Author's response: Baartman et al., Isotope discrimination of carbonyl sulfide (^{34}S) and carbon dioxide (^{13}C, ^{18}O) during plant uptake in flow-through chamber experiments

Response to editor comments:

Line 73 (previous version manuscript): "biofosfaat" changed to "biophosphate"

Table 1: added the international isotope reference scale for each reported isotope ratio

Table 2 (in previous version manuscript): removed this table in section 2.4. Table 3 from the previous version of the manuscript is now Table 2.

Equation (5): corrected the typographical mistakes and for all following equations (including the equations in appendix B), we changed the notations of entering and ambient air to be consistent. Entering air now always has a subscript "$_e$" and the ambient air in the chamber has the subscript "$_a$".

All equations and in text: we changed all occurrences of "C" to be capitalized, for consistency

Table 3 (previous version manuscript)/Table 2 (new version manuscript): Added "apparent" to $^{18}\Delta$. Switched the units for A^s and A^C. We also added new data to this table: number of samples, stomatal conductance, total conductance to COS, total conductance to CO_2 and C_i^C/C_a^C, according to suggestions from the reviewers. We included errors on the COS and CO_2 fluxes and the LRU. We also updated the Table caption, according to the suggestions from the reviewers.

Equation (B2): removed from Appendix B and subsequent equation numbering was updated. We instead refer to Equation (5).

In order to be more transparent and consistent about the uncertainties, we have changed the manuscript to have all the reported uncertainties on the averages represent the standard error of the mean (SEM), taking into account the errors on the individual measurements. And when we report the uncertainty on single measurements, we have indicated this.

We have implemented all the revisions to the manuscript that we committed to in our responses to the referee comments below.

Response to comments by Referee #1

We thank the reviewer for taking the time to read our manuscript and for providing useful suggestions for improvement. The reviewer comments and questions are in black and our responses are in blue below each comment.

The paper deals with an important and exciting topic. The use of COS as a unique tracer of photosynthesis and the rare measurements of the isotopic discrimination, D34S, associated with COS uptake. The paper presents a unique measurement system for gas exchange, COS, and isotopic analysis, and it is well-written.

However, the paper has some rather significant issues that need attention. This is partly so as there seems to be a gap between the impressive analytical measurements and the experimental, plant gas exchange, part . Below are some of the concerns noted as I was reading the paper (i.e., in no special order) that I hope will help to improve the paper.

In general, the motivation is to introduce D34S to “provide useful information on the COS uptake process and help to constrain the COS budget” (upfront in the abstract). However, at the end, the paper does not tell us what we learned in either aspect. **At least some discussion of these aspects is needed, or these should be strongly toned down.**

In fact, the paper goes on to declare another much more modest and specific goal: **To verify the published D34S data obtained in a ‘closed system’ (Davisson et al.) in their new ‘open steady-state system’**. The paper generally confirms the earlier data but in a way that does not provide additional confidence due to the experimental difficulties. In its present form, therefore, it is uncertain whether the paper will advance the field in that respect. **Better focusing on what exactly is the bottom line/take-home message for D34S is needed.**

In the revised manuscript, we will clarify the goal of the manuscript with the reviewer’s helpful comments. We agree that measuring plant COS discrimination poses technological and interpretational challenges, but we believe it holds potential to inform us about processes occurring across scales, from the leaf (e.g., test if COS is a unidirectional flux into the leaf) to the globe (e.g., to partition COS oceanic and anthropogenic sources using isotope constrained COS tropospheric mass balances (Davidson *et al.*, 2021)). Additionally, more observational data are required to improve and test mechanistic models of plant COS discrimination. The objective of this work was to design a system to simultaneously measure COS and CO_2 gas exchange and isotope discrimination using a continuous-flow plant chamber. We describe this system, present a first set of measurements in C_3 and C_4 plants, and compare them with the only other available dataset obtained with a closed plant chamber (Davidson *et al.*, 2021; Davidson *et al.*, 2022).

To improve clarity and balance, we will revise the manuscript to better reflect study’s objectives and the scope of the results. We will include a perspectives section (see suggestions from Referee #2) with ideas for future research. In a subsequent paper that we are currently writing, we use all available measurements to develop a mechanistic model for COS plant discrimination. In the revised manuscript, we will use some of the information from this accompanying paper to provide context for the measurement results.

