From: Getachew Agmuas Adnew and co-authors

To: - Dear Dr. Aninda Mazumdar

Thank you for editing and facilitating the review process of our manuscript.

We thank both the anonymous reviewers for their valuable and constructive feedbacks. The answers for each reviewer’s question are included under each question of the reviewer.

Referee: 1

The answers to the questions/ comments and suggestions are stated below each comment.

As far as I understand the justification for the great effort required in measuring $^{17}$O and its “access” (or anomaly), is the discovery of significant mass independent oxygen isotope effects in the stratosphere that is conserved to some extent in the troposphere (seems to be true both for atmospheric $O_2$ and $CO_2$). The extent to which this anomaly is conserved in the troposphere depends on the CO$_2$ (or O$_2$) cycling through the biosphere, which erases it by exchange with water. Thus, if the stratospheric production of the anomaly is known and it is relatively constant, the residual signal in the troposphere should reflect the biosphere productivity (GPP). This is exiting application considering the uncertainty around GPP.

This summary by the reviewer is correct. We would like to emphasize that if we reliably want to estimate GPP from $\Delta^{17}$O, we need to know the precise effect of photosynthesis and respiration on $\Delta^{17}$O, in the words of the referee, how does the $\Delta^{17}$O signature actually look after being "erased" by exchange with the biosphere.

ALL the processes associated with the Biosphere, including leaf gas exchange studied here, seems to be mass dependent and are FULLY covered by the conventional $^{18}$O studies.

The referee is correct that in principle $^{18}$O indeed cycles through the same biological system, and undergoes the same (bio)physical processes. However, we would like to nuance the idea that $\delta^{18}$O can help us FULLY understand ALL processes of interest. This is because conventional $\delta^{18}$O studies have a number of distinct disadvantages. Notably, the $\delta^{18}$O -signature of all water pools in the system must be known to use $\delta^{18}$O as a carbon cycle tracer. In addition, significant changes in $\delta^{18}$O can occur due to processes that are not of primary interest to understanding GPP, e.g., leaf evaporation, or soil equilibration.
Δ¹³O variation due to kinetic and equilibrium fractionation effects is much smaller and is better defined. This is because conventional bio-geo-chemical processes that modify δ¹³O and δ¹⁸O follow a well-recognized isotope fractionation slope. In earlier studies many assumptions had to be made, because the effect on Δ¹³O had never been quantified precisely. This is now accomplished through our study.

The only exception may be the small variations observed in the lambda factor that define the expected ratio of ¹⁸O to ¹⁷O mass dependent discrimination (∼0.5), which is not studied here.

The reviewer correctly identifies that the small variations in λ values can impact the Δ¹³O we measure. These effects have been studied previously and the three isotope slopes have been established and are used in our study. We have included an additional figure (reproduced below) in the revised manuscript, which shows conceptually how the three-isotope slopes differ between the various processes and how they affect the observed Δ¹³O signals. In addition, we also include for the first-time experiments and model studies that involve artificially ¹⁷O labeled CO₂. We demonstrate how the resulting differences in Δ¹³O between CO₂ and leaf water affect the results, and that experiments with ¹⁷O labeled CO₂ actually increase the signal to (measurement) noise ratio.

Figure 1. Schematic representation of the processes that affect the Δ¹³O of CO₂ and H₂O during photosynthetic gas exchange (not to scale). The triple oxygen isotope slope for transpiration at h=75 % relative humidity is \( \theta_{\text{trans}} = 0.522 - 0.008 \times h = 0.516 \) (Landais et al., 2006). The triple isotope slope of CO₂-H₂O exchange is \( \theta_{\text{CO₂-H₂O}} = 0.5229 \) (Barkan and Luz, 2012). The triple isotope slope for diffusion of CO₂ is \( \theta_{\text{CO₂-diff}} = 0.509 \) (Young et al., 2002) and for diffusion of water vapor \( \theta_{\text{H₂O(v)-diff}} = 0.518 \) (Barkan and Luz, 2007). The three isotope slope for equilibration between liquid (l) and gaseous (v) water is \( \theta_{\text{H₂O(l)-H₂O(v)}} = 0.529 \) (Barkan and Luz, 2005). ε¹⁸O is the enrichment or depletion in ¹⁸O due to the corresponding isotope fractionation process.
And so, while the present paper goes through an impressive exercise of gas exchange and isotopic measurements and calculations, I fail to see the purpose and merit of this exercise, beyond a test that verifies that indeed the $^{17}$O measurements are consistent with the $^{18}$O studies. The occlusions as much as I can see are already fairly well-known form $^{18}$O studies and, in fact, much of the calculations here still depends on the $^{18}$O measurements.

We appreciate that the referee acknowledges the considerable analytical effort that was made to produce our results. As mentioned above, we think that $^{18}$O measurements alone are not sufficient to study all aspects related to gas exchange between plants and the atmosphere and to quantify GPP. Thus, we posit that an alternative independent tracer is still very useful, and in fact $^{17}$O has been repeatedly suggested and already used as independent and potentially even superior tracer. We nevertheless realize from the comment that the merit of our study was not communicated well, and we have considerably strengthened the motivation. The key point is that so far, the three-isotope slope of each of the processes that participate in plant-atmosphere gas exchange has been studied individually in an idealized experiment. The overall effect of all processes, which work together in complex interaction, on $^{17}$O has never been evaluated in a real plant exchange experiment. This is what is achieved in the research described in our manuscript and it is explicitly stated in the revised version.

Specifically, the results communicated in our manuscript

1) demonstrate that the established theory is applicable to $^{17}$O-CO$_2$ exchange at leaf-level.
2) experimentally quantify for the first time the effect of photosynthesis on $^{17}$O of atmospheric CO$_2$
3) quantify of the dependence of this effect on critical parameters

4) provide an independent bottom-up $^{17}$O-isoflux estimate based on these lab experiments.

Furthermore, we have now demonstrated that such studies are possible with IRMS methods, with considerable effort, but they may actually become more widely accessible thanks to novel laser instrumentation in the near future (McManus et al., 2005).

For example, the key results indicated in the Abstract are: “Our results demonstrate that two key factors determine the effect of gas exchange on the $^{17}$O of atmospheric CO2. The relative difference between $^{17}$O of the CO2 entering the leaf and the CO2 in equilibrium with leaf water, and the back-diffusion flux of CO2 from the leaf to the atmosphere, which can be quantified by the Cm/Ca ratio”. Isn’t it that these ‘basic principles’ of leaf gas exchange are already fairly well known from previous CO2 and the $^{18}$O studies?

We clearly acknowledge in our paper that the processes affecting $^{18}$O and $^{17}$O are indeed the same, and in fact we use the established conceptual models, with appropriate references. Nevertheless, this is the first experimental leaf-scale study where the applicability of these theoretical concepts to $^{17}$O is actually demonstrated.
It seems also that the notion of “discrimination against Δ17O of atmospheric CO2” is not clear. If this is confused with D in leaf photosynthesis as for D18, then again 17O is predictable and has no clear additional information (other than perhaps the reflection of the possible variations in the lambda factor). The final estimate of global 17O discrimination anomaly is back of the envelope calculation based on these known principles and literature values. I am not sure what new insights are provided.

We realize from this comment that we have not explained clearly enough the difference of measuring δ17O and Δ17O. What the referee calls “ΔA in leaf photosynthesis as for ΔA18O” would be ΔA17O. This was also shown in our original paper for consistency, but does indeed not provide additional information. Only the combination of ΔA18O and ΔA17O to ΔAΔ17O provides the independent information. In the revised manuscript, we only present the results for ΔA18O and ΔAΔ17O. Some of the confusion may have to do with the notation, because the plant communities and atmospheric communities have used the symbol Δ for different quantities that are both used here.

Our final estimate of GPP is not dependent on the individual δ17O and δ18O values, but only on ΔAΔ17O. It is indeed a box model calculation, but to incorporate more variability, the entire mechanism would need to be incorporated in a global model. We are considering to implement this in the future, but for the box model presentations in this paper we have used global estimate of Δ17O of CO2 and leaf water from a recent 3D global Δ17O study (Koren et al., 2019).

And so, while the experimental setup, measurements, and going through the isotopic theory are impressive and seems to be well done on first look, I think the authors have to re-think the presentation and provide a better justification of what in these measurements takes advantage of any mass independent effects (as declared), and in what ways this goes beyond a sophisticated confirmatory report.

We realized already in the preparation of the manuscript that the presentation was difficult, and the referee comment confirms this. Nevertheless, we still think that the four conclusions identified above (copied below) make this a valuable study, whereas the referee sees only point 1 as significant merit.

1) demonstrate that the established theory is applicable to Δ17O-CO2 exchange at leaf-level
2) experimentally quantify for the first time the effect of photosynthesis on Δ17O of atmospheric CO2
3) study of the dependence of this effect on critical parameters
4) provide an independent bottom-up Δ17O-isoflux estimate based on these lab experiments.

Referee 2

Thank you for your constructive comments. We carefully went through all the comments and suggestions and have adjusted the manuscript according to the comments made. The answers to the questions/ comments and suggestions are stated below each comment.

The triple oxygen isotopic composition of CO2 (Δ17OCO2) had been regarded as spatiotemporally constant in the troposphere because of its short residence time (e.g., Luz et al., 2000). Recently, significant
seasonal and temporal variations of Δ17OCO2 were first revealed in the atmosphere near the surface by Hofmann et al. (2017) and Liang et al. (2017), respectively, both of which were mainly controlled by the interaction of CO2 between the atmosphere and biosphere. These studies were then followed by the three dimensional simulation study with an atmospheric physico-chemical model (Koren et al., 2019), to quantify the global CO2 budget. The next step, therefore, must be the process study involving oxygen isotope fractionations in association with individual CO2 fluxes.

This study by Adnew, Pons, Koren, Peters, Röckmann, aims to quantify the Δ17OCO2 change during photosynthetic CO2 removal from the atmosphere, caused by tiny difference of 17O-18O relationship between kinetic and equilibrium isotope fractionations inside the leaf.

To my knowledge, this is the first experimental study for Δ17OCO2 at the leaf-scale; thus, their results provided must be important. However, I am frustrated and feel difficult to plough through the manuscript because 1) the structure of the manuscript (context) seems scattered, 2) experimental results (raw data) were not shown although values in all graphs were processed, 3) there appears a lot of faults in equations or figure number in the main text, and 4) it’s a mixture of lengthy and in-short explanations. I strongly recommend the authors to revise the manuscript more simply and concisely.

We thank the referee for acknowledging the relevance of our study. We realize ourselves that the manuscript is quite difficult. We therefore thank the referee for the concrete suggestions below (including the suggesting for shortening), which helped us to improve the general storyline and readability.

**General comments**

# It spent 11 of 18 pages (until conclusion) from the Introduction to “Materials and methods (M&M).” It seems too dominant; in other words, Results and Discussion seem too short. There appears a lengthy description in M&M, and the description for experimental results is too short.

In the revised manuscript, we reduced the description of materials and methods section.

- The introduction, from line 91 to 106 was shortened and combined with the previous paragraph
- We shortened the materials and methods section and moved part of it to the supplementary material
- Section 2.1 was shortened based on the recommendation of the referee
- We shortened the theory part, line 170 to 201 in section 2.2 was moved to supplementary material.
- Section 2.3 was moved to the discussion section
- Section 2.4 was moved to the supplementary material

- We have also extended the results section following the concrete suggestions as described below.

# L84-90: This block appears the center of your motivation; however, there is no specific description of what the problem or limitation exists currently. Until this block (and perhaps in previous studies), you mentioned the Δ17O is free from any terrestrial MDF processes and made readers believe that Δ17O be
a more robust tracer for estimating GPP. You must describe what actual problems lying among previous studies such as inconsistency, uncertainty, speculation, assumption and so on. Without this explanation, readers could not have motivations to read the next pages. I strongly recommend adding descriptions for the different slopes of three-isotope plots due to the different MDF processes.

Thank you very much for the suggestion. Indeed, different MDF processes with different three-isotope slopes are involved, and in the revised manuscript we incorporated the following schematic figure to illustrate this point and to illustrate the objective of our study.

Figure 1 Schematic of the process that affects the $\Delta^{17}O$ of CO2 and H2O during the photosynthetic gas exchange (not to scale). The three-isotope slopes $\theta$ for the individual isotope fractionation processes (both kinetic and equilibrium fractionation) are $\theta_{\text{trans}}=0.522-0.008 \times h$ for transpiration at $h = 75$ % relative humidity (Landais et al., 2006), $\theta_{\text{CO2-H2O}}=0.5229$ for isotope exchange between CO2 and H2O (Barkan and Luz, 2012), $\theta_{\text{co2-diff}} = 0.509$ for the diffusion of CO2 (Young et al., 2002), $\theta_{\text{H2O(v)-H2O(l)}} = 0.529$ for the equilibrium between gas phase (v) and liquid (l) water, (Barkan and Luz, 2005) and $\theta_{\text{H2O(v)-diff}}$ for the diffusion of water vapor (Barkan and Luz, 2007). $\epsilon_{18}O$, indicated along the x-axis, is the isotope fractionation in 18O due to the responding process.

Furthermore, we have reformulated our motivation. The key point is that so far, the three-isotope slope of each of the processes that participate in plant-atmosphere gas exchange has been studied individually in an idealized experiment. The overall effect of all processes, which work together in complex interaction, on $\Delta^{17}O$ has never been evaluated in a real plant exchange experiment. This is what is achieved in the research described in our manuscript and it is explicitly stated in the revised version.
I strongly recommend the authors to revise the Theory part completely. The structure is scattered and forces readers to jump frequently between the main text, Appendix and Supplementary Materials (SM).

