Response to the comments on "Stomatal control of leaf fluxes of carbonyl sulfide and CO_2 in a *Typha* freshwater marsh"

Wu Sun, on behalf of all coauthors

In this response, the text is formatted as: referees' comments (indented blocks), authors' response, **applied changes in the manuscript**. Locations in the revised manuscript are referenced by "P{#X}L{#Y}", meaning "Line Y on Page X".

Reply to comments by Teresa E. Gimeno (Referee #1)

> Sun et al. present here the first field dataset for COS and CO_2 leaf relative uptake (LRU) collected in situ during continuous measurement over the peak of a growing season. The authors chose a typical wetland plant (*Typha latifolia*) and report continuous measurements of CO_2 and COS uptake under varying environmental conditions, mainly light (photosynthetically active radiation) and vapour pressure deficit (VPD). They demonstrate that the strong dependency of LRU with PAR observed under laboratory conditions is also observed under natural conditions. The authors explain that strong stomatal control of both processes (COS and CO_2 uptake) underlies the observed patterns. Interestingly, the authors report lower LRU values in natural conditions than those previously measured under laboratory conditions. This constitutes a very valuable contribution as it is the first dataset of LRU collected at the leaf level, in situ, under natural conditions and for more than a month. The paper is very clearly written and the figures and results are nicely presented.

We thank Dr. Gimeno for her evaluation of our manuscript. We have made corrections and clarifications to concerns raised in the comments. Below please find a detailed point-by-point response.

> Yet, I do not believe that this paper deserves to be published as regular 'research paper', rather these results would be more appropriately presented as a technical note or rapid report. The reason is that the authors report data from one chamber that measured continuously one single set of six leaves. Their results and conclusions are relevant as they constitute a strong proof of concept, but continuous measurements

> over a period with limited climatic variability (one campaign with homogenous meteorological conditions, P6L11–15) on a single set of leaves from the same plant (presumably) are not sufficient to constitute a whole research paper. Even more so taking into account the environmental heterogeneity of the light environment (P6L19–21).

We acknowledge these limitations in data collection; however, none of them should weaken the main conclusions or disqualify the manuscript from a full research paper. In fact, the experiments were designed to characterize leaf relative uptake (LRU) variability in response to environmental controls, particularly on the diurnal timescale. It represented the first continuous measurement of leaf fluxes under field conditions, and therefore the first validation of theory and lab measurements. This experimental design in turn served the higher purpose of COS-based ecosystem GPP estimation by providing accurate LRU parameters.

An ideal experimental design entails randomization and replication, yet field conditions and the resources available can often be restrictive. We were limited by the sampling time on the QCLS analyzer, because in each hour, 45 minutes were allotted to eddy covariance measurements (unpublished) and the rest was divided between a soil chamber (unpublished) and a leaf chamber (reported here). To support COS-based GPP estimation *on the hourly timescale*, LRU measurements must have the same resolution in time. This was the main reason that a high sampling frequency was chosen at the expense of having multiple leaf chambers. Nevertheless, we had a large sample size ($N > 300$) to support a robust analysis of LRU variability.

The similarity in day-to-day meteorological conditions was a blessing rather than a defect of the study, because it means that, other than PAR and vapor deficit, little else was changing, creating an ideal situation for testing LRU responses to PAR and vapor deficit. Indeed, diurnal variations of fluxes and LRU in response to PAR and vapor deficit were well characterized with a high sampling frequency. Besides, there were a few overcast days that caused the daytime mean LRU to increase; this was made clear in Fig. 7. The PAR sensor used in the study was collocated with the chamber (see P5L3–L4 added in the revised text), and this should have properly accounted for the light microenvironment around the chamber.

> In addition I have some major technical concerns and another major concern related to the result interpretation and theoretical framing of the study.

We have made revisions and added the requested information in the Methods section to address these technical concerns. We have provided stomatal conductance estimates to

improve data interpretations and to better support the main messages of this study.