On the methodological side, it is not clear how many plants were used as replicates. In the Method, three papyrus cuttings and “a sunflower plant” were noted. In Fig. 3, n=2 is indicated (with SE…). In Fig. 4, no replication is indicated; in Fig. 5, 6, some individual replications are plotted, each with its own SE. While the information is incomplete and confusing, the impression is that only a few actual replications were made, and there is no clear distinction between the precision (repeating the measurements) and replications. There is also missing information on Blank Testing of the chambers, which seems to be critical in COS experiments. The inlet COS concentration (2-3 ppb) is 4-5 times the atmospheric level) is indicated but not the chamber ambient concentrations (outlet). [BTW, in the Abstract, fluxes are reported in pmo mol-1, which are not flux units.] **More information seems to be required.**

We will include the requested information in the revised document. The data are from two experiments; one with a single sunflower and one with an assemblage of papyrus leaves. At each light level, duplicate samples were collected within the same experiment. We report their averages and standard errors. A few samples were likely affected by contamination and excluded from the analysis.

For the papyrus under dark conditions (PAR=0), the COS uptake was too low to calculate a discrimination value. As a result, these data points are not shown in the discrimination plots but are included in the flux figures. We will clarify the meaning of the duplicates, missing values and the errors in the Methods section and in the caption of Table 3 and relevant figures. Table 3 will also be expanded to include chamber mole fractions and stomatal conductance for each plant and treatment, as suggested in a later comment.

Blank (empty chamber) tests were conducted prior to the plant measurements, though not previously mentioned. We will add a description and the results of these tests in the revised manuscript.

The flux units in the Abstract should have been pmol m^{-2} s^{-1}, this was a typographical error, which we will correct.

The aspects noted above are significant as many of the observations are somewhat unexpected or uncharacteristic, and a range of particular explanations are required, such as non-uniform light level (“low light” in parts), “not optimal behavior”; “stomata not fully open”; increasing Ci with increasing light and increasing A, no response of COS assimilation to light, mostly constant D34S, constant Cm, etc. **In fact, the feeling is that more measurements would help to get more conventional results.**

We agree that more measurements would have strengthened our study, but logistic constraints limited extending the measurements. Furthermore, the isotope system’s capacity further constrained the amount of samples that we could measure (given that it takes a full day to measure only 3 - 4 samples).

We did conduct a follow-up experiment with the goal of expanding the dataset. However, when the experiments were finished, the COS isotope system needed extensive maintenance, after which we were limited in time and personnel. In addition, storage issues may have had an influence on these samples as COS can be unstable during longer storage.

Despite these limitations, we believe that the current dataset and innovation in methodology provide valuable insights, given the scarcity COS isotope discrimination data. At present, additional measurements are not feasible. The COS isotope system in Utrecht is non-operational and a new system in Bordeaux is still under development.

Fig. 3 presents a nearly complete insensitivity of COS uptake to light level (in sharp contrast to CO2 uptake), and it is explained by the light in-sensitivity of carbonic anhydrase. However, COS should still respond to light for the same CA activity because of its effect on conductance (g). **No information on conductance is given in this paper**.

We will provide the values for stomatal conductance and total conductance in the revised manuscript. We appreciate the reviewer’s point - information on these parameters is needed to explain the (lack of) variability with light level of the uptake fluxes.

Briefly, in non-dark conditions (PAR>0), stomatal conductance remained above 0.25 mol m^{-2} s^{-1}, suggesting that stomatal opening was sufficient to maintain COS uptake, even at lower light levels. This is also supported by the relatively small changes observed in Ci^s and Ci/Ca^s, between the highest and the lowest light setting. A more detailed explanation will be included in the revised manuscript.

Details of leaf gas exchange equations are presented, including conductance, internal concentrations, etc. However, all those were developed strictly for the leaf scale, which may not apply here. The photo in the Appendix shows that this was a rather ‘dense canopy scale’ experiment. The authors note this can explain some of the non-typical observations, but there is no discussion on how to scale from leaf to canopy (or vice versa). The photo clearly indicates no uniformity in conditions and, in turn, in activities. **This scaling gap should be addressed, and if it can be overcome, it should be explained in more detail**. By the way, it seems there are some publications on branch scale measurements, which can be helpful to compare (likely also Yang et al. 2017 or 2018 who tried to scale between leaf to canopy).