We revised the theory part of the manuscript and incorporated your suggestions in the modified manuscript.

Appendix should be moved to SM.

In the revised manuscript, the appendix is moved to the supplementary material.

The term “fractionation” should be replaced to “isotope fractionation” for all.

We use isotope fractionation instead of fractionation alone throughout the revised manuscript.

My major concern is the relation between dots of “Farquhar model” and curves in Figs 4 and 5a) and related description in Section 3.6. If I were not misunderstanding, both are results calculated from the “Farquhar model.” Dots were obtained by giving several observed results and curves were simulated by giving similar boundary conditions to the experimental setting. Is the former necessary? This is very confusing.

We are sorry for the confusion, but the two are not the same. The curves are based on the leaf cuvette model which we implemented for this study and the blue diamonds were the results for the individual experiments using the Farquhar model. In the revised manuscript, we excluded the blue diamond points because this is not really necessary for our line of argumentation.

I strongly recommend the authors to provide “List of symbols.” for all parameters used and defined.

In the revised manuscript, the list of symbols for all parameters used in this study is provided.

The parameter cm seems one of the most important numbers in this study. For obtaining this, only δ18O and α18 values were used concerning isotope ratio, though. Is it possible to use Δ17O and λ values to evaluate cm instead? At least does it make sense to test its feasibility?

Yes, it is possible to calculate the mole fraction of CO₂ at the CO₂-H₂O exchange site (cm) using the Δ¹⁷O and λ values. Since this requires development of yet another complicated set of equations and detailed discussion of the process of assimilation from a plant physiology point of view, it would make our paper even more complex and less focused. A companion manuscript with detailed description and derivation of the cm using Δ¹⁷O and λ values is under preparation.

As shown in Figure 5, the discrimination of Δ17O of CO2 during photosynthesis varies widely, and controlled by the magnitude of oxygen isotope equilibration at the CO2-H2O site, that is to say, the
relative contribution of kinetic (diffusion) and equilibrium isotope fractionation. This conclusion is almost identical to the knowledge using conventional $\delta^{18}O$ results. Moreover, in the last paragraph of Discussion, authors mentioned that the main uncertainty is cm/ca ratio, which may be same as the main uncertainty of $\delta^{18}O$. My impression after reading this manuscript is that the intra-MDF variation dominate that of MIF signature on tropospheric CO2, which weakens the merit to study $\Delta^{17}O$ of CO2. What is an advantage to use $\Delta^{17}O$ instead of $\delta^{18}O$? Please provide suggestions or implications to general biogeochemists.

The referee is correct that the processes that affect $\delta^{18}O$ are the same that affect $\Delta^{17}O$. Nevertheless, the quantitative evaluation of $\Delta^{17}O$ is largely independent of $\delta^{18}O$. The limitation of using $\delta^{18}O$ of atmospheric CO$_2$ as a tracer is its dependency on the $\delta^{18}O$ value of different water reservoirs and fractionation processes in the hydrological cycle, water isotopic inhomogeneity and dynamics, which are difficult to ascertain (Hoag et al., 2005). Unlike $\delta^{18}O$, $\Delta^{17}O$ variation is much smaller and is better defined (Miller, 2018). This is because conventional bio-geo-chemical processes that modify $\delta^{17}O$ and $\delta^{18}O$ follow well-defined three-isotope fractionation slope. Consequently, the formulation of the CO$_2$ budget using $\Delta^{17}O$ is a lot simplified, compared to using $\delta^{18}O$. Furthermore, unlike $\delta$’s, $\lambda$ is insensitive to temperature (Cao and Liu, 2011; Bao et al., 2016; Hofmann et al., 2012; Dauphas and Schauble, 2016; Miller, 2018).

**Specific comments**

L41: “replaced using…” What this means? Be more specific.

In the revised manuscript it is replaced with “replicated based on cross-consistency checks with atmospheric inversions, sun-induced fluorescence (SIF) and dynamic global vegetation models”

L47: “see equation (1)” instead of “see below”

In the revised manuscript we used “see equation (1)”

L51: "the latter term" I guess it should be "the former term," which means photosynthetic CO2 uptake.

Thank you, in the revised manuscript corrected it to “the former term”

L53: “variable $\delta^{18}O$ gradient” I think "significant $\delta^{18}O$ variation" is more appropriate.

In the revised manuscript we used significant $\delta^{18}O$ variation

L56: Delete "the isotopically exchanged"
In this block, you should use the term "isotope fractionation" with its definition for the subsequent block. More desirably, the term "mass-dependent isotope fractionation (MDF)" with its definition.

In the revised manuscript, we included “These physico-chemical processes change $^{17}\text{O}/^{16}\text{O}$ by approximately half the corresponding change in $^{18}\text{O}/^{16}\text{O}$, a process called mass-dependent isotope fractionation (see equation 2). This is because the mass difference between $^{17}\text{O}$ and $^{16}\text{O}$ (1.0042 amu) is approximately half as large as the mass difference between $^{18}\text{O}$ and $^{16}\text{O}$ (2.0042 amu). “at the end of the paragraph.

L63: "mass-dependent fractionation" should be “mass-dependent isotope fractionation” with its definition in detail.

We excluded this paragraph, L62-64 in the revised manuscript since it does not add additional information to the paragraph mentioned above.

L62-64: Need revision because the latter paragraph is just a refrain of the former.

We excluded this paragraph, L62-64 in the revised manuscript since it does not add additional information.

L65: Describe a specific value instead using "considerable"

In the revised manuscript, instead of “considerable $\delta^{17}\text{O}$” we used “the $\delta^{17}\text{O}$ of CO$_2$ is 1.7 to 2.2 times $\delta^{18}\text{O}$ of CO$_2$ (Wiegel et al., 2013)”

L60-71: In this block, you should use the term "mass-independent isotope fractionation (MIF)” with its definition, and associate it with "photochemical isotope exchange”

In the revised manuscript, we included the following paragraph

In nature, it was believed all processes that modify the oxygen isotope distribution are mass-dependent isotope fractionation until the discovery of a deviation from the assigned mass-dependent three-isotope fractionation line in meteorites (Clayton et al., 1973; Clayton et al., 1976) and ozone formation (Thiemens, 1983; Heidenreich and Thiemens, 1983, 1986), called mass-independent isotope fractionation (see
The $\Delta^{17}O$ of ozone can be transferred to other oxygen bearing molecules via direct chemical reaction with ozone or via $O(1D)$.

and rearranged the whole paragraph

L70-71: This is not sufficient because exchanges with soil and ocean water are also nonenzymatic processes.

The isotope exchange in the atmosphere is negligible due to lower liquid water content, lower residence time and the absence of carbonic anhydrase (Mills and Urey, 1940; Johnson, 1982; Miller et al., 1971; Silverman, 1982). We incorporated this sentence in the updated section.

It is true that CO$_2$-H$_2$O exchange with ocean water is a non-enzymatic processes, but CO$_2$-H$_2$O exchange with soil water is controlled by carbonic anhydrase (Wingate et al., 2009), similar to the exchange with leaf water.

L78: "The $\Delta^{17}O$ of CO2" instead of "The 17O-excess of CO2 ($\Delta^{17}O$) (equation 4)"

In the revised manuscript, we only used $\Delta^{17}O$

L80: Clarify "well-known three-isotope slope." “Non three-isotope person” cannot understand what this means.

In the revised manuscript, we included the three-isotope fractionation slope of 0.5229, and the figure above.

L92-106 and Figure 1: The explanation is this block is too general, should reduce to a few sentences. Detail description may be required if you would like to discuss the difference of results due to the different types in the Discussion. As for Figure 1, not this scheme but simpler scheme in Figure S6 was actually used in this study. Therefore, it seems more appropriate to delete Figure 1 and insert S6 here.

In the revised manuscript, we merged the necessary information with the other paragraphs and we agree that Figure 1 is not necessary, so it is left out. We excluded the general description of plant types. We only kept the following three sentences

“The mole fraction of CO$_2$ at the CO$_2$-H$_2$O exchange site ($c_{0w}$) is an important parameter to determine the effect of photosynthesis on the triple oxygen isotope composition of atmospheric CO$_2$. In C$_3$ plants, CA is found in the chloroplast, cytosol, mitochondria and plasma membrane (Fabre et al., 2007; DiMario et al., 2016) and the CO$_2$-H$_2$O exchange can occur anywhere between the plasma membrane and the
chloroplast. For C₄ plants, CA is mainly found in the cytosol, the CO₂-H₂O exchange occurs in the cytosol (Badger and Price, 1994)."

380 L108-109: What is "leaf level"?
In the revised manuscript we changed it from leaf level to leaf scale

385 L116-117: "Δ¹⁷O" instead of "triple oxygen isotopic composition"
Changed accordingly

390 Equations 1 and 2: Should be merged such as, δn O = n Rsample/ n RVSMOW – 1, n refers 17 or 18 or simpler, δ = Rsample/RVSMOW – 1.

Thank you, in the revised manuscript we used the first suggestion.

395 L134: I recommend "The MDF factor" instead of "The factor"
Changed accordingly

400 L135-137: Delete “This relation…, respectively.
In the revised manuscript we excluded the sentence, based on the suggestion above, we already defined mass dependent isotope fractionation.

405 L137: “variations" instead of "values." "Small delta value" is meaningless.
In the revised manuscript we changed values to variations. And at the end of the paragraph we introduced “Equation 4 can be linearized to Δ¹⁷O = δ¹⁷O − λ × δ¹⁸O (Miller, 2002), but this approximation causes an error that increases with δ¹⁸O." for more clarity.

410 L139-140: I recommend “Note that Δ¹⁷O changes not only by MIF processes, but also MDF processes with a different λ value from the definition,"

Changed according
L145-146: "which was obtained by the observation of" instead of "the value associated with"

Changed accordingly

L147-148: Delete "Note that ... δ18O."

Changed accordingly

L150-258 (Section 2.2-2.4): Revise completely.

In the revised manuscript,
- we moved most of section 2.2 to the supplementary material
- we moved section 2.3 to the discussion
- we moved section 2.4 to the supplementary material

Equation 5: Use n (18 or 17) or simpler expression as above, then revise or delete

In the revised manuscript, we implemented the suggestion expression

L158 and L163. Equation 12: Move after equation 5 with related sentences.

Changed accordingly

L163-168: Delete "We note that...itself."

The sentence is excluded from the revised manuscript.

L170-200 and Section 2.4: Integrate and locate in new section such like “Extension of Farquhar-Lloyd model to oxygen triple isotopes. Eqs. 6 and 11 are almost identical so that they should be merged.

Equation 15: Use n (18 or 17) or simpler expression, then revise or delete

This section is moved to the supplementary material and revised in the new version of the manuscript

L208-213 and Figure 2: Move to SM.

Changed accordingly

Section 2.3: I recommend moving this section to the Discussion.
L217: Delete “which is a net sink,”

This section is moved to the discussion part, and “which is a net sink,” is removed in the revised manuscript.

L230: Specify which model is used.

In the revised manuscript this section is moved to the discussion section and revised entirely.

L241-259: Here detail but still insufficient description was made only for δm, on the other hand, no description for ci and δi which were driven away to Appendix. This seems out of balance and forces readers to jump here and there. I recommend moving this block to SM.

In the revised manuscript, we moved this part to the supplementary material

L256-257 and related sentences in Appendix A3. No definition of ci.

In the revised manuscript, definition for all parameters is included as a table in the appendix

L262-265: Could it be shorter?

In the revised manuscript we shortened this part by excluding the sentence from line 263 to line 265,

“The dwarf type sunflowers were grown until the first leaf pair that was used for the experiments reached the final size, which is about 4 weeks.” We did the same for line 267 to 268, i.e. “After at least 6 weeks in the growth chamber, leaves that had developed and matured there were used for the experiment” is excluded in the revised manuscript.

L268-269: “The 4th or higher…” Is this sentence an explanation for maize or all species?

In the revised manuscript, we write “For maize, the 4th or higher …. “

Section 3.2: Need the model and the manufacturer for halogen lamp, neutral filters, dewpoint meter (the model).

The models and manufacturers are included in the revised manuscript.
Section 3.3: Could this section be shorter to several sentences? The description for $\delta D$ and obtaining optimum setting seem appropriate in SM.

In the revised manuscript this section has been considerably shortened and part of it is moved to the supplementary material.

L349: Water was converted to O2

In the revised manuscript L349 - L 354 has been deleted to make the manuscript more concise.

Section 3.5: In previous section, unit of $\Delta^{17}O$ is‰. Here ppm is used. Use a uniform manner.

In the revised manuscript, all numbers are given in ‰.

Section 3.6: See related general comment

The leaf cuvette model is described here for the first time, as a result we cannot make it shorter than this.

L403: The last sentence is a refrain.

In the revised manuscript, we excluded the sentence.

Results: Show experimental results (raw data) such as c, $\delta$, $\Delta$, w, for entering and leaving from the cuvette, etc. Show table of them and describe them.

In the revised manuscript, we provided the raw data for the gas exchange parameters in the beginning of the results section

L414-415: Delete this sentence

Changed accordingly

Section 4.2: Avoid using “$17O$-excess” in the title and L433 for uniformity

Changed accordingly
L477-493: I could not understand this block. If the authors applied different lambda values to individual results, the vertical axis in Figure 8 would be meaningless, and one could not evaluate the graph and related description at all.

