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# *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 #1 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 thio-



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cyanate concentrations. Using RNA-based community profiling methods, the authors report significant differences 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.

We thank referee #1 for highlighting the nice trends in OCS fluxes as a function of soil moisture with biome and land use and the novelty of our approach combining gas flux measurements with molecular analysis. We addressed all comments and improved the manuscript accordingly. In general for qPCR an 3-fold difference is commonly significant considering usual variability of DNA extraction. We refer our results to nanogramm extracted DNA to correct for the extraction bias (Degelmann et al., 2010). Nonetheless, we performed technical replicates for the extractions and thus, we interpret the results for bacterial and archaeal populations more carefully in the revised manuscript. The separation of net production and consumption of a certain trace gas requires fumigation with those gases at different mixing ratios combined to a stable isotope approach. Since this would be out of scope for our study, we focused on the report of the effect of OCS fumigation on NO exchange rate. The data for CO exchange rate have been moved into the supplementary information. The used primer systems have been well evaluated in various previous studies and thus deliver robust results. We agree that it would be advantageous to additionally quantify gene copy numbers of carbonic an-

hydrase and thiocyanate hydrolase genes. Since there were no well evaluated primer systems we decided not to address these genes by qPCR. Interestingly a new metatranscriptome study (Meredith et al., 2018) on the link of different carbonic anhydrase enzymes and OCS and CO18O concludes that measurements of other enzymes that may consume OCS (CS2 hydrolase, RubisCO, CO dehydrogenase, and nitrogenase) are needed. In agreement with previous studies, we added in table 1 nutrient concentrations to demonstrate a correlation of nitrate and OCS exchange rate (Kaisermann et al., 2018;Melillo and Steudler, 1989). The main focus of our study is to provide evidence that the analysis of multiple gases is a useful tool to better understand the contribution of microbial groups and to highlight a connection of OCS and NO (and potentially CO) exchange. It has been reported already e.g. by Bender and Conrad (1994) that nitrifying and methanotrophic populations oxidize not only their specific substrates but also other compounds.

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 fashion at some paragraphs contain multiple, unrelated concepts. Check that both () are given

We thank the R#1 for the valuable input and we present an improved version of the manuscript.

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.

We include that in the updated manuscript.

3) Because CO- and NO-free air were used in the experiments, it is difficult to evaluate





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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.

We agree to the reviewer and modified the discussion accordingly. Fig. 6 was excluded and we focus our discussion on the correlation of OCS and NO in Fig. 5 and 6. Furthermore we present data for more soils and moved the CO data into the supplementary information.

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

So far it is unknown if the concentration of CA and other enzymes (e.g. RubisCO) in soil is changing in a similar or different pattern across drying out conditions. Recent studies suggest that different classes of CA will get active (Meredith et al., 2018; Sauze et al., 2017). However, these studies did not investigate the role of RubisCO and conclude at the end that measurements of other enzymes that may consume OCS (CS2 hydrolase, RubisCO, CO dehydrogenase, and nitrogenase) are needed (Meredith et al., 2018). Whelan et al. (2018) pointed out the importance of RubisCO for OCS exchange from soils. RubisCO (and PepCO) was already discussed to react with OCS in a study where a CA specific inhibitor was used (Teusch et al., 1999). Unfortunately we took only one soil sample for molecular analysis at 1000 ppt OCS. With respect to the small difference in OCS mixing ratio due to fumigation and considering a 3-fold difference as usual variability of DNA extraction, we exclude the qPCR results at 1000 ppt OCS. Instead we focus on the robust change in cbbL, AOA amoA and AOB amoA over drying out at 50 ppt OCS. The changes for normalized red-like cbbL gene copy numbers





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support the idea of recent studies (e.g. Whelan et al., 2017; Meredith et al., 2018b) that other enzymes are involved in OCS exchange from soil. Thus, we discuss that more carefully in the improved version of the manuscript.

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.

We agree to R#1 and changed the interpretation of our results for the qPCR data respectively in the improved manuscript. The increase of cbbL, AOA amoA and AOB amoA follows clearly the OCS and NO release rates. Considering many previous studies on detectable gene differences referred to nanogramm soil, we are convinced that detected differences are significant, although we used only technical replication of extractions.

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-auseful-measure-of-microbial-activity/

We are aware of the limitations of using RNA as a proxy for microbial activity. Thank you for the comment. We have added a sentence in the improved manuscript and an additional reference, respectively.