We recognize that our gas-exchange approach is relatively simple and involves certain assumptions. We applied a *big leaf* approach, which we consider the most appropriate for our setup, given the use of small plants and a well-ventilated chamber thereby - minimizing boundary layer effects.

We also recognize that likely not all leaves received the same amount of PAR because of shading. However, given the precision at which COS isotope discrimination can be currently determined, it would seem too complex to go beyond a *big leaf* approach in our study. In addition, we did not obtain the gas-exchange data for the stem of the plants, so these could not be included in our calculations.

We will add a section to the Methods in the revised manuscript explaining our choice of the *big leaf* approach and the assumptions involved.

Along these lines, internal concentrations (Ci) are estimated using the leaf-scale equations, and Cm is calculated based on the D34S estimates. **The difference between the Ci and Cm is interesting but not defined or discussed**, except that very different values are reported for Ci/Ca and Cm/Ca.

We calculated the Cm/Ca ratios for COS as the mesophyll cell is the end-point for COS assimilation (the location of CA). We were also interested in whether Cm/Ca values could help explain the limited variation in COS flux across light levels, as. Indeed, we observed little variability in Cm/Ca with changing light. We will expand the discussion on the differences between Ci/Ca and Cm/Ca in the revised manuscript.

Note also that the physiological calculations of conductance, g, based on E and ci, depend on leaf temperature and water vapor saturation assumption. This is tricky in the present study, which uses a dense canopy in a different light and temperature in the chamber. It seems that COS flux, as long as it is based on the assumption of near-zero internal concentrations (i.e., **no compensation point, an issue that is ignored in this paper**), may offer a simpler alternative to total conductance, which could at least be compared (i.e., As=gCa…).

We appreciate the reviewer's thoughtful concerns. The equations by von Caemmerer and Farquhar (1981) do assume saturation of the leaf internal airspaces with water vapor - an assumption that may not hold under high evaporative demands (Cernusak *et al.*, 2018; Cernusak *et al.*, 2024), though such conditions were not present during our measurements.

Our calculations of conductance to COS represent a canopy average and may carry uncertainties related to leaf temperature. However, estimating the conductance under the assumption of zero COS concentration in the mesophyll could introduce even greater uncertainty

As mentioned earlier, we are preparing a companion paper that presents a modelling framework for COS isotope discrimination in plants. This model can account for non-zero internal COS concentrations and emissions, and will allow us to explore their effects on observed isotope discrimination.

Cernusak, L. A., Ubierna, N., Jenkins, M. W., Garrity, S. R., Rahn, T., Powers, H. H., ... & Farquhar, G. D. (2018). Unsaturation of vapour pressure inside leaves of two conifer species. *Scientific reports*, *8*(1), 1-7.

Cernusak, L. A., Wong, S. C., Stuart-Williams, H., Márquez, D. A., Pontarin, N., & Farquhar, G. D. (2024). Unsaturation in the air spaces of leaves and its implications. *Plant, Cell & Environment*, *47*(10), 3685-3698.

The LRU estimates are important. However, it is clearly sensitive to the high ambient COS used as COS uptake can generally be linearly related to Ca-cos, and it also seems that some information on this response may be available in the literature. In this case, **the effect could be estimated to some extent, and an attempt to correct the LRU for comparison with literature values at ambient COS could be made,** and discussed. In fact, a good agreement on the uncorrected LRU does not add confidence, as noted above.

Although the primary goal of our experiments was not to quantify LRU under natural conditions, we appreciate this suggestion. Applying such a correction would rely on a limited dataset (Stimler et al. 2011) and could introduce additional assumptions and uncertainties. Nonetheless, we will acknowledge in the revised manuscript that the LRU values reported may not fully represent natural conditions due to the use of higher-than-ambient COS mole fractions in the chamber air.

Response to comments by Referee #2

We thank the reviewer for reviewing our manuscript and for providing useful suggestions for improvement. The reviewer comments are in black and our responses are in blue below each comment.

General

The paper presents the first isotopic measurements of COS and CO2 made in flow-through chamber for one papyrus, a C4 plant and sunflower, a C3 plant. The setting allows them to derive the COS and CO2 plant uptake, the internal and mesophyll concentrations of CO2 and COS respectively, the photosynthetic discrimination against 13CO2, C18O2 and to explore how they respond to increasing light intensity. They conclude about a distinct behavior was observed between the C3 and the C4 plant.