In the revised manuscript we incorporated the reference triple oxygen isotope fractionation slope ($\lambda$), also in the caption. Sorry for the confusion we did not mention it clearly. When we described $\Delta^{17}O$ in the theory section, we clearly mentioned which lambda value we used ($\lambda=0.528$).

Section 5.2: Avoid using “$^{17}O$-excess” for uniformity

Figure 3: Add individual flow direction.

Figure 4: Panel b seems unnecessary. Delete and insert Figure 5a here.

Figure 5: Move Panel a to Figure 4 as above

Figure 6: Is it important to plot both of blue diamonds and curve. Should the curve be improved by blue diamonds?

The curves are based on the leaf cuvette model which we implemented for this study. The blue diamonds were the results for the individual experiments using the Farquhar model. In the revised manuscript, we excluded the blue diamond points.

Typographic errors

Space inserted after semicolon (e.g., L33)

L42: Welp et al. (2011)  
Corrected

L45: The concept of the latter study..  
Corrected
L60: equation 4)
Corrected, now it is “see equation 2”

L207: Figure 2
Now Figure S1

L237: “Following (Farquhar…..)” Need grammatical correctness
Corrected to: “The CO₂ mole fraction at the site of CO₂-H₂O exchange is calculated as shown in equation S10 following (Farquhar and Cernusak, 2012; Barbour et al., 2016; Osborn et al., 2017).

This section has also been moved to the supplementary material.

L267: Maize
In the revised manuscript, “Mays” is corrected to “Maize”

L279, L297: Need grammatical correctness.
Line 279
In the revised manuscript “of” is replaced by “for”, now it reads as follow:
A schematic for the gas exchange experimental setup is shown in Figure 2

L297
In the revised manuscript, we deleted the phrase “as described in detail in” since it does not change the meaning of the sentence. Now it reads as:
“The isotopically enriched CO₂ was prepared by photochemical isotope exchange between CO₂ and O₂ under UV irradiation (Adnew et al., 2019).

Section 3.2: “Figure 3” instead of “Figure 2” (If Figure 2 were moved to SM, they are accidentally correct, though)

Thank you, this is corrected in the revised manuscript
References: I found typo. in Barbour et al. (2016) and Caemmerer and Farquhar (1981). There may be more. Confirm all.
Thank you very much. In the revised manuscript we corrected all of them. All of them related to the name von Caemmerer.
L950: “entering and leaving” instead of ”leaving and entering"
In the revised manuscript, we re-ordered it chronologically. The appendix has also been moved to the supplementary material.

Equation A1.4: If the referred article (Caemmerer and Farquhar, 1981) was correct, the denominator must be (gt ac + E/2).

Corrected
Thank you very much, all the Typographic errors are corrected in the revised manuscript.

References


Johnson, K. S.: Carbon dioxide hydration and dehydration kinetics in seawater 1, Limnology and Oceanography, 27, 6, 1982.


Track change marked up Manuscript
Leaf-scale quantification of the effect of photosynthetic gas exchange on \(^{\Delta 17}O\) of atmospheric CO\(_2\)

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Abstract

Understanding the processes that affect the triple oxygen isotope composition of atmospheric CO\(_2\) during gas exchange can help constrain the interaction and fluxes between the atmosphere and the biosphere. We conducted leaf cuvette experiments under controlled conditions, using three plant species. The experiments were conducted at two different light intensities and using CO\(_2\) with different \(^{\Delta 17}O\). We directly quantify for the first time the effect of photosynthesis on \(^{\Delta 17}O\) of atmospheric CO\(_2\). Our results demonstrate the established theory for \(^{\delta 18}O\) is applicable to \(^{\Delta 17}O\)-CO\(_2\) at leaf-level and we confirm the two key factors determine the effect of photosynthetic gas exchange on the \(^{\Delta 17}O\) of atmospheric CO\(_2\). The relative difference between \(^{\Delta 17}O\) of the CO\(_2\) entering the leaf and the CO\(_2\) in equilibrium with leaf water, and the back-diffusion flux of CO\(_2\) from the leaf to the atmosphere, which can be quantified by the \(c_w/c_a\) ratio where \(c_a\) is the CO\(_2\) mole fraction in the surrounding air and \(c_w\) the one at the site of oxygen isotope exchange between CO\(_2\) and H\(_2\)O. At low \(c_w/c_a\) ratio the discrimination is governed mainly by diffusion into the leaf, and at high \(c_w/c_a\) ratio by back-diffusion of CO\(_2\) that has equilibrated with the leaf water. Plants with a higher \(c_w/c_a\) ratio modify the \(^{\Delta 17}O\) of atmospheric CO\(_2\) more strongly than plants with a lower \(c_w/c_a\) ratio. Based on the leaf cuvette experiments, the global value for discrimination against \(^{\Delta 17}O\) of atmospheric CO\(_2\) during photosynthetic gas exchange is estimated to be \(-0.57\pm0.14\%\) using \(c_w/c_a\) values of 0.3 and 0.7 for \(C_4\) and \(C_3\) plants, respectively. The main uncertainties in this global estimate arise from variation in \(c_w/c_a\) ratios among plants and growth conditions.
1. Introduction

Stable isotope measurements of CO$_2$ provide important information on the magnitude of the CO$_2$ fluxes between atmosphere and biosphere, which are the largest components of the global carbon cycle (Farquhar et al., 1989; Ciais et al., 1997a; 1997b; Flanagan and Ehleringer, 1998; Yakir and Sternberg, 2000; Gillon and Yakir, 2001; Cuntz et al., 2003a; 2003b). A better understanding of the terrestrial carbon cycle is essential for predicting future climate and atmospheric CO$_2$ mole fractions (Booth et al., 2012). Gross primary productivity (GPP), the total carbon dioxide uptake by vegetation during photosynthesis, can only be determined indirectly and remains poorly constrained (Cuntz, 2011; Welp et al., 2011). For example, Beer et al. (2010) estimated global GPP to be 102-135 PgC yr$^{-1}$ (85% confidence interval, CI) using machine learning techniques by extrapolating from a database of eddy-covariance measurements of CO$_2$ fluxes. This estimate has since then been widely used as target for terrestrial vegetation models (Sitch et al., 2015), and replicated based on cross-consistency checks with atmospheric inversions, sun-induced fluorescence (SIF) and global vegetation models (Jung et al., 2020). As an alternative, Welp et al. (2011) estimated global GPP to be 150-175 PgC yr$^{-1}$ using variations in $\delta^{18}$O of atmospheric CO$_2$ after the 1997/98 El Nino event; see equation 1 for definition of the $\delta$ value.

The concept behind the latter study was that atmospheric CO$_2$ exchanges oxygen isotopes with leaf and soil water, and this isotope exchange mostly determines the observed variations in $\delta^{18}$O of CO$_2$ (Francey and Tans, 1987; Yakir, 1998). Following the 97/98 ENSO event, the anomalous $\delta^{18}$O signature imposed on tropical leaf and soil waters was transferred to atmospheric CO$_2$, before slowly disappearing as a function of the lifetime of atmospheric CO$_2$. This in turn is governed by the land vegetation uptake of CO$_2$ during photosynthesis, as well as soil invasion of CO$_2$ (Miller et al., 1999; Wingate et al., 2009). For the photosynthesis term, the equilibration of CO$_2$ with water is an uncertain parameter in this calculation, partly because the $\delta^{18}$O of water at the site of isotope exchange in the leaf is not well defined. Importantly, a significant $\delta^{18}$O variation can occur in leaves due to the preferential evaporation of H$_2^{18}$O relative to H$_2^{16}$O (Gan et al., 2002; Farquhar and Gan, 2003; Gan et al., 2003; Cernusak et al., 2016), which induces a considerable uncertainty in estimating $\delta^{18}$O of CO$_2$. Similar considerations for the transfer of the $\delta^{18}$O signature of precipitation into the soils, and then up through the roots, stems, and leaves make $^{18}$O of CO$_2$ a challenging measurement to interpret (Cuntz et al., 2003a; 2003b; Peylin et al., 1999).

Classical isotope theory posits that oxygen isotope distributions are modified in a mass-dependent way. This means that the $^{18}$O/$_{16}$O ratio changes by approximately half of the corresponding change in $^{18}$O/$_{16}$O (equation 2), and it applies to the processes involved in gas exchange between atmosphere and plants. However, in 1983 Thiemens and co-workers (Thiemens, 1983; Heidenreich and Thiemens, 1983; 1986) reported a deviation from mass-dependent isotope fractionation in ozone (O$_3$) formation called mass-independent isotope fractionation ($\Delta^{18}$O, equation 3). In the stratosphere, the $\Delta^{18}$O of O$_3$ is transferred to CO$_2$ via isotope exchange of CO$_2$ with O($^{17}$D) produced from O$_3$ photolysis (Shaheen et al., 2007; Yung et al., 1991; Yung et al., 1997), which results in a large $\Delta^{18}$O in stratospheric CO$_2$ (Wiegel et al., 2013; Thiemens et al., 1991; 1995; Lyons, 2001; Lüammerzahl et al., 2002; Thiemens, 2006; Kawagucci et al., 2008).
Once \( \Delta^{17}O \) has been created in stratospheric CO\(_2\), the only process that modify its signal is isotope exchange with leaf water, soil water and ocean water at the Earth’s surface, after CO\(_2\) has re-entered the troposphere (Boering, 2004; Thiemens et al., 2014; Liang and Mahata, 2015; Hofmann et al., 2017). Isotope exchange with leaf water is more efficient relative to ocean water due to the presence of the enzyme carbonic anhydrase (CA), which effectively catalyzes the conversion of CO\(_2\) and H\(_2\)O to HCO\(_3^-\) and H\(^+\) and vice versa (Francay and Tans, 1987; Friedli et al., 1987; Badger and Price, 1994; Gillon and Yakir, 2001). The isotope exchange in the atmosphere is negligible due to lower liquid water content, lower residence time and the absence of carbonic anhydrase (Mills and Urey, 1940; Johnson, 1982; Miller et al., 1971; Silverman, 1982; Francay and Tans, 1987).

\( \Delta^{17}O \) of CO\(_2\) has been suggested as an additional independent tracer for constraining global GPP (Hoag et al., 2005; Thiemens et al., 2013; Hofmann et al., 2017; Koren et al., 2019; Liang et al., 2017b), because the processes involved in plant-atmosphere gas exchange are all mass-dependent. Therefore, \( \Delta^{17}O \) at the CO\(_2\)-H\(_2\)O exchange site in the leaf will vary much less than \( \delta^{18}O \). Nevertheless, mass-dependent isotope fractionation processes with slightly different three-isotope fractionation slopes are involved, which have been precisely established in the past years. Figure 1 shows how the different processes affect \( \Delta^{17}O \) of the H\(_2\)O and CO\(_2\) reservoirs involved. The triple isotope slope of oxygen in meteoric waters is taken as reference slope, \( \lambda_{\text{ref}}=0.528 \) (Luz and Barkan, 2010; Meier and Li, 1998; Barkan and Luz, 2007; Landais et al., 2008; Uemura et al., 2010) and we assume that soil water is similar to meteoric water. Due to transpiration and diffusion in the leaf, \( \Delta^{17}O \) of leaf water gets modified following a humidity dependent three-isotope slope \( \lambda_{\text{trans}}=0.522-0.008 \times h \) (Landais et al., 2006). Exchange of oxygen isotopes between leaf water and CO\(_2\) follows \( \lambda_{\text{CO}_2-H_2O}=0.5229 \) (Barkan and Luz, 2012) which determines the \( \Delta^{17}O \) of CO\(_2\) inside the leaf at the CO\(_2\)-H\(_2\)O exchange site. Finally, the \( \Delta^{17}O \) of the CO\(_2\) is modified when CO\(_2\) diffuses into and out of the leaf with \( \lambda_{\text{diff}}=0.509 \) (Young et al., 2002).

In the first box model study of Hoag et al. (2005), the small deviations in \( \Delta^{17}O \) of CO\(_2\) due to differences in three-isotope slopes were neglected and exchange with water was assumed to reset \( \Delta^{17}O \) to 0. Hofmann et al. (2017) included the different isotope effects shown in Figure 1 in their box model. Koren et al. (2019) incorporated all the physico-chemical processes affecting \( \Delta^{17}O \) of CO\(_2\) in a 3D atmospheric model and investigated the spatiotemporal variability of \( \Delta^{17}O \) and its use as tracer for GPP. Using these and other similar models, numerous measurements of \( \Delta^{17}O \) in atmospheric CO\(_2\) from different locations have been performed and used to estimate GPP (Liang et al., 2006; Barkan and Luz, 2012; Thiemens et al., 2014; Liang and Mahata, 2015; Laskar et al., 2016; Hofmann et al., 2017). The three-isotope slopes of the processes involved in the gas exchange (Figure 1) have been precisely determined in idealized experiments. In the advanced models mentioned above it is assumed that when all the pieces are put together they result in a realistic overall modification of \( \Delta^{17}O \) of CO\(_2\) in the atmosphere surrounding the leaf. However, this has not been confirmed by measurements previously.
In this study we report the effect of photosynthesis on $\Delta^{17}O$ of CO$_2$ in the surrounding air at the leaf scale. We measured $\Delta^{17}O$ of CO$_2$ entering and leaving a leaf cuvette to calculate the isotopic fractionation associated with photosynthesis for three species that are representative for three different biomes. The fast-growing annual herbaceous C$_3$ species Helianthus annuus (sunflower) has a high photosynthetic capacity ($A_N$) and high stomatal conductance ($g_s$) and is representative for temperate and tropical crops (Fredeen et al., 1991). The slower growing perennial evergreen C$_4$ species Hedera hyperborea (ivy) is representative of forests and other woody vegetation and stress subjected habitats (Pons et al., 2009). The fast-growing, agronomically important crop Zea mays (maize) is an herbaceous annual C$_4$ species with a high $A_N$ and a low $g_s$, typical for savanna type vegetation (Weijide et al., 2013).

