

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**

Revisions 31-07-2025

Reviewer's suggestions:

"The paper has seen significant improvements since the initial draft. The authors have indeed worked significantly to enhance the narrative and contextualize their findings within the framework of existing studies. The method is also clarified. I only have minor comments that can improve the manuscript, based on the lines of the track change.

Line 21 : Add the word "Irreversibly" catalyzed

This has been added

Line 32 : Add a S to conductanceS

This has been added

Line 55 : Add "For both C3 and C4 plants, for CO₂, a negative relationship..."

This has been added

Line 56 : Rephrase the first part of the sentence "The CO₂ uptake ... plant," to "... The CO₂ uptake and C₁₃ and C₁₈ discriminations of sunflower have expected values for a C₃ plant"

We rephrased this beginning of the sentence to "The CO₂ uptake and $^{13}\text{CO}_2$ and $\text{C}^{16}\text{O}^{18}\text{O}$ discriminations of sunflower have expected values for a C₃ plant,..."

Reply to reviewer 2 in reference to line 43: Please report these lines in the introduction to better explain the motivations.

We added the following to the sentence from lines 53 – 54 (tracked changes version of manuscript): "solar-induced chlorophyll fluorescence (SIF), near infrared reflectance of vegetation (NIRv) and inverse atmospheric modeling studies (Kettle et al., 2002; Ma et al., 2021; Remaud et al., 2022)."

And in line 57: "or, in the case of modeling studies, prior information on location and magnitude of the fluxes."

These additions are in line with our response to the comment from reviewer #2 on the introduction.

Line 125 : The addition of "and as the reaction with CA is supposed to be irreversible" is inconsistent with lines 161 which explain that, in C₄ plants, the CA activity is low. Cho et al. (2024) showed that CA activity depends on temperature and reaches a maximum at a specific temperature (<https://bg.copernicus.org/articles/21/3735/2024/bg-21-3735-2024.html>). How does the dependency affect h?

We thank the reviewer for this question as this is still one of the uncertainties/questions in the field of COS isotope discrimination in plants. Davidson et al. (2022) mention that they suspect that COS is able to diffuse out of the leaf again in C₄ plants as C_i increases due to the lower CA activity. This

means that part of the COS is not hydrolyzed “fast enough” by CA, COS builds up to higher concentrations inside of the leaf (compared to C3 plants) and may partly diffuse out again. This COS that diffuses out would then have some fingerprint of the CA discrimination against the heavier isotopologue ^{34}S as the lighter one ^{32}S would be preferred to go into the reaction. Hence the COS inside of the leaf would be slightly enriched in the heavier CO^{34}S , thus a stronger discrimination would be observed in a C₄ plant than compared to a C₃. But this is still theoretical and needs to be confirmed with experimental data.

In equation (4), h represents the discrimination of CA against CO^{34}S , which we assume to be a constant value, for now. But how much this term h influences the overall observed discrimination $^{34}\Delta$ depends on the Cm/Ca ratio, which we think would be higher in C₄ species, following the line of reasoning above.

In terms of temperature dependency, we expect that a higher temperature leading to higher CA activity would then lower the Cm/Ca ratio and following Eq. (4) would then also decrease the observed discrimination. And at a maximum CA activity, Cm/Ca would be close to zero, which leads to $^{34}\Delta = \bar{a}$ (line 119 in current version of manuscript). Since we did not do a temperature response experiment ourselves, we choose not to go into the details of the CA activity – temperature dependency.

Implemented changes:

→ We removed the part “and as the reaction with CA is supposed to be irreversible” in order to avoid confusion.

→ We rephrased the sentence in lines 127-128, which now reads “In the case of non-zero C_m^S , enzymatic fractionation during COS fixation by CA (h) will affect the observed $^{34}\Delta$ (Eq. (4)).”

Part 2.4 As you show the values gsw in Table 2, the formula of gsw should also be shown in this part.

We agree that we need to provide calculations for gsw, but since we would then have to include three equations for full clarity and we do not wish for this paragraph to become too lengthy, we refer the reader now to Appendix B, Equations (B3), (B4) and (B5), where we provide all detailed calculations. We hope the reviewer agrees with this solution.

Line 449: For which kind of plants the mentioned values from Stimlers apply? C3 or C4?

We mentioned these data in a more specific way by adding “ranging between around 15 to 30 $\text{pmol m}^{-2} \text{s}^{-1}$ for the C₄ species maize, sorghum and amaranthus, under a light intensity of 500 $\mu\text{mol m}^{-2} \text{s}^{-1}$,...” (lines 328-329 in new version of the manuscript)

Appendix C: Why are the values of PAR from Davidson (2022) so low in the Table?"

We were also surprised by these very low light intensity in their experiments, but these are the numbers that Davidson et al. (2022) provide in their supplementary material. Since they only used one (small) lamp at the top of their chamber, and used species with a large leaf area, we expect that this is why the light intensity diminished so drastically within their chamber.

Editor's suggestions:

Line 111: Change “ribulose-1,5-biphosphate” to “ribulose-1,5-bisphosphate”

This has been changed

Line 394: Please also mention in the header of Table 2 that 18Δ indicates the apparent discrimination.

This has been added

Line 557: I think you meant “slightly higher LRU values”

Indeed, this has been changed

Line 565: Add “plants” after “C3 and C4”

The word “plants” has been added

Additional edits:

- Missing period was added in line 88.
- As per request from the editorial support, we changed the direction of page 15 back to portrait mode and rotated the table, so that we can still include the entire table with all the necessary information in the main text of the manuscript. We hope the layout works like this and otherwise we will discuss other options.
- We edited the reference list to be in compliance with the Copernicus formatting.