The strength of the paper stands on the perspectives that such isotopic measurements could provide insights into the underlying processes of the COS and CO2 plant uptake. Especially, the isotopic discrimination of COS gives insight into the CA activity. However, the paper needs to be written in a rush, and the authors should add more contexts and perspectives. In short, I would recommend publishing this paper with major revisions, which consist in improving the storyline. The comments below go around those lines.

The storyline (objectives, method, conclusion) needs to be clarified: . Which complementary piece of information each isotope discrimination can bring? The benefits of using isotopes discrimination of carbon and COS are not clearly explained. Also, in the method section, the photosynthetic discriminations against 13CO2 and C18O2 are not presented. The author should also some perspectives in their conclusion.

In the revised manuscript, we will clarify the overall storyline and more explicitly define the objectives and benefits of combining CO_2 and COS isotope discrimination measurements. Additionally, we will provide more detail on discrimination against $^{13}CO_2$ and $C^{18}O^{16}O$. However, we intend to keep the primary focus on COS isotope discrimination and will refer to relevant literature for in-depth discussions on CO_2 isotope discrimination in plants.

Clarity of the Figures: The green and the dark green colors are hard to distinguish. I would recommend putting the same colors for each PAR for papyrus and sunflower. The number of plant replicate is confusing. What is a replicate? I would suggest making a tabular with the number of measurement/replicates for each measured or computed quantity. Likewise, the observations made at PAR equal to 0 are not shown on Figure 4.

We thank the reviewer for the suggestions. We will include the information on the number of measurements/replicates for each measured plant and treatment.

Regarding the replicates, these were air samples for isotope analysis taken consecutively during the same treatment (PAR level) for the same plant. We will clarify this in the revised manuscript.

For the figures, we would still like to maintain the distinction between the different PAR levels for the two plant species, as this provides valuable information that would otherwise be missed. In the revised manuscript, we will improve the color scheme for clarity.

The data for PAR = 0 are not included in Figure 4 because the figure plots the CO_2 uptake against Ci^s/Ca^s, and in the dark, the plant was not taking up CO_2 uptake, but respiring it. We will address the absence of this data in the caption of Figure 4 in the revised manuscript.

Representativeness of the experiments: The papyrus, a C4 plant, in the experiment grows in tropical swamps and in arid light saturated environment. How this C4 plant is representative of all the C4 plants? The experiment is far from reality as, because of time constrain, the author did not repeat the experiments with higher light intensity than PAR=400. The effects of soil water level, VPD and nutrients availabilities are also not discussed. Likewise, the chamber was well aerated, which results in infinite boundary layer conductance. Is it realistic, especially at night when the boundary layer becomes stable?

We acknowledge that papyrus is not a widely studied C4 species and may not be fully representative of typical C4 plants. Given the constraints we faced, we used what was available and feasible. We did attempt to grow and measure several other C4 species including *Zea mays* (a widely studied C_4 species), but encountered significant experimental challenges. For example, successfully quantifying COS isotope discrimination in our flow-through gas-exchange system, required significant COS uptake while maintaining an adequate flow rate to minimize boundary layer and condensation issues. We therefore needed relatively large leaf areas, which we were not able to obtain from other C4 species.

We agree that future experiments would benefit from using representative C_4 species and we will include this suggestion in the outlook section of the revised manuscript.

Due to the time-consuming nature of the COS isotope measurements (a maximum of 3 samples per day under optimal conditions), we limited the number of samples to 18.

Regarding the dark measurements and the infinite boundary layer conductance; we were not aiming to perfectly replicate night conditions, but rather to observe the effect of stomatal closure on COS and CO_2 fluxes and isotope discrimination.

More in-depth analysis of the results is needed: Stomatal and total conductances are needed to explain the results for the C3 and C4 plants (as done in Stimler et al. 2011). The paper only presents experimental results and lacks interpretation in terms of processes.

We agree with the reviewer that a more detailed analysis, including stomatal conductance, would strengthen the paper. In the revised manuscript, we will expand the Results & Discussion to incorporate the conductance data and provide a more detailed interpretation of the isotope discrimination results, as also mentioned in our response to Referee #1.

Specific comments

Abstract

Line 24 – “does not exit the leaf again”. Confusing, sounds like it is the same CO2 molecule that enters and leaves the leaf.

We will rephrase this

Line 25 – Precise why performing such isotopic measurements and how can isotopic discrimination of COS **and CO2.** Only the isotopic discrimination of COS is mentioned.