> In the methods, the authors claimed that they used a 'flow-through (dynamic) chamber' (P4L5). Yet, during the 5-minute measurement period the chamber acted as a static enclosure (P4L13) and the authors calculated COS and CO_2 uptake and transpiration from the slope of the progressive drawdown (or accumulation) of the different species over time (P5L4–15). This approach should be valid provided good mixing, but that is hard to achieve for a > 10 L chamber without a fan. The authors need to provide the flow rate entering the cuvette over the measurement period and discuss to what extent they can warranty thorough mixing inside their chamber. In addition, the authors report that they characterised the blank fluxes of (presumably) the same chamber and that these were negligible (methods P4L27). Still, they do not specify how often were these characterised and under what conditions. More important, although it is not stated specifically, it appears that they authors calculated transpiration rates from H_2O vapour concentration measured with the QCLS. If that is the case, I assume the authors did not operate their QCLS coupled to a Nafion drier (or other type of water trap) and thus they need to correct for the interference associated with the water absorption line (Kooijmans et al. 2016 Atmospheric Measurements Techniques 9: 5293–5314).

These technical concerns have been addressed in §2.2 Experimental setup.

The leaf chamber was not operated as a static enclosure. There was always airflow passing through the chamber, supplied by a vacuum pump. During the measurement phase, the flow rate entering the enclosure was the same as that leaving. To clarify that the chamber was always an open system, **the label of the measurement phase has been changed from "ch closed" to "ch meas" in Fig. 1b**.

There were two fans running in the chamber, one for ventilation, and the other for mixing. During the measurement phase indicated in Fig. 1b, the ventilation fan was turned off, but the chamber nevertheless was still a flow-through system, because (i) the pump was pulling air from the chamber and (ii) the opening of the ventilation fan served as the inlet. The mixing fan was kept running continuously to make sure the air inside the chamber was always mixed. **This paragraph has been rewritten.** See P4L8–L13 in §2.2 Experimental setup.

The median flow rate through the chamber was 6.4 slm, which translated to a chamber turnover

time of 1.5 minutes. **This information has been added in P4L17–L18.**

Blank chamber effects were negligible: 0.05 ± 0.29 pmol m^{-2} s^{-1} for COS and 0.02 ± 0.15 µmol m^{-2} s^{-1} for CO_2. **This information has been added in P4L32–L34.** Please also see the Supplement for a detailed description.

We did not use a Nafion dryer or any other water trap. We applied the water broadening corrections supplied by TDLWintel—a data acquisition software on the QCL—using default correction factors. **We have now described the water correction procedure in P4L23–L27.** In addition, we have also discussed the potential influences of the uncertainty in the CO_2 water correction factor (see the Supplement).

> The authors claim that simultaneous stomatal control of both CO_2 and COS uptake underlies the coupling between these processes and the changes in LRU observed under low light and high VPD. Indeed, stomatal control lies at the heart of the discussion and the theoretical framing of the paper, but no data are shown. Also, the authors claimed that they monitored leaf temperature (P4L29–30) and they also had transpiration fluxes (Figure 3c). Still, no calculations of stomatal conductance have been performed. Later in the discussion, some calculations of stomatal conductance are mentioned (P8L20, P9L24), but the authors do not detail how these were obtained. Given that the authors have all the ingredients to calculate stomatal conductance, but yet these are missing, I wonder if this is due to poor mixing inside the chamber, which would have affected all other measurements. This needs to be clarified. In addition, comparing estimates of stomatal conductance derived from COS-uptake measurements with independent quantifications of stomatal conductance from transpiration and leaf temperature would allow to further demonstrate the tight stomatal control of COS uptake. These issues need to be clarified.

Diurnal patterns of the stomatal conductance of H_2O and the total conductance of COS have now been presented in the new Figure 6. Interpretations and discussions of the results have been added. See §3.2 in the Results and §4.1 in the Discussions. We have also described how stomatal conductance of H_2O and the total conductance of COS are calculated in §2.5.1 in the Methods.

> In addition to these major issues, I have some additional concerns:

Title Remove the term 'Stomatal control' unless you decide to include stomatal conductance measurements, otherwise I suggest "Effects of light and vapour pressure deficit on the coupling of leaf fluxes of carbonyl sulphide and CO_2 in a *Typha* freshwater marsh under natural conditions", or something similar.

We have included stomatal conductance estimates. The title is kept unchanged.

Abstract

P1L3–4 I think, here, you could be more specific with respect to what we have learned so far: 'LRU is known to increase under low light'.

Revised. See P1L4.