The mole fraction of CO$_2$ at the CO$_2$-H$_2$O exchange site ($c_w$) is an important parameter to determine the effect of photosynthesis on $\Delta^{17}O$ of CO$_2$. In C$_3$ plants, the CO$_2$-H$_2$O exchange can occur anywhere between the plasma membrane and the chloroplast since the catalyzing enzyme CA has been found in the chloroplast, cytosol, mitochondria and plasma membrane (Fabre et al., 2007; DiMario et al., 2016). For C$_4$ plants, CA is mainly found in the cytosol and the CO$_2$-H$_2$O exchange occurs there (Badger and Price, 1994). In our experiments, sunflower and ivy are used to cover the wide $c_w/c_a$ ratio range among C$_3$ plants and maize represents the $c_w/c_a$ ratio for the C$_4$ plants. Using our results from the leaf scale experiments, we estimated the effect of terrestrial vegetation on $\Delta^{17}O$ of CO$_2$ in the global atmosphere.

1. Theory

1.1. Notation and definition of $\delta$ values

Isotopic composition is expressed as the deviation of the heavy to light isotope ratio in a sample relative to a reference ratio and is denoted as $\delta$, expressed in per mill ($\%$). In the case of oxygen isotopes, the isotope ratios are $^{18}R = [^{18}O]/[^{16}O]$ and $^{17}R = [^{17}O]/[^{16}O]$, and the reference material is Vienna Standard Mean Ocean Water (VSMOW).

$$\delta^{18}O = \frac{n^{18}\text{Sample}}{n^{18}\text{VSMOW}} - 1, \text{n refers to 17 or 18}$$  (1)

For most processes, isotope fractionation depends on mass, and therefore the fractionation against $^{17}O$ is approximately half of the fractionation against $^{18}O$ (equation 3).

$$\ln(\delta^{17}O + 1) = \lambda \times \ln(\delta^{18}O + 1)$$  (2)

The mass-dependent isotope fractionation factor $\lambda$ ranges from 0.5 to 0.5305 for different molecules and process (Matsuhisa et al., 1978; Young et al., 2002; Thiemens, 1999; Cao and Liu, 2011). $\Delta^{17}O$ is used to quantify the degree of deviation from equation (2) (see equation 3). Note that $\Delta^{17}O$ changes not only by mass independent isotope fractionation processes, but also by mass dependent isotope fractionation processes with a different $\lambda$ value from the one used in the definition of $\Delta^{17}O$ (Barkan and Luz, 2005; Landais et al., 2006; 2008; Luz and Barkan, 2010; Barkan and Luz, 2011; Pack and Herwartz, 2014).
The choice of $\lambda$ is in principle arbitrary and in this study, we use $\lambda = 0.528$, which was established for meteoric waters (Meijer and Li, 1998; Landais et al., 2008; Brandt et al., 2010; Barkan and Luz, 2012; Sharp et al., 2018). Equation 3 can be linearized to (Miller, 2002)

$$\Delta^{17}O = \ln(\delta^{17}O + 1) - \lambda \times \ln(\delta^{18}O + 1)$$

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1.2. Discrimination against $\Delta^{17}O$ of CO$_2$

060 The overall isotope fractionation associated with the photosynthesis of CO$_2$ is commonly quantified using the term discrimination as described in (Farquhar and Richards, 1984; Farquhar et al., 1989; Farquhar and Lloyd, 1993). We use the symbol $\Delta$ for discrimination due to assimilation in this manuscript since the commonly used $\Delta$ is already used for the definition of $\Delta^{17}O$ (see equation 3). $\Delta$ quantifies the enrichment or depletion of carbon and oxygen isotopes of CO$_2$ in the surrounding atmosphere relative to the CO$_2$ that is assimilated (Farquhar and Richards, 1984). It can be calculated from the isotopic composition of the CO$_2$ entering and leaving the leaf cuvette (Evans et al., 1986; Gillon and Yakir, 2000a; Bao et al., 2016) as:

$$\Delta A^{17}O_{obs} = \frac{n_{B_{obs}}}{n_{A_{obs}}} - 1 = \frac{\delta^{17}O_{B} - \delta^{17}O_{A}}{1 + \delta^{17}O_{A}}$$

070 where the indices e, a and A refer to CO$_2$ entering and leaving the cuvette and being assimilated, respectively. $\zeta = \frac{c_{e}}{c_{a} - c_{e}}$, where $c_{e}$ and $c_{a}$ are the mole fractions of CO$_2$ entering and leaving the cuvette.

075 For quantifying the effect of photosynthesis on $\Delta^{17}O$, three experiments, the $\Delta A^{17}O$ is calculated from $\Delta^{13}O$ and $\Delta^{18}O$ using the three-isotope slope $\lambda_{3I} = 0.528$, similar to equation 3. In previous studies slightly, different formulations have been used to define the effect of photosynthesis on $\Delta^{17}O$, and a comparison of the different definitions is provided in the supplementary material (equation S37-S40).

080 It is important to note that when the logarithmic definition of $\Delta^{17}O$ or $\Delta A^{17}O$ is used, values are not additive (Kaiser et al., 2004). In linear calculations, the error gets larger when the relative difference in $\delta^{18}O$ between the two CO$_2$ gases increases regardless of the $\Delta^{17}O$ of the individual CO$_2$ gases (Figure S1). Therefore, $\Delta A^{17}O$ values have to be calculated from the individual $\Delta A^{13}O$ and $\Delta A^{18}O$ values, and not by linear combinations of the $\Delta^{17}O$ of air entering and leaving a plant chamber.

2. Materials and methods

2.1. Plant material and growth conditions
Sunflower (*Helianthus annuus* L. cv “sunny”) was grown from seeds in 0.6 L pots with potting soil (Primasta, the Netherlands) for about four weeks. All leaves appearing above the first leaf pair were removed to avoid shading. Established juvenile *ivy* (*Hedera hibernica* L.) plants were pruned and planted in 6 L pots for 6 weeks. *Ivy* leaves that had developed and matured were used for the experiments. *Maize* (*Z. mays* L. cv “saccharate”) was grown from seed in 1.6 L pots for at least 7 weeks. *For maize*, the 4th or higher leaf number was used for the experiments when mature. A section of the leaf at about 1/3 from the tip was inserted in the leaf cuvette. They were placed on a sub-irrigation system that provided water during the growth period in a controlled environment growth chamber, air temperature 20°C, relative humidity 70%, and CO₂ mole fraction of about 400 ppm. The photosynthetic photon flux density (PPFD) was about 300 μmol m⁻² s⁻¹ during a daily photoperiod of 16 hours measured with a PPFD meter (Licor LI-250A, Li-Cor Inc., Nebraska, USA).

### 2.2. Gas exchange experiments

Gas exchange experiments were performed in an open system where a controlled flow of air enters and leaves the leaf cuvette similar to the setup used by (Pons and Welschen, 2002). A schematic for the gas exchange experimental setup is shown in Figure 2. The leaf cuvette had dimensions of 7 x 7 x 7 cm³ (lwxh) and the top part of the cuvette was transparent. The temperature of the leaf was measured with a K type thermocouple. The leaf chamber temperature was controlled by a temperature-controlled water bath kept at 20°C (Tamson TLC 3, The Netherlands). A halogen lamp (PRADOVIT 253, ERNST LEITZ WETZLAR GMBH, Germany) in a slide projector was used as a light source. Infrared was excluded by reflection from a cold mirror. The light intensity was varied with spectrally neutral filters (PRADOVIT 253, ERNST LEITZ WETZLAR GMBH, Germany).

The CO₂ mole fraction of the incoming and outgoing air was measured with an infrared gas analyzer (IRGA, model LI-6262, LI-COR Inc., Nebraska, USA). The isotopic composition and mole fraction of the incoming and outgoing water vapor were measured with a triple water vapor isotope analyzer (WVIA, model 911-0034, Los Gatos Research, USA). Compressed air (ambient outside air without drying) was passed through soda lime to scrub the CO₂. The CO₂ free air could be humidified depending on the experiment conditions (see Figure 2). The humidity of the inlet air was monitored continuously with a dewpoint meter (HYGRO-ML, General Eastern, Watertown, MA, USA). Pure CO₂ (either normal CO₂ or isotopically enriched CO₂) was mixed with the incoming air to produce a CO₂ mole fraction of 500 ppm. The isotopically enriched CO₂ was prepared by photochemical isotope exchange between CO₂ and O₂ under UV irradiation (Adnew et al., 2019).

An attached leaf or part of it was inserted into the cuvette, the composition of the inlet air was measured, and both IRGA and WVIA were switched to measure the outlet air. Based on the CO₂ mole fraction of the outgoing air the flow rate of the incoming air to the cuvette was adjusted to establish a drawdown of 100 ppm CO₂ due to photosynthesis in the plant chamber. The water vapor content entering the cuvette was adjusted depending on the transpiration rate relative to CO₂ uptake to avoid condensation (Figure 2). The outgoing air was measured continuously until a steady state was reached for CO₂ and H₂O mole fractions and δD and δ¹⁸O of the water vapor. After a steady state was established, the air was directed to...
the sampling flask while the IGRA and WVIA were switched back to measure the inlet air. The air passed through a Mg(ClO$_3$)$_2$ dryer before entering the sampling flask.

After sampling, the leaf area inside the cuvette was measured with a LI-3100C area meter (LI-COR, Inc. USA). Immediately afterward, the leaf was placed in a leak tight 9 mL glass vial and kept in a freezer at -20°C until leaf water extraction.

### 2.3. Calibration of the Water Vapor Isotope Analyzer (WVIA) and leaf water analysis

The WVIA was calibrated using five water standards provided by IAEA (Wassenaar et al., 2018) for both $\delta^{18}$O and $\delta^D$ ([Figure S2](#fig:s2)). We did not calibrate the WVIA for $\delta^{17}$O, so the $\delta^{17}$O data are not used in the quantitative evaluation. The isotopic composition of the water standards ranged from -50.93 to 3.64‰ and -396.98 ‰ to 25.44 ‰ for $\delta^D$ and $\delta^{18}$O, respectively. The detailed characterization and calibration of the water WVIA is provided in the supplementary material ([Figure S2 to S4](#fig:s2-s4)).

Leaf water was extracted by cryogenic vacuum distillation for 4 h at 60°C following a well-established procedure as shown in Figure S5 ([Wang and Yakir, 2000; Landais et al., 2006; West et al., 2006](#bib:17)). Details are provided in the supplementary material. The $\delta^18$O and $\delta^{18}$O of leaf water was determined at the Laboratoire des Sciences du Climat et de l’Environnement laboratory using a fluorination technique as described in ([Barkan and Luz, 2005; Landais et al., 2006; 2008](#bib:13)).

### 2.4. Carbon dioxide extraction and isotopic analysis

CO$_2$ was extracted from the air samples in a system made from electropolished stainless steel ([Supplementary Figure S6](#fig:s6)). Our system used four commercial traps (MassTech, Bremen, Germany). The first two traps were operated at dry ice temperature (-78°C) to remove moisture and some organics. The other two traps were operated at liquid nitrogen temperature (-196°C) to trap CO$_2$. The flow rate during extraction was 55 mL min$^{-1}$, controlled by a mass flow controller (Brooks Instruments, Holland). The reproducibility of the extraction system was 0.030 ‰ for $\delta^{18}$O and 0.007 ‰ for $\delta^{13}$C determined on 14 extractions (1σ standard deviation, [Supplementary Table S1](#bib:13)).