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?

The differences in storage were only some weeks and the difference in incubation time was less than 30 minutes. Thus, it seems unlikely that sample conditions impacted the result. We interpret the qPCR data now more carefully. See also our answer to comment 4.

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.

We present the result on the class level in figure 3 and 4. The sequence depth is not enough for conclusions, but we agree with the reviewer that wash out trends on the phylum level can occur.

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 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.

Thank you for this comment. Using ITS as proxy for fungal protein biosynthesis is a well established method and has been used in other studies ((Žifčáková et al., 2016; Baldrian et al., 2012). We added the references and a sentence in the introduction.

Specific comments: Make sure the term 'red-like' is defined.

We added (in nongreen algae and $\alpha$- and $\beta$-Proteobacteria, Selesi et al. 2005) in L180.

L195: elemental

Corrected in final manuscript.

L196-200: check that chemical names are accurate

Corrected typo.

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.

We improved the description for clarity in the revised manuscript version. Technical replication indicates that we did not access true biological variability because soil samples from different points in the field already were mixed and homogenized by sieving. Please see also our first comment where we refer to a 3-fold difference in qPCR results as significant considering usual variability of DNA extraction.

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?

Corrected. Soil samples were rewetted until saturation occurred and a thin film of water was visible.

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

Small fluctuations in the mixing of CO2 (which we denoted by $\sim$ 400 ppm) are reported also from other works (e.g. Kaisermann et al., 2018). In similar manner we added now the standard deviation of our experiments of 8 ppm.

Table 1 should be referenced (in full, not abbreviated), especially before referring to abbreviated sample names _L214.

Modified in final manuscript.

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

Reference Behrendt et al., 2014 moved to L280 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-

Corrected.

L236: We can't see Bourtsoukidis et al., submitted and it is not included in your references, so this procedure should be suitably described here. What is ts?

Msoil (ts) is the soil mass at the time when the experiment was stopped. The detailed formula's for the calculation of the soil moisture are given in Behrendt et al., 2014 which is cited in L233.

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

The time after wetting until start of the experimental dry out was about 30 minutes.

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?

Our study has 3 different focal points: (1) screening of a large number of soils for OCS exchange under 500 ppt OCS (ambient conditions), (2) correlation of OCS exchange to other trace gases (NO and CO release rates), and (3) link to microbial community. Due to pratical limitation we only investigated some soils for all three aspects. We included a statement in the discussion.

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)?

We moved the OCS release rates into the supplementary information (S. 1). Some soil properties were adapted from other studies (see Table 1). All other data are newly created and have not been published.

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 anal-

ysis 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.

The sub-samples refer to the molecular analysis. We rephrased as follows '. . .the OCS and NO (and potentially CO) exchange rates suggested that cbbL, AOA and AOB amoA functional genes associated with their turnover might actively be expressed.'

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

Changed into flushed with OCS free air

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.

We included accuracy and precision now.

Equation 2: define Msoil

Msoil equals the dry mass of soil after dried for 48h at 105°C and is included in the improved manuscript.

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

Incubation time was added in Table 1.

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.

The objective was to see if differences in microbial gene expression were linked to different OCS mixing ratios in inlet air (50 and 1000 ppt). 50 ppt OCS was chosen to minimize OCS consumption compared to OCS production while 1000 ppt was chosen to maximize OCS consumption. We agree that it would be advantageous to study more than one sample under OCS consumption. However, we were not sure if we can resolve overall differences in microbial gene expression based on OCS mixing ratio. Bunk et al. 2017 has been included.

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

Changed to all agricultural soils, since even the agricultural soil under sugar beet cultivation produced OCS above the noise criteria of 1.09 pmol g-1 h-1.

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

Replaced by "switched".

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.

Incubation time is given in Table 1.

L368: Spell out 'less than' instead of <.

Changed into "less than".

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

Changed into "agricultural".

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

The texture for the desert soils was determined according to ISO 11277 as sand (WRB classification).

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.

Reference was removed from the result section.

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.

Moved into the result section.

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

About changed into below.

L390: Stay in past tense.

“Are” changed into “were”.

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

Archaea has been included in 3.2 and 4.2 headline and a topic sentence has been added in 3.2.

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

Changed into “which could suggest”

L406: give significance of trend.





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Sorry, p-value now included.

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.

