and UOCS from A1 soil under different OCS fumigation regimes”. We evaluate the molecular results more carefully in the modified version. The p-value for Chytridiomycota is 0.07 and for Glomeromycota is 0.765. Chytridiomycota is weakly significant, Glomeromycota not significant.

Line 417-420 Not sure what this means or is if it is supported by the data.

Given the complexity of the correlation of OCS and CO exchange, we removed the CO data into the supplementary information. We focus on the discussion of OCS and NO release data.

Line 439-441 this seems like the most interesting and novel result

Yes we agree. For improving the robustness of the result, we added ammonium and nitrate data (see Table 1) and highlighted “The highest net OCS release rates were correlated with highest nitrate concentrations in a sandy soil from a desert (D2) and a soil sample originated from a cornfield (A2).

Line 452-454 repetition of results

Deleted in the new version.

Line 469 ambiguous

We modified the discussion and included Meredith et al., 2018a.

Line 474 what evidence do you have for this statement?

We included the following references “Both inorganic and organic S availability control OCS production rates in general (e.g. Meredith et al., 2018a; Banwart and Bremner, 1976; Banwart and Bremner, 1975; Flöck et al., 1997; Lehmann and Conrad, 1996),

but rates of OCS consumption are controlled by different parameters (e.g. Kaisermann et al., 2018).”

Line 475 I think it is possible to model the moisture response of consumption quite well see Ogee et al., 2016

See reply to comment line 65.

Line 494-496 this statement is not supported by the results as net flux was not partitioned

In our study we manipulated the OCS mixing ratio of the background air which was flushed into the soil chambers. Since such studies over the whole range of soil moisture require a lot of resources, we were not able to perform the experiment under various OCS mixing ratios. As shown in other studies this would be required for partitioning into OCS production and consumption (Kaisermann et al., 2018). However, it is known that under OCS-free air the OCS production dominates, whereas under fumigation with OCS (1000 ppt) the OCS consumption is the predominant process. We reformulated, but keep our statement in the improved manuscript.

Line 503 not sure of the relevance of this statement

See reply to R#1 L502.

Line 504-507 I am not sure I would jump so quickly to this explanation when it is clear soil texture has a strong control on the soil moisture response of COS

We thank R#2 to point out the strong control of soil texture on the soil moisture response of OCS. Despite we analyzed soils highly variable in soil texture a quite uniform and reproducible pattern of OCS exchange with respect to soil moisture was observed (figure 1) A recent study found that soil OCS exchange rates vary with diversity of CA forms (Meredith et al., 2018b). However, the role of archaea and bacteria for OCS exchange from soils is not yet understood. Thus, we excluded our explanation.

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Line 516 onwards this does not make much sense

We improved the wording of this paragraph.

Line 608 cannot find this ref cite Kaisermann et al 2018 and Melillo & Steudler, 1989 instead

In improved manuscript references were replaced.

Text on graphs too small, the combination of red and green symbols/bars is not colour-blind friendly and also green on green symbols and lines is impossible to read too. Fig 5b should have another panel.

We improved all figures according to the suggestions from the reviewers.

Referring to A, F or D is also inconsiderate to the reader I don't want to have to memorise labels to read a paper.

We agree to the reviewer and changed the main text accordingly.

In addition the table describing the soils gives very little detail about the soil characteristics necessary to understand the main drivers of the moisture response such as texture and bulk density. I would get rid of fig 6 and 7

We thank the reviewer for the comment and added nutrient concentrations to table 1 and removed figure 6 and 7.

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Interactive comment on SOIL Discuss., <https://doi.org/10.5194/soil-2018-7>, 2018.

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