P1L15–17 reduce the emphasis on the role of stomatal control.

Since stomatal conductance data have been added to the revised manuscript, the emphasis on the role of stomatal control is appropriate.

Introduction

This section is interesting and very clearly written.

P2L2–10 maybe consider shortening this section, these concepts have already been amply discussed in the literature.

We have shortened this paragraph by 25%. See P2L2–L8.

P2L2 'COS has been shown to be a unique tracer'.

Changed to "Carbonyl sulfide (COS) is a unique tracer for . . .". See P2L2.

P2L8 'The approach to estimate photosynthesis from COS fluxes'

This sentence has been removed for conciseness.

P2L12 'COS and CO_2 follow the same diffusional pattern'

We think that 'pathway' is a more suitable word. A search in Google Ngram (https://books.google.com/ngrams/) finds no result of the phrase 'diffusional pattern'.

P2L22 'environmental variables that regulate diffusional limitations, mainly stomatal conductance, including photosynthetically active radiation (PAR) (. . .) and vapour pressure deficit (VPD)' Also provide a citation here (e.g. Leuning 1995 that you already cite).

Revised according to the reviewer's suggestion. See P2L18–L21.

P2L25 'In contrast to the CO_2 flux, at night, COS uptake might continue. . .'

Revised to "At night, in contrast to the CO_2 emission, COS uptake may continue . . .". See P2L22.

P2L27–28 'Night time COS uptake has been observed..'

Revised: 'found' —> 'observed'. See P2L24.

L29–31 This is not entirely clear. I think here what you mean is that the cited studies inferred vegetation COS uptake from ecosystem-scale measurements instead of direct measurements.

The sentence has been improved to clarify the point. See P2L26–L27.

Also, please note that both Maseyk et al. (2014) and Commane et al. (2015) found not only evidence for COS uptake, but also emission, this should be briefly mentioned here.

Emissions reported in Maseyk et al. (2014) came from soils and mature grain heads. And those in Commane et al. (2015) were not found in subsequent years of their campaign (Wehr et al., 2017). Since the scope of this study is leaf scale COS exchange, non-foliar sources of COS are only of peripheral importance and are hence not elaborated here.

P3L16 ‘We need direct measurements of how LRU. . .’

Revised. See P3L12.

P3L19–23 Please try to specify the research objectives more clearly, or even better formulate two hypotheses (e.g. LRU will decrease under low light in natural conditions) instead of stating the questions that motivated the study.

The research objectives have been rephrased in terms of clearly-defined hypotheses. See P3L14–L20.

Methods

This section is also very clear and nicely written, but some key details are missing (see major concerns above).

The missing details have been added.

P4L5–14 Could you please provide a schematic drawing of the gas-exchange chamber?

A schematic diagram of the chamber has been added in panel (a) of the new Figure 1.

P4L29–30 Where are the data for leaf temperature?

Leaf temperature data are now shown in Figure S3 in the Supplement and are added to the online dataset. In addition, vapor deficit shown in Fig. 3f has been corrected with respect to leaf temperature.

P5L17–18 ‘Conspicuously unrealistic data points in the meteorological data were removed.’
P5L18 ‘independent criteria to filter measurements’
P5L21 ‘were also discarded’
P5L23 ‘these filtering criteria’

Revised following the referee’s suggestions. See P5L21, L22, L25, and L27.

Results

P6L2 so if LRU was only calculated during the daytime, why do present the 24-h mean LRU in figure 6?

We have now clarified how the instantaneous LRU (i.e., the commonly referred 'LRU' in the literature) and the time-integrated LRU are calculated in §2.5.2 in the Methods. The use of time-integrated LRU is relevant to large-scale applications, as is discussed in §4.3 in the Discussions.

The results section is very clear and I only have one minor comment: no need to repeat the definition of LRU (P7L1).

We have removed the redundant definition.

Discussion and conclusions

In my opinion, this section turned out to be the least interesting of the paper. It is nicely written, but it only consists on a mere repetition of the results and ideas previously presented in the introduction. It can be shortened significantly and I believe the results and discussion section should be merged into one, which would be a much more adequate format for a technical paper. I provide some further specific details below.