We will include a sentence to mention the motivation behind measuring both COS and CO_2 fluxes and isotope discrimination.

Line 30 -35: Mention what are the implications of these results

We will add a sentence on the implication of the results

Line 35: “The papyrus was not … experiments” Explain a bit better. How is the C4 plant supposed to behave? And how does the papyrus behave in the experiment?

We will add an explanation of what would be expected from a typical C4 plant in terms of CO_2 fluxes and isotope discrimination.

Introduction

- You only describe the processes and the equations underlying the isotope discrimination of COS. As the title mentions it, you should also describe , here or in the method, the processes underlying the isotope discrimination of CO2.

As the primary focus of this paper is COS isotope discrimination, a relatively newly studied topic, we chose to introduce the relevant calculations and definitions in the introduction. CO_2 discrimination values are to complement and contextualize the COS data. Given that CO_2 isotope discrimination is a well-established field with widely understood methodologies, we decided not to go into detail on its method.

We agree, however, that the mention of CO_2 isotope measurements in the final paragraph of the introduction (lines 110-114), comes slightly out of the blue. In the revised manuscript, we will clarify the benefit of including CO_2 isotope data alongside the COS measurements in the introduction.

- You should add a tabular or a section showing which piece of information each isotope discrimination or molecule can give to guide the reader.

We appreciate the suggestion of the reviewer to clarify what information can be gained from each isotope discrimination value (COS and CO_2). However, we believe a table may not be the most effective way to present this, as the interpretation of each discrimination value varies between C_3 and C_4 species. Additionally, COS isotope discrimination research is still in its early stages, and it may be difficult to summarize in a table exactly what information each isotopologue could provide. For instance, variations in observed sulfur-34 discrimination in COS result from a combination of factors, including conductances and CA activity.

In the revised manuscript, we will include a section in the introduction highlighting the potential insights that can be gained from each isotope discrimination value.

- Make a tabular with the Davidson et al. (2022) to compare their measurements with yours, add a column for the experimental conditions (CO2, COS mole fractions ec)

We appreciate this suggestion and we will include a table in the Appendix of the revised manuscript. While Davidson et al. (2021; 2022) used different chamber conditions - such as CO_2 and COS mole fractions, as well as different temperatures, and significantly lower PAR - which may limit direct comparisons, we agree that providing an overview of all currently available data can be valuable for the reader.

Line 40 Add a citation

We will do this

Line 43 The SIF, satellite retrievals of GPP, net co2 fluxes estimated by atmospheric inversion are not mentioned. Which advantage has COS plant uptake compared to these mentioned methods?

We appreciate this, however we specifically chose to limit to and name methods based on gas exchange and flux. We indeed need to study GPP from various perspectives. Our point is not so much that COS is more advantageous, but that COS may be a potential valuable independent proxy that can further constrain existing estimations of GPP. Promising approaches to estimate GPP on the regional and global scale include SIF, NirV, and atmospheric inversions e.g. using COS (Ma et al., 2021; Remaud et al. 2022). However, the latter method requires the use of prior information. Therefore, studies on ecosystem and plant scales are needed to obtain a better mechanistic understanding of the combined exchange of water, CO_2, and COS.

Ma, J., Kooijmans, L. M., Cho, A., Montzka, S. A., Glatthor, N., Worden, J. R., ... & Krol, M. C. (2021). Inverse modelling of carbonyl sulfide: implementation, evaluation and implications for the global budget. *Atmospheric Chemistry and Physics*, *21*(5), 3507-3529.

Remaud, M., Chevallier, F., Maignan, F., Belviso, S., Berchet, A., Parouffe, A., ... & Peylin, P. (2022). Plant gross primary production, plant respiration and carbonyl sulfide emissions over the globe inferred by atmospheric inverse modelling. *Atmospheric Chemistry and Physics*, *22*(4), 2525-2552.

Line 45 Add Wehr et al. 2015 (https://www.sciencedirect.com/science/article/abs/pii/S0168192315007145) who quantified respiration from GPP thanks to isotopic measurements.

We will do this

Line 49 Lack of fundamental papers about COS

We will add these

Line 52 Cite Montzka et al. 2007 https://agupubs.onlinelibrary.wiley.com/doi/full/10.1029/2006JD007665

We will do this

Line 56 «Assuming that there is no COS emissions" Discuss the validity of this assumption: it has been shown that Beliso et al. (2018) (https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0278584) showed that rapeseed emit COS. Some plants in swamps also emit COS.