The $\Delta^{13}$O of CO$_2$ was determined using the CO$_2$-O$_2$ exchange method ([Mahata et al., 2013; Barkan et al., 2015; Adnew et al., 2019](#bib:13)). The CO$_2$-O$_2$ exchange system used at Utrecht University is described in ([Adnew et al., 2019](#bib:13)). In short, equal amounts of CO$_2$ and O$_2$ were mixed in a quartz reactor containing a platinum sponge catalyst and heated at 750°C for 2hrs. After isotope equilibration, the CO$_2$ was trapped at liquid nitrogen temperature, while the O$_2$ was collected with 1 pellet of 5Å molecular sieve (1.6 mm, Sigma Aldrich, USA) at liquid nitrogen temperature. The isotopic composition of the isotopeically equilibrated O$_2$ was measured with a Delta+XL isotope ratio mass spectrometer in dual inlet mode with reference to a pure O$_2$ calibration gas that has been assigned values of $\delta^{18}$O = 9.254 ‰ and $\delta^{17}$O = 18.542 ‰ by E. Barkan at the Hebrew University of Jerusalem. The reproducibility of the $\Delta^{13}$O measurement was better than 0.01 ‰ ([Supplementary Table S1](#bib:13)).
2.5. Leaf cuvette model

We used a simple leaf cuvette model to evaluate the dependence of $\Delta_{A}^{17}$O on key parameters. In this model, the leaf is partitioned into three different compartments: the intercellular air space, the mesophyll cell, and the chloroplast. In the leaf cuvette model, we used a 100 ppm down-draw of CO$_2$, similar to the leaf exchange experiments, i.e., the CO$_2$ mole fraction decreases from 800 ppm in the entering air ($c_{a}$) to 400 ppm in the outgoing air ($c_{o}$), which is identical to the air surrounding the leaf ($c_{c}$) as a result of thorough mixing in the cuvette. The assimilation rate is set to 20.0 µmol m$^{-2}$s$^{-1}$. The leaf area and flowrate of air are set to 30 cm$^2$ and 0.7 L min$^{-1}$, respectively. The isotope composition of leaf water at the site where the H$_2$O-CO$_2$ exchange occurs is $\delta^{17}$O = 5.39 ‰ and $\delta^{18}$O = 10.648 ‰, which is the mean of the measured $\delta^{17}$O and $\delta^{18}$O values of bulk leaf water in our experiments. The leaf water temperature is set to 22°C (similar to the experiment). In the model, the $\delta^{18}$O of the CO$_2$ entering the cuvette is set to 30.47 ‰ for all the simulations, as in the normal CO$_2$ experiments, but the assigned $\Delta^{17}$O values ranges from -0.5 ‰ to 0.5 ‰, which encompasses both the stratospheric intrusion and combustion components. The corresponding $\delta^{17}$O of the CO$_2$ entering the cuvette is calculated from the assigned $\Delta^{18}$O value (30.47 ‰) and $\Delta^{17}$O values (-0.5 ‰ to 0.5 ‰). For the calculations with this model, we assumed an infinite boundary layer conductance. The leaf cuvette model is illustrated in the supplementary material (Figure S7) and the detailed code and description is available at https://git.wur.nl/leaf_model/.

3. Results

3.1. Gas exchange parameters

Table 1 summarizes the isotopic composition and mole fraction of the CO$_2$ used in this study for sunflower, ivy, and maize. The $\Delta^{18}$O of CO$_2$ used in this study varies from -0.215 ‰ to 0.44 ‰ while the $\delta^{18}$O value is close to 30 ‰ for all the experiments. For all the experiments, the mole fraction of CO$_2$ entering the leaf ($c_{d}$) is 400 ppm whereas the mole fraction of the CO$_2$ in the intercellular air space ($c_{i}$), at the CO$_2$-H$_2$O exchange site ($c_{m}$), and in the chloroplast ($c_{c}$) varies depending on the assimilation rate and metabolism type of the plants. Estimating the mesophyll conductance is described in the companion paper. A detailed description for estimating $c_{d}$ and $c_{c}$ is provided in the supplementary material. A list of variables and parameters used in this study are summarized in table 3.

3.2. Discrimination against $^{18}$O of CO$_2$

Figure 3a shows discrimination against $^{18}$O associated with photosynthesis ($\Delta_{A}^{18}$O) for sunflower, ivy, and maize as a function of the $c_{m}/c_{d}$ ratio. $\Delta_{A}^{18}$O varies with $c_{m}/c_{d}$, as found in previous studies (Gillon et al., 2014).
and Yakir, 2000a; Barbour et al., 2016). For sunflower, we observe $\Delta_{18}O$ values between 29‰ and 64‰ for $cw/c_o$ between 0.54 and 0.86. Ivy shows a relatively little variation of $\Delta_{18}O$ around a mean of 22‰ for $cw/c_o$ between 0.48 and 0.58. For maize, $\Delta_{18}O$ is lower than for the C$_3$ plants measured in this study, with values between 10‰ and 20‰ for $cw/c_o$ between 0.15 and 0.37.

For sunflower changing the irradiance from 300 µmol m$^{-2}$s$^{-1}$ (low light, hereafter LL) to 1200 µmol m$^{-2}$s$^{-1}$ (high light, hereafter HL) leads to a clear decrease in $\Delta_{18}O$ (average 22‰). For maize, the $\Delta_{18}O$ change is only 4.4‰ on average. For ivy, changing the light intensity does not significantly change the observed $\Delta_{18}O$. The solid lines in Figure 3a show results of leaf cuvette model calculations, where the dependence of $\Delta_{18}O$ on $cw/c_o$ is explored for a set of calculations with otherwise fixed parameters. The model agrees well with the experimental results except for ivy, where the model overestimates the discrimination.

### 3.3. Discrimination against $\Delta^{17}O$ of CO$_2$

The discrimination of photosynthesis against $\Delta^{17}O$ of CO$_2$ ($\Delta_{\Delta^{17}O}$) is shown in Figure 3b. $\Delta_{\Delta^{17}O}$ is negative for all experiments and it depends strongly on the $cw/c_o$ ratio and $\Delta_{\Delta^{17}O}$ increases with $cw/c_o$ ratio. For instance, for $\Delta^{17}O$ of CO$_2$ entering the cuvette of -0.215‰, $\Delta_{\Delta^{17}O}$ is -0.25‰ for maize with $cw/c_o$ ratio of 0.3, -0.3‰ for ivy with $cw/c_o$ ratio of 0.5‰ and -0.5‰ for sunflower with $cw/c_o$ ratio of 0.7 (Figure 3b). For sunflower and ivy, $\Delta_{\Delta^{17}O}$ also shows a strong dependence on the $\Delta^{17}O$ of CO$_2$ supplied to the cuvette, whereas no significant dependence is found for maize. For an increase in $\Delta^{17}O$ of CO$_2$ entering the cuvette from -0.215‰ to 0.435‰, $\Delta_{\Delta^{17}O}$ increases from -0.3‰ to -0.9‰ at $cw/c_o$ ratio of 0.5 for ivy. For sunflower, an increase in $\Delta^{17}O$ of CO$_2$ entering the cuvette from -0.215‰ to 0.31‰ increases $\Delta_{\Delta^{17}O}$ from -0.8‰ to -1.7‰ at $cw/c_o$ ratio of 0.8. The leaf cuvette model results illustrate the shape of the dependence on the $cw/c_o$ ratio and agree well with the experiments. For the leaf cuvette model, the $\Delta^{17}O$ value of the water is assigned a constant value of -0.122‰ (average $\Delta^{17}O$ value for the bulk leaf water).

Figure 4b shows the same values of $\Delta_{\Delta^{17}O}$ as a function of the difference between $\Delta^{17}O$ of CO$_2$ entering the leaf and the calculated $\Delta^{17}O$ of leaf water at the evaporation site where CO$_2$-H$_2$O exchange takes place ($\Delta^{17}O_a - \Delta^{17}O_{w,s}$), for different $cw/c_o$ ratios. The leaf cuvette model results (solid lines in Figure 4b) suggest a linear dependence between $\Delta_{\Delta^{17}O}$ and ($\Delta^{17}O_a - \Delta^{17}O_{w,s}$). The experimental results agree with the hypothesis that $\Delta_{\Delta^{17}O}$ is linearly dependent on $\Delta^{17}O_a - \Delta^{17}O_{w,s}$ at a certain $cw/c_o$ ratio. Figure 4a shows the corresponding relation where $\Delta_{\Delta^{17}O}$ is divided by $\Delta^{17}O_{w,s}$. All the values follow the same relationship as function of the $cw/c_o$ ratio, which can be approximated quite well by an exponential function (equation 5). This function quantifies the dependence of $\Delta_{\Delta^{17}O}$ on $cw/c_o$ and thus the effect of the diffusion of isotopically exchanged CO$_2$ back to the atmosphere, which increases with increasing $cw/c_o$ ratio.

$$\frac{\Delta_{\Delta^{17}O}}{\Delta^{17}O_{a} - \Delta^{17}O_{m}} = -0.150 \times \exp(3.707 \times c_w/c_o) + 0.028$$ (5)
Figure 5a and c show results from the leaf cuvette model that illustrates in more detail how Δ$^{17}$O and Δ$^{13}$O of CO$_2$ that has undergone isotopic equilibration in the mesophyll diffuses back to the atmosphere, and therefore Δ$^{17}$O stays close to the incoming Δ$^{17}$O, modified by the fractionation during CO$_2$ diffusion through the stomata (Figure 5a). Figure 5c confirms that indeed at low $c_m/c_a$, Δ$\lambda$Δ$^{17}$O approaches the fractionation constant expected for diffusion, -0.170 ‰. This diffusional fractionation is independent of the isotopic composition of the CO$_2$ entering the leaf, and therefore at low $c_m/c_a$, the Δ$\lambda$Δ$^{17}$O curves for the different values of the anomaly of the CO$_2$ entering the leaf converge. For a high $c_m/c_a$ ratio, the back-diffusion flux of CO$_2$ that has equilibrated with water becomes the dominant factor, and in this case, the isotopic composition of the outgoing CO$_2$ converges towards this isotope value, independent of the isotopic composition of the incoming CO$_2$ (Figure 5a). This can lead to a very wide range of values for the discrimination against Δ$^{17}$O, because now the effect on Δ$^{17}$O of the ambient CO$_2$ depends strongly on the difference in isotopic composition between incoming CO$_2$ and CO$_2$ in isotopic equilibrium with the leaf water.

In the model calculations shown in Figure 5b and d, the isotopic composition of the water was changed from Δ$^{17}$O$_{wes} = -0.122\%_o$ to 0.300 ‰, whereas all other parameters were kept the same. The value of Δ$^{17}$O for which Δ$\lambda$Δ$^{17}$O does not depend on $c_m/c_a$ is shifted accordingly, again being similar to Δ$^{17}$O$_{wes}$. At low $c_m/c_a$, Δ$\lambda$Δ$^{17}$O converges to the same value as in Figure 5c, confirming the role of diffusion into the stomata as discussed above.

Figure 6 shows how $\delta^{18}$O and Δ$^{17}$O vary in key compartments of the leaf cuvette system that determine the oxygen isotope effects associated with photosynthesis, based on the previously established three-isotope slopes of the various processes (Figure 1). The irrigation water has a Δ$^{17}$O value of 0.017. The measured bulk leaf water is 6-16 ‰ enriched in $^{18}$O and its Δ$^{17}$O value is lower by -0.075 to -0.200 ‰ (mean value -0.121 ‰) than the irrigation water, calculated using a three-isotope slope of λ$_{trans}$ = 0.516 at 80% humidity (Landais et al., 2006, Figure 1). Δ$^{17}$O of leaf water at the evaporation site, calculated from the transpired water, has slightly lower Δ$^{17}$O, with values between -0.119 ‰ and -0.237 (average -0.184 ‰). Note that the bulk leaf water was not measured for all the experiments. For the experiments where the bulk leaf water is measured, Δ$^{17}$O of leaf water at the evaporation site ranges from -0.160 ‰ to -0.231 with an average value of -0.190 ± 0.020 ‰. The calculated isotopic composition of water at the exchange site was thus similar, but slightly lower in Δ$^{17}$O than the values measured for bulk leaf water. CO$_2$ exchanges with the water in the leaf with a well-established fractionation constant (see equation S17, supplementary material) and a three-isotope slope of λ$_{CO2,H2O}$ = 0.5229 (Barkan and Luz, 2012, Figure 1), leading to the lower Δ$^{17}$O values of the equilibrated CO$_2$. In our experiments, the Δ$^{17}$O value of CO$_2$ in equilibrium with leaf water is lower than the Δ$^{17}$O value of CO$_2$ entering the leaf. The Δ$^{17}$O of the CO$_2$ in the intercellular air space is a mixture between two end members, the Δ$^{17}$O of the CO$_2$ entering the leaf and Δ$^{17}$O of the CO$_2$ in equilibration with leaf water. This explains why the observed values of Δ$\lambda$Δ$^{17}$O are negative for the experiments performed in this study.
4.1. Discrimination against $^{18}$O of CO$_2$

The higher $\Delta_{\text{obs}}^{18}$O values for sunflower compared to maize and ivy (Figure 3a) are mainly due to a higher back-diffusion flux ($c_\text{w}/(c_\text{C} - c_\text{m})$). The back-diffusion flux is higher for the C$_4$ plants sunflower and ivy than for the C$_3$ plant maize, a consequence of the lower stomatal conductance and higher assimilation rate of C$_4$ plants (Gillon and Yakir, 2000a; Barbour et al., 2016). In C$_4$ plants most of the CO$_2$ entering the stomata is carboxylated by PEPC resulting in a lower CO$_2$ mixing ratio in the mesophyll which results in a lower back-diffusion flux. The increase of assimilation rate with higher light intensity decreases the $c_\text{w}/c_\text{C}$ ratio and thus leads to a lower back-diffusion flux, which explains the decreases of $\Delta_{\text{obs}}^{18}$O for maize and most clearly for sunflower. A similar trend of increase in $\Delta_{\text{obs}}^{18}$O with an increase in $c_\text{w}/c_\text{C}$ ratio has been reported in previous studies (Gillon and Yakir, 2000b; a;Osborn et al., 2017). For ivy, $\Delta_{\text{obs}}^{18}$O and $\Delta^{17}$O$_\text{obs}$ do not decrease with an increase in irradiance, because the change in assimilation rate with irradiance is small. Thus, $c_\text{w}$ will not decrease strongly and the effect on the back diffusion is smaller than the variability in $\Delta_{\text{obs}}^{18}$O of different leaves of the same plant.