We have restructured and greatly abridged the discussion to strive for a balance between conciseness and clarity. The former §4.1 has been removed, because most of its original contents are now addressed in the Results. The former §4.3 on LRU environmental control has been completely rewritten, explained in terms of the stomatal vs. internal conductance competition. Other parts of the discussion have been condensed significantly.

For the optimal flow of the text, we did not merge the Discussion into the Results. This is simply because each part of the Discussion may rely on multiple pieces of information from the Results.

P7L19 Provide a citation to support light-independency of COS hydrolysis.

Added Protoschill-Krebs et al. (1996). See P10L15.

P7L17–26 This paragraph is a long compilation of ideas presented already in the results and in the introduction.

Removed.

P7L27–P8L2 this paragraph belongs to the results section.

Moved to §3.1 in the Results. See P8L28–L33.

P8L20 provide the details for these calculations in the methods.

This is intended as a back-of-the-envelope calculation for discussion only. Strictly speaking, the obtained value is an estimate derived from the data, so it is not appropriate to document the calculations in the methods. **We have detailed the calculations in the Supplement instead.**

P9L6–10 Shorten this section, most of these ideas are repeated elsewhere in the paper.

Removed.

P9L16–22 Again repeated ideas, this belongs to the introduction.

This part has been completely rewritten. See P10L20–L30 in §4.1.

P9L23 The discussion is not a section appropriate for introducing new equations, move this to the methods.

P9L24 detail how this was calculated in the methods.

This part has been removed since it is no longer essential for the discussion. However, generally, there is no rule or guideline to discourage the use of equations in the discussion, if they are well explained.

P10L8–10 this discussion on the variation of LRU among species is very relevant. Note that *T. latifolia* has a very particular physiological behaviour, often exhibiting very

high rates of carbon uptake (e.g. Yavitt & Knapp 1998, 139:495–503 or Jespersen et al. 2017 Functional Plant Biology 44:774–784). Thus it is not surprising to find lower LRU value than those previously reported for other plants. Maybe also consider comparing your measurements of leaf CO_2 uptake with previous measurements as you seem to have measured much lower values than those previously reported, although this might be simply due to the differences in environmental conditions among studies, most likely light environment.

The reviewer raised an interesting point regarding the link between LRU and photosynthetic parameters. **We have added a brief explanation to the low LRU of the *T. latifolia* in P11L33–P12L3 in §4.3.**

P10L11–24 I do not think it is relevant to discuss the differences between day-time LRU and 24-h averaged LRU. The parameter LRU is useful to estimate GPP from COS uptake, thus it is only relevant during day-time. Please remove this section and the corresponding values from figure 6.

The all-day mean LRU is relevant to large-scale applications, because regional COS drawdown patterns are time-integrated features. **This is discussed in P12L7–L15 in §4.3.**

P11L2 remove 'that is only stomatal conductance limited'.

Removed.

P11L4–6 rephrase, are 'midday' and 'early afternoon' the same? Because you use them interchangeably here!

This sentence has been removed from the conclusion. We have taken care to use these terms consistently in other parts.

P11L7–9 I am not quite sure I understand the logic behind this statement. In the afternoon, presumably, PAR does not limit stomatal opening, instead stomatal opening would be limited by high VPD and thus COS and CO_2 would both be constraint and hence LRU would not respond to VPD.

This is because COS uptake is more stomatal-conductance-limited than CO_2 uptake due to the

much higher enzyme activity of CA in catalyzing COS hydrolysis (k_{cat}/K_m of CA > k_{cat}/K_m of RuBisCO). **We have added a discussion of this issue in P10L25–L29 in §4.1.**

> In fact, I cannot appreciate a change in LRU at midday in figure 3d. I think this conclusion might be a bit misguided by an earlier interpretation of the measurements.

The y-axis range of Figure 3d has been adjusted to emphasize the variations of LRU. A "dip" of LRU between 15:00 and 18:00 should be clearly visible now.

> P11L10–15 this should be the opening, not the closing paragraph of the discussion.

This paragraph no longer exists. **The conclusion has been rewritten.**

> Figure 4. The data points do not appear colored.

The figure has been revamped to resolve the issue experienced by the reviewer. The problem was likely caused by the aliasing of the edges of data points under low-resolution conditions.