After reading the valuable comments from Reviewer #2, we decided to highlight the effect of OCS fumigation on NO release rates, add nutrient data and NO release rates from 2 additional soils and moved the CO data into the supplementary information. Section was renamed to effect of [OCS] on NO release rate and topic sentence was included.

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.

We agree that from our experiments it is not possible to conclude if CO production or CO consumption was affected. The mean values and standard deviation were calculated from replicates in time, i.e. from the last five time points. This information has been added now in Section 2.3. Additionally, we moved the CO exchange rate data into the supplementary information.

L428: Cite Figure here.

Included.

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

See our first comment. In general for qPCR an 3-fold difference is commonly significant considering usual variability of DNA extraction. Given the low variability in OCS





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concentration (50 to 1000 ppt) we excluded the data for 1000 ppt OCS and show only the robust trend for 50 ppt OCS normalized to nanogramm extracted DNA to correct for the extraction bias (Degelmann et al., 2010).

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.

We performed for each measurement (n=3) technical replicates and the variability at OCS 1000 ppt cbbL was smaller than the symbol and therefore not visible. However, since the difference in cbbL qPCR measurements under 1000 to 50 ppt is not significant given a 3-fold difference (considering usual variability of DNA extraction), we excluded the qPCR data at 1000 ppt OCS.

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

We applied an OCS treatment (50 ppt and 1000 ppt, each constant over a dry-out experiment) and measured NO, thus it is likely that OCS affects NO release rate, but the mechanism is unclear.

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.

We referenced other studies to point out that other precursors are involved in OCS production (e.g. Banwart and Bremner, 1976; Banwart and Bremner, 1975; Lehmann and Conrad, 1996). Our result that thiocyanate concentration is inversely related to OCS production and demonstrates that thiocyanate plays a minor role. We therefore added Meredith et al., (2018) to highlight the importance of S-containing amino acids for OCS production.

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.

The total sulfur content (Table 1) shows that in the sandy desert soils (D1 and D2) the total sulfur content was enriched. However, we did not perform further analysis and crusts were not visible. Therefore, we excluded that point in the improved manuscript.

L465: Low concentrations of what?

Changed into 'low microbial abundance'.

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

Thank you for the suggestion which we added in the new version of the manuscript.

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

Since the focus of our manuscript is on OCS, we deleted "CO18O and".

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).

We now improved our explanation: "A possible explanation for the large differences in POCS and UOCS among the various soils investigated here might be a separation (here: soil moisture) of gene expression and activity maxima under different moisture conditions for different OCS-converting enzymes: At high soil moisture the production OCS by hydrolysis of organic S compounds might be the dominant process, while at moderate soil moisture consumption of OCS by CO2 assimilation might be the predominant process."

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.

We now use the terms ITS RNA relative abundance and gene transcripts for qPCR data.

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)."

We agree with the reviewer that this is not relevant and removed it from the manuscript.

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

We agree to the reviewer and excluded the sentence.

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

We clarified the statement in the improved manunscript.

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.

We improved the discussion on this crucial point.

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.

We improved the discussion.

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.

The full conversion of 1000 ppt OCS to H2S leads to ∼43 pmol l-1 in the gas phase. Even when considering that the respective mols are converted in one gramm of soil with about 106 active cells the amount is much to less to conserve enough energy for cell growth. However, this rough estimate needs to further investigated with pure cultures. Also, toxicity at this low level of H2S concentration is unlikely.

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.

We improved the table in the new version of the manuscript. Results for NO and CO (see supplementary information) exchange under 50 and 1000 ppt OCS for two more samples, F3 and Mainz corn dried, were added. For easier understanding 50 ppt OCS are referred to as 'zero-air' and 500 ppt OCS 'ambient', and 1000 ppt OCS 'elevated'. + and – indicate for which soil samples CO and/or NO exchange in addition to OCS exchange have been measured. We included "and measured OCS, CO and NO exchange rates (+ measured and – not measured)".

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

In the improved version of the manuscript Figure 1 is spelled out, LM, MM and HM is defined and it is pointed out in the caption that y-axis scales of subfigures are different.

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 2 was changed accordingly and fit was removed in new version.

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?

We improved the figures, and corrected the wording in the figure legend.

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

Figure 5 was improved accordingly.

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.

We agree and cut the section. Instead we followed the comment from reviewer #2 and added a section about the correlation of OCS to nitrogen cycle.

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

We understood this comment as a support to leave that part of discussion in a revised version of the manuscript. So we had done.

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.

We improved the discussion section.

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