Indeed, recent evidence suggests that certain species and/or environmental conditions may lead to COS emissions or the existence of a COS compensation point. We will add a brief discussion of this in the revised manuscript.

Line 62: How negligible is the daytime respiration? (https://www.sciencedirect.com/science/article/abs/pii/S0168192315007145)

Daytime respiration is indeed not always negligible. One could also account for daytime respiration when using LRU as a tool for estimating GPP. We will adjust this sentence accordingly. However, a detailed discussion of the broader caveats associated with using LRU for GPP estimation falls outside the scope of this manuscript.

Figure 70 Please make the Figure more understandable. What is the blue and red lines in the middle? What are the zigzag lines in the middle? Add the name of the conductance's. Some accolades near the name of the space could help to separate the cell/space.

We will revise this figure to make it more understandable and include labels for the conductances and other parameters. We will also include a more thorough explanation in the figure caption.

Line 75 The first two sentences, "COS discrimination…factors" and "The discrimination …" are not logical. You should explain which beneficial information COS isotope discrimination can give based on literature and go to the next line, for the definition.

We agree that this section would benefit from a clearer explanation of the value of COS isotope discrimination data. We will revise the manuscript to include this, as also noted in our responses to earlier comments.

Line 88 Add As the reaction with CA is supposed to be irreversible,

We will add this

Line 93 Explain why this may be a too crude simplification of the diffusion processes taking place based on literature studies (https://bg.copernicus.org/articles/20/2573/2023/bg-20-2573-2023.pdf)

We will add this

Line 95 In this case = for C4 species?

We intended to refer to the case where mesophyll COS concentration is not zero. We will rephrase this in the revised manuscript.

Line 101 "ambient COS and CO2 concentrations". You just said above the experiments were caried at high CO2 and COS mole fractions. And how high?

Davidson et al. (2021; 2022) conducted several experiments using both elevated and ambient COS and CO_2 concentrations. We will include the relevant concentration values in the revised manuscript. The elevated concentration measurements were specifically designed to estimate the carbonic anhydrase (CA) isotope fractionation.

Line 165 Why do you use to instruments to measure both air in and out of the chamber? What is the specifities of each instrument?

We measured both inlet and outlet air to calculate the fluxes. The LI-COR instrument was part of the existing chamber setup and was used specifically to measure the H_2O fluxes. Since the LI-COR cannot detect COS, we used a second instrument, the QCLS, which measures COS mole fractions with high precision. We will make this more explicit in the improved manuscript.

Line 182 Why choosing these temperatures? Are they representative of the place where these two plants live or is it associated with maximal CA activity?

We selected relatively high temperatures to obtain sufficient COS uptake fluxes for isotope discrimination analysis, while also avoiding condensation in the system. Throughout the experiments, we maintained the temperature as consistently as possible.

Line 185 remove the space

We will do this

Line 189 add coma

We will do this

Line 194 There is a mistake in the formula, please verify. Wa is not wo?

There is indeed a typographical mistake here. We will make sure to fix it

Line 261 – 264 These two sentences should be in the method. This is not clear either. Why some sample must be treated as duplicate?

We will move this section to the Methods. At each treatment, we collected two air samples for isotope analysis. Under light conditions (PAR > 0), fluxes remained stable, so these samples were treated as duplicates and averaged. However, under dark conditions (PAR = 0), the plant was still adjusting, and fluxes were gradually changing. As a result, the two samples captured different states and were treated as separate data points rather than duplicates. We will clarify this approach more explicitly in the revised manuscript.

Line 264 Can you compare your COS uptake fluxes with those from the literature by making a distinction between C3/C4 plant? Can you find some COS uptake flux measured at the ecosystem level (fluxnet type)?

We thank the reviewer for the suggestion. We will include a brief literature comparison section distinguishing between C_3 and C_4 COS fluxes. However, we note that there is limited COS flux data available for C_4 species, particularly at the ecosystem scale.

Line 266 Some measurements are also made at the ecosystem level and not in controlled chamber. Please mention that.

The sentence mentions that some studies are at the ecosystem scale, but it indeed does not distinguish between studies at the canopy scale and those at the ecosystem scale. We will clarify this distinction in the revised manuscript.