4.2. Discrimination against the $\Delta^{17}$O of CO$_2$

The leaf cuvette model includes the isotope fractionations of all the individual processes that have been quantified in dedicated experiments previously (Figure 1). The good agreement of the model results with the measurements (Figure 3a) demonstrates that when all these processes are combined in the quantitative description of a gas exchange experiment, they actually result in a correct quantification of the isotope effects associated with photosynthesis. This has already been demonstrated before for $\Delta_{\text{obs}}^{18}$O but has now been confirmed for $\Delta_{\text{obs}}^{17}$O.

Unlike ivy and sunflower, maize does not show a significant change in $\Delta^{17}$O when CO$_2$ gases with different $\Delta^{17}$O are supplied to the plant. The C$_4$ plant maize has a small back-diffusion flux due to its high assimilation rate and low stomatal conductance, leading to a low $c_\text{w}/c_\text{C}$ ratio. At low $c_\text{w}/c_\text{C}$ ratios, $\Delta_{\text{obs}}^{17}$O is expected to be close to the weighted fractionation due to diffusion through boundary layer and stomata. In general, the effect of diffusion on $\Delta^{17}$O of atmospheric CO$_2$ can be expressed as follows:
\[ \Delta^{17}O_{\text{Modified}} = \Delta^{17}O_a + (\lambda_{RL} - \lambda_{\text{diffusion}}) \times \ln \alpha_{\text{diffusion}} \] (6)

where \( \Delta^{17}O_a \) is the \( \Delta^{17}O \) of the CO\(_2\) surrounding the leaf, \( \Delta^{17}O_{\text{Modified}} \) is the \( \Delta^{17}O \) of the CO\(_2\) modified due to diffusional fractionation and \( \lambda_{\text{diffusion}} \), \( \lambda_{RL} \) and \( \Delta^{17}O_a \) are the oxygen three-isotope relationships during diffusion from the CO\(_2\)-H\(_2\)O exchange site to the atmosphere, the reference slope used and the fractionation against \( ^{18}O \) for CO\(_2\) during diffusion through the stomata. Using the values \( \lambda_{RL} = 0.528 \), \( \lambda_{\text{diffusion}} = 0.509 \) (Young et al., 2002, Figure 1) and \( \alpha_{\text{diffusion}} = 0.9912 \) (Farquhar and Lloyd, 1993), the effect of diffusional fractionation on the \( \Delta^{17}O \) of atmospheric CO\(_2\) is -0.168 ‰ regardless of the anomaly of the CO\(_2\) entering the leaf, and the model results confirm this at low \( c_{wv}/c_a \) ratio (Figure 5 c and d, inset).

At a high \( c_{wv}/c_a \) ratio, \( \Delta^{17}O_a \) is dominated by the back-diffusion flux of CO\(_2\) that is equilibrated with water. As a consequence, \( \Delta^{17}O_a \) converges to a common value that is independent of the anomaly of the CO\(_2\) entering the cuvette and is determined by the isotopic composition of leaf water. Figure 5 confirms that the end member is equal to the \( \Delta^{17}O \) of CO\(_2\) in equilibrium with leaf water, \( \Delta^{17}O_{\text{m}} \). In fact, when \( \Delta^{17}O_a = \Delta^{17}O_{\text{m}} \), \( \Delta^{17}O_a \) does not change with \( c_{wv}/c_a \), indicating that in this case the \( \Delta^{17}O \) of the CO\(_2\) diffusing back from the leaf is the same as the \( \Delta^{17}O \) of CO\(_2\) entering the leaf.

\( \Delta^{17}O_{\text{m}} \) is the overall discrimination occurring during the diffusion of \( ^{12}C^{18}O^{16}O \) from the ambient air surrounding the leaf to the CO\(_2\)-H\(_2\)O exchange site (see Table 3 for the list of variables). In our study \( \Delta^{17}O_{\text{m}} \) ranges from 5 ‰ to 7.2 ‰, lower than the literature estimate of 7.4 ‰ (Farquhar et al., 1993). \( \Delta^{17}O_{\text{m}} \) depends on the ratio of stomatal conductance, which is associated with a strong fractionation of 8.8 ‰ to mesophyll conductance with an associated fractionation of only 0.8 ‰. Therefore, the higher the ratio \( (g_s/g_m) \) the lower the \( \Delta^{17}O_{\text{m}} \) (Table S2, supplementary material). The difference in \( \Delta^{17}O_{\text{m}} \) of 2.4 ‰ between the literature value of 7.4 ‰ and the lowest \( \Delta^{17}O_{\text{m}} \) estimate in this study will introduce an error of only 0.046 ‰ in the \( \Delta^{17}O \) value (see equation 6). The uncertainty in \( \Delta^{17}O_{\text{m}} \) has lower influence on the \( \Delta^{17}O \) of C\(_3\) plants compared to C\(_4\) plants since the diffusional fractionation is less important at the higher \( c_{wv}/c_a \) ratio where C\(_3\) plants operate.

**4.3. Global average value of \( \Delta \Delta^{17}O \) and \( \Delta^{2}O \) isoflux**

We can use the established relationship between \( \Delta \Delta^{17}O \) and \( \Delta^{17}O_{\text{m}} \) for a certain \( c_{wv}/c_a \) ratio to provide a bottom-up estimate for the global effect of photosynthesis on \( \Delta^{17}O \) in atmospheric CO\(_2\), based on data obtained in real gas exchange experiments. For this, we use results from a recent modeling study, which provides global average values for CO\(_2\) and leaf water (\( \Delta^{17}O \)(CO\(_2\)) = -0.168 ‰, \( \Delta^{17}O \)(H\(_2\)O leaf) = -0.067 ‰; (Koren et al., 2019); Figure S13 and S14, supplementary material). The \( \Delta^{17}O \)(CO\(_2\)) values agree well with the limited amount of available measurements (Table 1).

To extrapolate \( \Delta \Delta^{17}O \) determined in the leaf scale experiments to the global scale, the **global average** \( c_{wv}/c_a \) ratios of 0.7 and 0.3 are used for C\(_3\) and C\(_4\) plants, respectively, similar to previous studies (Hoag et al., 2005; Liang et al., 2017b). From SIBCASA model results we obtained an annual variability of \( c_{wv}/c_a \) values, with a standard deviation of 0.12 and 0.17 for C\(_3\) and C\(_4\) plants respectively (Figure S18, supplementary material).
The δ¹⁸O value of atmospheric CO₂ (21.53 ‰) is calculated from the global δ¹⁸O and Δ¹⁷O values (41.5 ‰ and -0.168 ‰, respectively) (Koren et al., 2019). The δ¹⁸O and δ¹⁷O values of global mean leaf water are calculated from the soil water. A global mean δ¹⁸O value of soil water is -8.4 ‰ assuming soil water to be similar to precipitation (Bowen and Revuahugh, 2003; Koren et al., 2019). The δ¹⁸O value of soil water is -4.4 ‰, calculated using equation (7) (Luz and Barkan, 2010).

\[
\ln(\delta^{18}O_{\text{soil}} + 1) = 0.528 \times \ln(\delta^{18}O_{\text{water}} + 1) + 0.033
\]  

(7)

Δ¹⁷O and δ¹⁸O of leaf water are calculated from δ¹⁸O and δ¹⁸O of soil water with fractionation factors of 1.0043 and 1.0084, respectively (Hofmann et al., 2017; Koren et al., 2019). The fractionation factor for δ¹⁸O is calculated using \( \alpha^{18}O = (\alpha^{18}O)_{\text{trans}} \) with \( \alpha^{18}O_{\text{trans}} = 0.516 \), assuming relative humidity to be 75 ‰ (Lindai et al., 2006). The δ¹⁷O and δ¹⁸O values of global mean leaf water are then -0.136 ‰ and -0.131 ‰, respectively. Thus, the difference between global atmospheric CO₂ and leaf water is δ¹⁷O CO₂ - water = 21.666 ‰ and δ¹⁸O CO₂ - water = 41.631 ‰. This yields Δ¹⁷O CO₂ - water = -101 ‰, and this value is indicated as dashed black line in Figure 4. The grey shaded area indicates the propagated error using the standard deviation of the relevant parameters in 180 x 360 grid boxes for 12 months of leaf water and 45 x 60 grid boxes for 24 months for CO₂ (Koren et al., 2019). In Figure 4b, the intersection between the dashed black vertical line and the discrimination lines for the representative c_mw/c_o ratios of C₃ and C₄ plants correspond to the Δ₃Δ¹⁷O value of C₃ and C₄ plants. For C₃ plants (c_mw/c_o = 0.3) this yields Δ₃Δ¹⁷O = -0.3 ‰ (gray dashed line in Figure 4b) and for C₄ plants (c_mw/c_o = 0.7), Δ₃Δ¹⁷O = -0.65 ‰ (black dashed line in Figure 4b).

Three main factors contribute to the uncertainty of the extrapolated Δ₃Δ¹⁷O value. The first is the measurement error, which contributes 0.25 ‰ (standard error for individual experiments). The second factor is the uncertainty in the difference between Δ¹⁷O of atmospheric CO₂ and leaf water, and we use results from the global model to estimate an error. For Δ¹⁷O of atmospheric CO₂, statistics for all 45 x 60 grid boxes for 24 months (2012-2013) show a range of -0.218 ‰ to -0.151 ‰, with a mean of -0.168 ‰ and a standard deviation of 0.013 ‰ (Figure S13, supplementary material). For Δ¹⁷O of the leaf water statistics for all 180 x 360 grid boxes for 12 months show a range of -0.236 ‰ and -0.027 ‰ (Figure S14, supplementary material). The mean is -0.067 ‰ with a standard deviation of 0.041 ‰. From the combined errors we estimate the error in (Δ¹⁷O CO₂ - Δ¹⁷O leaves) to be 0.043 ‰. The third uncertainty in the extrapolation of Δ¹⁷O comes from the uncertainty in the c_mw/c_o ratio. For C₃ and C₄ plants, these errors are indicated by the light orange and light blue shadings in Figure 4b.
Taking these uncertainties into account leads to a mean value of $\Delta_A \Delta^{17}O = -0.3 \pm 0.18 \%$ for C4 plants and $\Delta_A \Delta^{17}O = -0.65 \pm 0.18 \%$ for C3 plants. The leaf scale discrimination against $^{17}O$ is then extrapolated to global vegetation using these representative values of $\Delta_A \Delta^{17}O$ and the relative fractions of photosynthesis by C4 and C3 plants, respectively as:

$$\Delta_A \Delta^{17}O_{\text{global}} = f_{c4} \times \Delta_A \Delta^{17}O_{c4} + f_{c3} \times \Delta_A \Delta^{17}O_{c3}$$  \hspace{1cm} (8)

where $f_{c4}$ and $f_{c3}$ are the photosynthesis weighted global coverage of C4 and C3 vegetation, $\Delta_A \Delta^{17}O_{c4}$ and $\Delta_A \Delta^{17}O_{c3}$ quantify the discrimination against $^{17}O$ by C4 and C3 plants, which are calculated using estimated values of $c_o / c_a$ from a model. Using assimilation weighted fractions of 23% for C4 and 77% for C3 vegetation (Still et al., 2003), the global mean value of $\Delta_A \Delta^{17}O$ obtained from equation 8 is -0.57$\pm 0.14 \%$.

Isoflux is the product of isotope composition and gross mass flux of the molecule. In the case of assimilation, the net flux $F_A = F_{AL} - F_{A}$ is multiplied with the discrimination associated with assimilation (Ciais et al., 1997a), $F_{AL}$ and $F_{A}$ are total CO2 fluxes from leaf to the atmosphere and from atmosphere to leaf, respectively. The global scale $\Delta^{17}O_{A}$ isoflux is calculated by multiplying the discrimination with the assimilation flux as:

$$F_A \times \Delta_A \Delta^{17}O = A \times (f_{c4} \times \Delta_A \Delta^{17}O_{c4} + f_{c3} \times \Delta_A \Delta^{17}O_{c3})$$  \hspace{1cm} (9)

where, $A = 0.88 \times $GPP is the terrestrial assimilation rate. The factor 0.88 accounts for the fraction of CO2 released due to autotrophic respiration (Ciais et al., 1997a). The $\Delta_A \Delta^{17}O$ isoflux due to photosynthesis is calculated using a GPP value of 120 PgCyr$^{-1}$ (Beer et al., 2010) and $A = 0.88 \times $GPP, resulting in an isoflux of -60$\pm 15 \%$ PgCyr$^{-1}$ globally. This is the first global estimate of $\Delta_A \Delta^{17}O$ based on direct measurements of the discrimination during assimilation. Our value is in good agreement with previous model estimates. Hofmann et al. (2017) estimated an isoflux ranging from -42 to -92 $\%$ PgCyr$^{-1}$ (converted to a reference line with $\lambda = 0.528$) using an average $c_o / c_a$ ratio of 0.7 for both C4 and C3 plants and $\Delta^{17}O$ of -0.147 $\%$ for atmospheric CO2. A model-estimated value from (Hong et al., 2005) is -47 $\%$ PgCyr$^{-1}$ (converted to our reference slope of $\lambda = 0.528$), derived with a more simple model and using $\Delta^{17}O$ of -0.146 $\%$ with $c_o / c_a$ ratio of 0.33 and 0.66 for C4 and C3 plants, respectively.