Reply to comments by Mary E. Whelan (Referee #2)

> Leaf relative uptake (LRU) of COS and CO_2 is a parameter that is often used to estimate plant CO_2 uptake from observed ecosystem fluxes of COS. There are other sources and sinks of COS in ecosystems, though they are typically small compared to uptake through plant stomata. One important exception is wetland soils, which tend to be a relatively large source of COS. In non-wetland or agricultural systems, measurements of net CO_2 and COS concentrations and fluxes are sufficient to make an estimate of GPP with an approximation of LRU.

We thank Dr. Whelan for her helpful and insightful comments. Indeed, COS fluxes from wetland soils—potentially large sources—need to be carefully constrained when using COS measurements to infer GPP. In this study, the chamber enclosure created a separate system for leaf gas exchange that was free from soil interference. When scaled up to the canopy, with soil COS budget constrained, the COS method for GPP estimation can still work reliably in a wetland ecosystem. The

treatment of soil COS budget in GPP estimation is out of the scope of this paper, but will be demonstrated in a manuscript on ecosystem-scale COS fluxes by our group (Seibt et al., in prep.).

> Here, Sun et al. present a dataset of H_2O, COS, and CO_2 flux and concentration measurements from a single leaf chamber in a wetland over about 36 days. This type of data is an important contribution and will be undoubtedly useful for other studies. However, the interpretation would be aided by greater attention to stomatal conductance, as the title implies, rather than LRU.

We have provided data and a figure of stomatal conductance estimates at the request of both reviewers. See Figure 6 and §2.5.1 in the revised manuscript.

We acknowledge that the previous version might have created misleading expectations for the study of LRU. **We have rewritten most of the Results and the Discussions to reorient the manuscript on how LRU varies in field conditions and how such behavior manifests stomatal responses.**

> The trouble with focusing on LRU is the matter of scale and applicability. Work by Hilton et al. (2015) demonstrated that, for regional GPP estimates, LRU is not the most important source of uncertainty. On the leaf scale, a direct measurement of CO_2 uptake can be made, though it includes photorespiration. At the tower-level scale (1 km^2), I am not sure that COS-based GPP estimates are more accurate than recent approaches relying on CO_2 measurements alone, though the Wehr et al. (2017) study in a temperate forest demonstrated COS-based estimates of canopy stomatal conductance were consistent with other measurement approaches in that system. In short, LRU is not the most important question on large scales, not employed in and of itself on leaf scales, and has some applicability still under development at the site scale. While having a better description of LRU variation with PAR would be an improvement, it is not the urgent next step that the text here describes.

At the ecosystem scale (~1 km^2), the COS-based method for GPP estimation is meant to supplement rather than replace conventional CO_2-based methods. In terms of accuracy, it is true that previous studies that applied the COS method on the ecosystem scale ended up getting similar—but not more accurate—results compared with the CO_2-based methods. Part of the reason was that LRU variability was unable to be treated properly due to the lack of concurrent leaf-level measurements. The uncertainty in LRU would further propagate into the GPP estimates. Recog-

nizing the problem, our study aims to contribute to its solution rather than circumvent it.

Yet the actual value-added benefit of COS tracer lies in the fact that it provides GPP estimates that are *independent* of assumptions on the temperature response of respiration and on the light response of photosynthesis—at least one of which is required in CO_2-based methods (Reichstein et al., 2005; Lasslop et al., 2010). In other words, uncertainties in the built-in assumptions of CO_2-based methods cannot be assessed unless other *independent constraints*—such as COS—are introduced. For example, COS-based GPP estimates may allow us to obtain daytime respiration straightforwardly, which further opens the possibility of studying the Kok effect (i.e., light inhibition of leaf respiration). Various ecophysiological applications of COS form an evolving frontier, and the usefulness of COS could not be overstated.

At large scales, currently available datasets are limited in spatial and temporal coverage, and the uncertainty in remotely retrieved COS concentrations would likely overwhelm the uncertainty of LRU in GPP-oriented applications (Whelan et al., 2017). But the research field likely would not stay there. Were better COS data products to be available in the future to allow for data assimilation at finer spatial and temporal scales—like a 'NOAA CarbonTracker' for COS—then LRU would become an issue. LRU responses to light and VPD would mean that synoptic weather events may shift regional estimates of daily averaged LRU. Without accounting for the relevant effects on LRU, GPP products derived from COS measurements could be biased. Although models like SiB can simulate LRU ab initio, the simulated LRU has yet to be validated with field studies and it is too early to put complete trust in model-generated LRU values.