Figure 3. Why the As is lower in C4 plant than in C3 plant at PAR=0?

The difference can be attributed to lower stomatal conductance for C4 at the time of sampling compared to the C3 species. Since we plan to include conductance data in the revised manuscript, we will also provide an explanation of this phenomenon in the paper.

Line 289 Would be nice to have a plot for LRU as well on Figure 3 (3 panels)

We appreciate this suggestion and will include this in the revised manuscript.

Line 294 Please make a distinction between C3 (LRU=1.68) and C4 plant (1.21). only 4 values for C4….

We thank the reviewer for pointing this out and we will make the distinction in the revised manuscript.

Line 297 Your results cannot be directly compared with Davidson 2022 as they used both high CO2 and high COS concentrations. For instance, Wu Sun 2021 showed that LRU increases with CO2 and that can also affect Davidson 2022 results.

We appreciate this suggestion and will mention this in the revised manuscript.

Line 302 Cite Wu Sun for the dependance of LRU to various environmental conditions and be more specific.

We will do this

Line 304 Explain first why the quantity ci/ca is interesting to study.

For CO_2, Ci/Ca is indeed not explicitly explained. We will include a brief explanation of the relevance of studying Ci/Ca for CO_2 in the revised manuscript.

Line 370 What is the typical PAR that other experiments use?

We are not entirely clear on the reviewer's question. Low PAR values are mentioned in the comparison with studies in line 368. Many studies involving plant chamber experiments typically perform light-response curves starting from around PAR = 100 (or similar) up to PAR = 1500 or 2000 μmol m^{-2} s^{-1}.

Line 371-377, not clear, explain better…As papyrus grow in swamps, this setting is closer to reality?

We acknowledge that the conditions under which we measured the papyrus leaves were less than ideal. We initially attempted to measure several other C_4 species, but the COS uptake was too low for precise isotope discrimination. Given the circumstances, we worked with what was available - papyrus from the tropical greenhouse at Wageningen University. It should be noted that these plants were grown in a relatively dry and warm environment, not a swamp. Due to the plant's size, we cut several stems with leaves and placed the stems in water to transport to the lab. Papyrus clearly thrived when kept in water and continued photosynthesis shortly after being placed in the chamber. However, we observed some unexpected CO_2 flux and isotope discrimination values that were atypical for a C_4 species. We hypothesize that some CO_2 may have been transported from the water into the chamber, and we will clarify this in the revised manuscript.

Line 435 Remind that CO2 assimilations in C4 plants are expected to be more efficient

We are not sure which sentence the reviewer is referring to, as line 435 is the caption of Figure A1 in the appendices. If the reviewer is referring to line 415, we can clarify this paragraph by specifically discussing the differences in CO_2 assimilation between our C_3 and C_4 species.

Line 421 These two sentences are contradictory. First, you sat that the CA reaction is light independent and then you say that the COS uptake was lower during the dark experiment.

We will rephrase this sentence to clarify that COS assimilation by carbonic anhydrase (CA) is light-independent. However, stomatal opening is still necessary for COS uptake, so when stomata partially close in the dark, COS uptake will decrease.

Conclusion

Lower discrimination against C18O2 is observed, which is consistent with previous measurements (Stimler et al., 2011). Why not for 14COS?

We are not entirely sure what the reviewer means by 14COS, but we assume they are referring to $CO^{34}S$. Additionally, we believe the reviewer is referring to our observation of lower $C^{18}O^{16}O$ in C_4 compared to C_3 species, which aligns with the findings of Stimler et al. (2011). The question seems to be why we did not observe the same pattern for $CO^{34}S$ discrimination. We address these unexpected results in lines 424-427 in the conclusion. We also observed atypical results for $^{13}CO_2$ discrimination in our C_4 papyrus, highlighting the value of measuring both COS and CO_2 isotope discrimination in a single experiment.

Can you bring some conclusions about the CA activity?

We did not directly measure CA isotope discrimination, and the cm/ca values we report are derived from the sulfur isotope discrimination, meaning they are not independent. Therefore, with our current dataset, we can only speculate on CA activity. To better constrain this in future studies, targeted measurements of $CO^{34}S$ isotope discrimination by the enzyme CA would be highly valuable. We will add this recommendation to the perspectives section of the revised manuscript.

It is necessary to add a perspective section to pave the way for future experiments.

We appreciate the suggestion and we will make sure to add this to the revised manuscript.