The main uncertainty in the extrapolation of $\Delta_A \Delta^{17}O$ from the leaf experiments to the global scale is the uncertainty in the $c_o / c_a$ ratio. The error from the uncertainty in $c_o / c_a$ ratio increases when the relative difference in $\Delta^{17}O$ between CO2 and leaf water increases (Figure 5b). It is difficult to determine a single representative $c_m$ value for different plants because this value would need to be properly weighted with temperature, irradiance, CO2 mole fraction and other environmental factors (Flexas et al., 2008;2012;Shrestha et al., 2019). Recent developments in laser spectroscopy techniques (McManus et al., 2005;Nelson et al., 2008;Tuzson et al., 2008;Kammer et al., 2011) might enable more and easier
measurements of \( \frac{c_{w}/c_d}{c_{a}} \) both in the laboratory and under field conditions. This could lead to a better understanding of variations in the \( \frac{c_{w}/c_d}{c_{a}} \) ratio among plant species and, temporally, spatially and environmentally.

5. Conclusions

In order to directly quantify the effect of photosynthetic gas exchange on the \( \Delta^{17}O \) of atmospheric CO\(_2\), gas exchange experiments were carried out in leaf cuvettes using two \( C_3 \) plants (sunflower and ivy) and one \( C_4 \) plant (maize) with isotopically normal and slightly anomalous (\(^17\)O-enriched) CO\(_2\). Results for \( \Delta^{17}O \) agree with results reported in the literature previously. Our results for \( \Delta^{17}O \) confirm that the formalism developed by Farquhar and others for \( \delta^{18}O \) is also applicable to the evaluation of \( \Delta^{17}O \). In particular, our experiments confirm that two parameters determine the effect of photosynthesis on CO\(_2\): 1) the \( \Delta^{17}O \) difference between the incoming CO\(_2\) and CO\(_2\) in equilibrium with leaf water and 2) the \( c_{w}/c_d \) ratio, which determines the degree of back-flux of isotopically exchanged CO\(_2\) from the mesophyll to the atmosphere. At low \( c_{w}/c_d \) ratios, \( \Delta_{\text{leaf}}^{17}O \) is mainly influenced by the diffusional fractionation. Under our experimental conditions, the isotopic effect increased with \( c_{w}/c_d \). e.g. \( \Delta_{\text{leaf}}^{17}O \) was -0.3‰ and -0.65‰ for maize and sunflower with \( c_{w}/c_d \) ratios of 0.3 and 0.7, respectively. However, experiments with mass independently fractionated CO\(_2\) demonstrate that the results depend strongly on the \( \Delta^{17}O \) difference between the incoming CO\(_2\) and CO\(_2\) in equilibrium with leaf water. This is supported by calculations with a leaf cuvette model.

Unlike \( \delta^{18}O \), \( \Delta^{17}O \) variation is much smaller and is better defined since conventional bio-geo-chemical processes that modify \( \delta^{17}O \) and \( \delta^{18}O \) follow a well-defined three-isotope fractionation slope. Consequently, the formulation of CO\(_2\) budget using \( \Delta^{17}O \) is a lot simplified compared to using \( \delta^{18}O \) which is largely affected by kinetic and equilibrium processes between CO\(_2\) and substrate water and also source water isotopic inhomogeneity and dynamics.

Results from the leaf exchange experiments were upscaled to the global atmosphere using modeled values for \( \Delta^{17}O \) of leaf water and CO\(_2\), which results in \( \Delta_{\text{global}}^{17}O = -0.57 \pm 0.14 \)‰ and a value for the \( \Delta^{17}O \) isoflux of -60±15‰ PgCy\(r^{-1}\). This is the first study that provides such an estimate based on direct leaf chamber measurements, and the results agree with previous \( \Delta^{17}O \) calculations. The largest contribution to the uncertainty originates from uncertainty in the \( c_{w}/c_d \) ratio and the largest contributions to the isoflux come from \( C_3 \) plants, which have both a higher share of the total assimilation and higher discrimination. \( \Delta_{\text{plant}}^{17}O \) is less sensitive to \( c_{w}/c_d \) ratios at lower values of \( c_{w}/c_d \), for instance for \( C_4 \) plants, maize.

\( \Delta^{17}O \) of tropospheric CO\(_2\) is controlled by photosynthetic gas exchange, respiration, soil invasion, and stratospheric influx. The stratospheric flux is well established and the effect of photosynthetic gas exchange can now be quantified more precisely. To untangle the contribution of each component to the \( \Delta^{17}O \) atmospheric CO\(_2\) we recommend measuring the effects of foliage respiration and soil invasion both in the laboratory and at the ecosystem scale.
Code and data availability.
The data used in this study are included in the paper either with figures or tables. The python code for the cuvette model is available at https://git.wur.nl/leaf_model/D17O_.

Author contributions.
GAA and TR designed the main idea of the study. GAA and TP designed the leaf cuvette setup. TP monitors plant growth. GAA and TR designed the CO\textsubscript{2} extraction and CO\textsubscript{2}-H\textsubscript{2}O exchange system. GAA conducted all the measurements. GK provided the leaf cuvette model. WP enabled the work within the ASICA project. All authors discussed the results at different steps of the project. GAA and TR prepared the manuscript with contributions from all the co-authors.

Competing interests.
The authors declare that they have no conflict of interest

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Δ¹⁷O ln(δ¹⁸O+1) = 0.529 θ_{diff,trans} = 0.509 θ_{H₂O(v)-H₂O(l)} = 0.529 θ_{H₂O(v)-diff} = 0.518 ln(δ¹⁸O+1) θ_{18O} = 0.522-0.008h θ_{18O} = 0.528 θ_{18O} = 0.5229 θ_{18O} = 0.528 θ_{H₂O} = 0.528 θ_{H₂O} = 0.528

Water vapor from leaf

CO₂ from leaf

Δ¹⁷O (leaf water) θ_{H₂O} = 0.522 θ_{H₂O} = 0.522 θ_{H₂O} = 0.522

Equilibrated water vapor

Leaf water

CO₂ equilibrated with leaf water

Δ¹⁷O (CO₂ equilibrated with leaf water) ε_{18O, H₂O-CO₂} ε_{18O, trans} ε_{18O, Soil water} ε_{18O, Leaf water}
Figure 1 Schematic for mass-dependent isotope fractionation process that affects the Δ\(^{17}\)O of the CO\(_2\) and H\(_2\)O during the photosynthetic gas exchange (not to scale). The triple oxygen isotope relationship for the individual isotope fractionation processes (both kinetic and equilibrium fractionation) are assigned with \(\theta\). \(\theta_{\text{trans}}=0.522-0.008 \times h\), where \(h\) is relative humidity (Landais et al., 2006), in this study the humidity is 75 %, \(\theta_{\text{trans}}=0.516\). \(\theta_{\text{CO}_2\text{-H}_2\text{O}}\) (Barkan and Luz, 2012), \(\theta_{\text{CO}_2\text{-diff}}\) (Young et al., 2002), \(\theta_{\text{H}_2\text{O(v)-H}_2\text{O(l)}}\) (Barkan and Luz, 2005) and \(\theta_{\text{H}_2\text{O(v)}\text{-diff}}\) (Barkan and Luz, 2007). Where \(v\) and \(l\) for vapor and liquid water, respectively; \(\varepsilon^{18}\)O is enrichment or depletion in \(^{18}\)O isotope composition due to the corresponding isotope fractionation process; \(\text{diff}\) and \(\text{trans}\) stand for diffusion and transpiration, respectively.
Figure 2 Schematic diagram of the leaf cuvette experimental setup. IRGA stands for the infrared gas analyzer, WVSS is the water vapor standard source, WVIA is the water vapor isotope analyzer, N-CO$_2$ is normal CO$_2$, E-CO$_2$ is $^{18}$O-enriched CO$_2$. 
Figure 3. a) $\Delta^{18}O_{\text{obs}}$ during photosynthesis for two C$_3$ plants, sunflower (circles) and ivy (triangles) and C$_4$ plant maize (stars) as a function of $c_m/c_a$. The solid lines show results from the leaf cuvette model, where $\delta^{18}$O of the CO$_2$ entering the cuvette is 30.47‰. b) $\Delta A^{17}O$ of CO$_2$ as a function of $c_m/c_a$ for isotonically different CO$_2$ gases entering the cuvette (color bar shows $\Delta^{17}O_e$) for sunflower (circles), ivy (triangles) and maize (stars). $\Delta A^{17}O$ values calculated using the leaf cuvette model are shown as solid lines in corresponding colors ($\Delta^{17}O_e$ values given in the legend). The shaded area indicates the $c_m/c_a$ range for C$_4$ and C$_3$ plants and the vertical dashed lines indicate the mean $c_m/c_a$ ratio used for extrapolating from the leaf scale to the global scale. Solid lines are leaf cuvette model results for the corresponding $c_m/c_a$ ratio.

Figure 3a: $\Delta^{18}O_{\text{obs}}$ vs. $c_m/c_a$ for sunflower (circles), ivy (triangles) and maize (stars).

Figure 3b: $\Delta A^{17}O$ vs. $c_m/c_a$ for sunflower (circles), ivy (triangles) and maize (stars).
**Figure 4** a) Dependency of $\Delta_3\delta^{17}O$ on the relative difference on the $\delta^{17}O$ of CO$_2$ entering the leaf and the $\Delta^{17}O$ of CO$_2$ in equilibrium with leaf water against $c_{mw}/c_{ca}$ ratio. b) Dependency of $\Delta_3\delta^{17}O$ on the difference between the $\Delta^{17}O$ of CO$_2$ entering the cuvette and the $\Delta^{17}O$ of leaf water at the evaporation site color-coded for different $c_{mw}/c_{ca}$ ratios. The solid lines are results of the leaf cuvette model for different $c_{mw}/c_{ca}$ ratios stated in the legend. The dashed vertical black line indicates the difference between the global average $\Delta^{17}O$ value for CO$_2$ (-0.168‰) and leaf water (-0.067‰) (Koren et al., 2019). The gray and yellow horizontal dashed lines indicate $\Delta_3\delta^{17}O$ of C$_4$ and C$_3$ plants for $c_{mw}/c_{ca}$ ratio of 0.3 and 0.7, respectively globally.

**Figure 5** a) and b) $\delta^{17}O$ as a function of $c_{mw}/c_{ca}$ for various values of $\Delta^{17}O$ (see legend) for $\Delta^{17}O_{wes} = -0.122$‰ in a) and $\Delta^{17}O_{wes} = 0.300$‰ in b). c) and d) show the corresponding values for $\Delta_3\delta^{17}O$. $\Delta^{17}O_{global}$ is the global average $\Delta^{17}O$ value for atmospheric CO$_2$ (Koren et al., 2019). When $\Delta^{17}O$ of CO$_2$ entering the cuvette is approximately 0.2‰ lower than the $\Delta^{17}O$ of leaf water at the CO$_2$-H$_2$O exchange site, $\Delta^{17}O$ of the CO$_2$ leaving the cuvette does not change when $c_{mw}/c_{ca}$ vary.
Figure 6. Isotopic composition of various relevant oxygen reservoirs that affect the $\Delta^{17}O$ of atmospheric CO$_2$ during photosynthesis: irrigation water (grey triangle), calculated leaf water at the evaporation site (brown circles), measured bulk leaf water (brown star), CO$_2$ entering the cuvette (black circles), CO$_2$ leaving the leaf cuvette (green circles), CO$_2$ equilibrated with leaf water at the evaporation site (blue circles), CO$_2$ equilibrated with bulk leaf water (blue stars). $\Delta^{17}O$ is calculated with $\lambda=0.528$. 