In short, the outstanding issues around LRU have been underappreciated; but it does not mean that LRU is well understood and it no longer begs for questions, nor would LRU be less useful with the presence of process-based models. LRU is still an indispensable tool linking COS and CO_2 uptake, because it is simple enough to provide an understanding of the relationship between COS and CO_2 uptake, which would otherwise be inscrutable.

> The second issue is applicability to other ecosystems. This dataset was collected from a chamber containing leaves of a plant typically found in wetlands. The COS–GPP tracer technique is not usually applied at the site level in wetlands because of often substantial COS production from wetland soils. Also, some wetland plants have interesting adaptations to tolerate suboxic soil environments. For example, *Typha* have well developed aerenchyma to allow oxygen to diffuse into the root zone. Aerenchyma can also transport reduced gas compounds to the surface, circumventing oxidation

in the water column. This has been shown for methane and Whelan et al., (2013) suggested a similar route for carbonyl sulfide. The data do not necessarily show COS release from the parts of the leaves enclosed in the chamber, but teasing apart uptake from other sources of COS in the system would probably be a challenge. It is confusing to carry out an LRU study in one of the few ecosystems where applying LRU to back out GPP is an exception to the simplicity of the approach.

During the same campaign, we had a soil chamber installed to characterize soil COS emissions. We have already attempted COS-based GPP estimation at the site. The COS-based GPP estimates (GPP_{COS}) agree well with traditional CO_2-based GPP estimates (GPP_{NEE}). Results from that study have been presented at the 2016 AGU Fall Meeting, and are currently being written up as a manuscript (Seibt et al., in prep.).

As for the aerenchymal COS transport, we did not have the means to measure its contribution to COS fluxes. However, the close resemblance between GPP_{COS} and GPP_{NEE} suggests that the aerenchymal COS transport does not constitute a significant missing source of COS, although its presence cannot be ruled out.

While using LRU is probably the most popular method of calculating GPP from COS measurements, it is not the only method. The SiB model, for instance, has a "mechanistic" uptake representation that does not rely on an LRU number. The applicability of COS measurements to carbon cycle studies does not depend solely on LRU.

This is a valid point. But the advantages of SiB shine better in large-scale applications, especially when a representative LRU is difficult to determine from the upscaling of field data. **We have revised the related discussion in the manuscript to reflect this point (P12L7–L15).**

Motivating this study interpretation with the vagaries of leaf conductances would be of greater interest. Already, Sun et al. show that nighttime stomatal conductance is occurring and that daytime conductances change with evaporative demand. Sect. 4.2 should be expanded to include the broader literature on nocturnal stomatal conductance, rather than restricting the discussion to focus only on COS studies. Graphically comparing an established method to the COS-based method of estimating stomatal conductance could reveal possible mismatches and highlight the strengths of each approach, even if leaf temperature was not measured precisely. Re-working the figures to this effect would be beneficial.

We thank Dr. Whelan's suggestions for improvement. The following changes have been made to address these issues:

- **Figure 6 (new) has been added to show diurnal trends of the stomatal conductance of water and the total conductance of COS.**
- **In §4.2, the nighttime stomatal conductance estimate has been corrected for an erroneous assumption that internal conductance is negligible. The discussion has also been improved.**

> Small technical concerns include publishing chamber blank results and also the exact equation that was used for the QCL water correction. There are a growing number of researchers using this make of QCL and water is a problem for the older models.

Blank chamber effects are now provided in §2.2 Experimental setup. See P4L32–L34. Please also see the Supplement for more details on the blank chamber effects.

We have added information on the QCL water correction. See P4L23–L27 in §2.2. A detailed description of the equations used for water vapor correction and their effects on flux uncertainty is given in the Supplement.

> In short, this is a good dataset, but the interpretation could perhaps avoid the concept of LRU entirely.

We have explained why LRU is useful in assessing the relationship between COS and CO_2 leaf uptake. Please see the reply to a previous comment on Pages 12–13 of this response.