$\lambda_{RL} = 0.528$
Table 1: Summary of gas exchange parameters and isotopic composition of maize, sunflower and ivy. Mole fraction at the site of exchange (\(c_m\)) is calculated assuming complete isotopic equilibrium with the water at the CO\(_2\)-H\(_2\)O exchange site. The water at the CO\(_2\)-H\(_2\)O exchange site is assumed the same as the isotopic composition at the site of evaporation. Number in the parenthesis are the standard deviation of the mean (1\(\sigma\)).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sunflower</th>
<th>Ivy</th>
<th>Maize</th>
<th>Irradiance ((\mu)mol m(^{-2}) s(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A_n)</td>
<td>(\mu)mol mol(^{-1}) m(^{-2})s(^{-1})</td>
<td>18(0.7)</td>
<td>12(0.7)</td>
<td>17(2)</td>
</tr>
<tr>
<td>(g_s)</td>
<td>mol m(^{-2})s(^{-1})</td>
<td>0.45(0.14)</td>
<td>0.11(0.02)</td>
<td>0.08(0.01)</td>
</tr>
<tr>
<td>(\delta^{18}O_e) (%)</td>
<td></td>
<td>27.26 to 31.80</td>
<td>28.28 to 30.48</td>
<td>27.26 to 30.48</td>
</tr>
<tr>
<td>(\Lambda^{17}O_e) (%)</td>
<td></td>
<td>-0.227 to 0.409</td>
<td>-0.215 to 0.435</td>
<td>-0.215 to 0.310</td>
</tr>
<tr>
<td>(\delta^{18}O_a) (%)</td>
<td></td>
<td>33.25 to 43.87</td>
<td>32.64 to 35.86</td>
<td>34.04 to 29.764</td>
</tr>
<tr>
<td>(\Lambda^{17}O_a) (%)</td>
<td></td>
<td>-0.333 to 0.163</td>
<td>-0.276 to 0.327</td>
<td>-0.270 to 0.296</td>
</tr>
<tr>
<td>(\Delta^{18}O_{obs}) (%)</td>
<td></td>
<td>57.12(4.70)</td>
<td>22.20(1.32)</td>
<td>17.23(1.32)</td>
</tr>
<tr>
<td>(\Delta\Lambda^{17}O_{obs}) (%)</td>
<td></td>
<td>-2.61 to -0.43</td>
<td>-1.03 to -0.19</td>
<td>-0.36 to -0.09</td>
</tr>
<tr>
<td>(\delta^{18}O_o) (%)</td>
<td></td>
<td>52.02(1.24)</td>
<td>47.17(1.17)</td>
<td>52.62(0.52)</td>
</tr>
<tr>
<td>(\Lambda^{17}O_{o}) (%)</td>
<td></td>
<td>-0.41(0.001)</td>
<td>-0.35(0.001)</td>
<td>-0.40(0.001)</td>
</tr>
<tr>
<td>(c_a) ppm</td>
<td></td>
<td>402 (3)</td>
<td>403 (3)</td>
<td>403 (3)</td>
</tr>
<tr>
<td>(c_i) ppm</td>
<td></td>
<td>357(10)</td>
<td>284(0.1)</td>
<td>194(20)</td>
</tr>
<tr>
<td>(c_c) ppm</td>
<td></td>
<td>277(15)</td>
<td>188(30)</td>
<td>300</td>
</tr>
<tr>
<td>(c_m) ppm</td>
<td></td>
<td>320(10)</td>
<td>229(10)</td>
<td>134(15)</td>
</tr>
</tbody>
</table>
Table 2: Summary of the parameters used for the extrapolation of leaf scale experiments to the global scale and the results obtained. Bottom part: overview of available Δ¹⁷O measurements.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPP</td>
<td>120 PgCyr⁻¹</td>
<td>(Beer et al., 2010)</td>
</tr>
<tr>
<td>fC₄</td>
<td>23 %</td>
<td>(Still et al., 2003)</td>
</tr>
<tr>
<td>fC₃</td>
<td>77 %</td>
<td>(Still et al., 2003)</td>
</tr>
<tr>
<td>c₃₀/c₃₃ (C₃)</td>
<td>0.7</td>
<td>(Hoag et al., 2005)</td>
</tr>
<tr>
<td>c₃₀/c₃₃ (C₄)</td>
<td>0.3</td>
<td>(Hoag et al., 2005)</td>
</tr>
<tr>
<td>Δ¹⁷O leaf water (global mean, modelled)</td>
<td>-0.067±0.04 ‰</td>
<td>(Koren et al., 2019)</td>
</tr>
<tr>
<td>Δ¹⁷O CO₂ (global mean, modelled)</td>
<td>-0.168±0.013 ‰</td>
<td>(Koren et al., 2019)</td>
</tr>
<tr>
<td>ΔΔ¹⁷O (global mean for C₄)</td>
<td>-0.3±0.18 ‰</td>
<td>(Figure 5b, for c₃₀/c₃₃ ratio of 0.3)</td>
</tr>
<tr>
<td>ΔΔ¹⁷O (global mean for C₃)</td>
<td>-0.65±0.18 ‰</td>
<td>(Figure 5b, for c₃₀/c₃₃ ratio of 0.7)</td>
</tr>
<tr>
<td>ΔΔ¹⁷O-isoflux (global mean for C₄)</td>
<td>-7.3±4 ‰PgCyr⁻¹</td>
<td>(Equation 14, only for C₄)</td>
</tr>
<tr>
<td>ΔΔ¹⁷O-isoflux (global mean for C₃)</td>
<td>-5.3±15 ‰PgCyr⁻¹</td>
<td>(Equation 14, only for C₃)</td>
</tr>
<tr>
<td>ΔΔ¹⁷O-isoflux (global mean for whole vegetation)</td>
<td>-60±15 ‰PgCyr⁻¹</td>
<td>(equation 14)</td>
</tr>
<tr>
<td>ΔΔ¹⁷O-isoflux (global mean for whole vegetation)</td>
<td>-47 ‰PgCyr⁻¹</td>
<td>(Hoag et al., 2005)</td>
</tr>
<tr>
<td>ΔΔ¹⁷O-isoflux (global mean for whole vegetation)</td>
<td>-42 to -92 ‰PgCyr⁻¹</td>
<td>(Hofmann et al., 2017)</td>
</tr>
</tbody>
</table>

Δ¹⁷O value of tropospheric CO₂

- For CO₂ samples collected in La Jolla-UCSD (California, USA) (1990 to 2000): -0.173±0.046 ‰ | (Thiemens et al., 2014) |
- For CO₂ samples collected in Israel: 0.034±0.010 ‰ | (Barkan and Luz, 2012) |
- For CO₂ samples collected in South China sea (2013-2014): -0.159±0.084 ‰ | (Li et al., 2017a; Li et al., 2017b) |
- For CO₂ samples collected in Taiwan (2012-2015): -0.156±0.080 ‰ | (Li et al., 2017a; Li et al., 2017b) |
- For CO₂ samples collected in California (USA) (2015): -0.177±0.029 ‰ | (Li et al., 2017a; Li et al., 2017b) |
- For CO₂ samples collected in Göttingen (Germany) (2010-2012): -0.122±0.065 ‰ | (Hofmann et al., 2017) |
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
<th>Unit/calculation/value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$A_{\text{CO}_2}$</td>
<td>Rate of CO$_2$ assimilation</td>
<td>$\frac{\mu g}{m^2\cdot s}$</td>
</tr>
<tr>
<td>$T$</td>
<td>Transpiration rate</td>
<td>$\frac{\mu g}{m^2\cdot s}$</td>
</tr>
<tr>
<td>$c_w$</td>
<td>Mole fraction of water vapour inside leaf</td>
<td>$p_{\text{atm}} \frac{(1/\text{saturated})}{(1/mol\cdot s)}$</td>
</tr>
<tr>
<td>$c_v$</td>
<td>Mole fraction of water vapour leaving the cuvette, leaf surrounding</td>
<td>$\text{WVIA (IRGA, mol mol)}^2$</td>
</tr>
<tr>
<td>$c_e$</td>
<td>Mole fraction of water vapour entering the cuvette</td>
<td>$\text{WVIA/IRGA, mol mol}^2$</td>
</tr>
<tr>
<td>$c_{\text{CO}_2}$</td>
<td>Mole fraction of CO$_2$ entering the cuvette</td>
<td>$\text{IRGA, mol mol}^2$</td>
</tr>
<tr>
<td>$c_{\text{CO}_2}$</td>
<td>Mole fraction of CO$_2$ in the leaf surrounding/leaving the cuvette</td>
<td>$\text{IRGA, mol mol}^2$</td>
</tr>
<tr>
<td>$F_e$</td>
<td>Flow rate of air entering the cuvette</td>
<td>mol s$^{-1}$</td>
</tr>
<tr>
<td>$A_{\text{leaf}}$</td>
<td>Surface area of the leaf inside the cuvette</td>
<td>m$^2$</td>
</tr>
<tr>
<td>$P$</td>
<td>Atmospheric pressure</td>
<td>bar</td>
</tr>
<tr>
<td>$T_{\text{leaf}}$</td>
<td>Leaf temperature</td>
<td>°C</td>
</tr>
<tr>
<td>$g_{\text{mm}}$</td>
<td>Stomatal conductance for water vapour</td>
<td>$\frac{\text{mol m}^{-1} \cdot \text{mol m}^{-1} \cdot \text{s}^{-1}}{\text{mol m}^{-1} \cdot \text{mol m}^{-1} \cdot \text{s}^{-1}}$</td>
</tr>
<tr>
<td>$g_{\text{lw}}$</td>
<td>Boundary layer conductance for water vapour</td>
<td>Calibrated for the cuvette we used</td>
</tr>
<tr>
<td>$E_{\text{lw}}$</td>
<td>Conductance for water vapor through the boundary layer and stomata</td>
<td>$\text{mol m}^{-1} \cdot \text{s}^{-1}$</td>
</tr>
<tr>
<td>$g_{\text{st}}$</td>
<td>Stomatal conductance for CO$_2$</td>
<td>$\text{mol m}^{-1} \cdot \text{s}^{-1}$</td>
</tr>
<tr>
<td>$g_{b}$</td>
<td>Boundary conductance for CO$_2$</td>
<td>$\text{mol m}^{-1} \cdot \text{s}^{-1}$</td>
</tr>
<tr>
<td>$g_{\text{co}}$</td>
<td>Conductance for CO$_2$ through the boundary layer and domain</td>
<td>$\text{mol m}^{-1} \cdot \text{s}^{-1}$</td>
</tr>
<tr>
<td>$E_{\text{co}}$</td>
<td>CO$_2$ compensation point</td>
<td>$\text{mol m}^{-1} \cdot \text{s}^{-1}$</td>
</tr>
<tr>
<td>$g_{\text{ai}}$</td>
<td>CO$<em>2$ conductance from intercellular air space to the site of carboxylation calculated using $\Delta</em>{\text{le}}$ C (for C$_3$ plants only)</td>
<td>$\text{mol m}^{-1} \cdot \text{s}^{-1} \cdot \text{bar}^{-1}$</td>
</tr>
<tr>
<td>$g_{\text{ai}}$</td>
<td>CO$_2$ conductance from intercellular air space to CO$_2$ H$<em>2$O exchange site calculated using $\Delta</em>{\text{co}}$ C$_2$</td>
<td>$\text{mol m}^{-1} \cdot \text{s}^{-1} \cdot \text{bar}^{-1}$</td>
</tr>
<tr>
<td>$g_{\text{ai}}$</td>
<td>CO$_2$ conductance from intercellular air space to CO$_2$ H$<em>2$O exchange site calculated using $\Delta</em>{\text{co}}$ C$_2$</td>
<td>$\text{mol m}^{-1} \cdot \text{s}^{-1} \cdot \text{bar}^{-1}$</td>
</tr>
<tr>
<td>$g_{\text{ai}}$</td>
<td>CO$_2$ conductance from intercellular air space to CO$_2$ H$<em>2$O exchange site calculated using $\Delta</em>{\text{co}}$ C$_2$</td>
<td>$\text{mol m}^{-1} \cdot \text{s}^{-1} \cdot \text{bar}^{-1}$</td>
</tr>
<tr>
<td>$c_{\text{CO}_2}$</td>
<td>Mole fraction of CO$_2$ in the intercellular air space</td>
<td>$\frac{(\text{mol m}^{-1} \cdot \text{mol m}^{-1})}{(\text{mol m}^{-1} \cdot \text{mol m}^{-1})}$</td>
</tr>
<tr>
<td>$c_{\text{CO}_2}$</td>
<td>Mole fraction of CO$_2$ at the leaf surface</td>
<td>$\frac{\mu g}{\mu g}$</td>
</tr>
<tr>
<td>$c_{\text{CO}_2}$</td>
<td>Mole fraction of CO$_2$ at the site of CO$_2$ H$_2$O exchange</td>
<td>mol mol$^{-1}$</td>
</tr>
<tr>
<td>$c_{\text{CO}_2}$</td>
<td>Mesophyll conductance to the chloroplast (for C$_3$ plants)</td>
<td>$\frac{\mu g}{\mu g}$</td>
</tr>
<tr>
<td>$E_{\text{co}}$</td>
<td>Ternary correction for $^{13}$CO$_2$</td>
<td>$\frac{\mu g}{\mu g}$</td>
</tr>
<tr>
<td>$E_{\text{co}}$</td>
<td>Ternary correction for C$_3$/C$_4$</td>
<td>$\frac{\mu g}{\mu g}$</td>
</tr>
<tr>
<td>$T_{\text{co}}$</td>
<td>Ternary correction for C$_3$/C$_4$</td>
<td>$\frac{\mu g}{\mu g}$</td>
</tr>
<tr>
<td>Symbol</td>
<td>Description</td>
<td>Formula</td>
</tr>
<tr>
<td>--------</td>
<td>-------------</td>
<td>---------</td>
</tr>
<tr>
<td>$R_D$</td>
<td>Dark respiration rate</td>
<td>$0.8 \text{ mmol m}^{-2} \text{ s}^{-1}$</td>
</tr>
<tr>
<td>$R_N$</td>
<td>Day respiration rate</td>
<td>$0.8 \text{ mmol m}^{-2} \text{ s}^{-1}$</td>
</tr>
</tbody>
</table>

### Oxygen and carbon isotope effects

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\delta^{18}O$</td>
<td>Kinetic fractionation of water vapour in air</td>
<td>$\frac{278 + 1918}{\theta_0 + R_N} \times 10^3$</td>
</tr>
<tr>
<td>$\delta^{18}C_1$</td>
<td>Equilibrium fractionation between liquid and gas phase of water vapor</td>
<td>$2.644 - 3.206 \left( \frac{\theta_0}{\theta_r} \right) + 1.534 \left( \frac{\theta_0}{\theta_r} \right)^2$</td>
</tr>
<tr>
<td>$\delta^{18}C_2$</td>
<td>Weighted fractionation for $^{18}CO$ as $CO_2$ diffuses through the boundary layer and stomata</td>
<td>$\frac{\theta_0 + R_N}{\theta_0 - \theta_r}$</td>
</tr>
<tr>
<td>$\delta^{18}C_3$</td>
<td>Weighted fractionation for $^{18}CO$ as $CO_2$ diffuses through the boundary layer and stomata</td>
<td>$\frac{\theta_0 + R_N}{\theta_0 - \theta_r}$</td>
</tr>
<tr>
<td>$\delta^{18}C_4$</td>
<td>Weighted fractionation for $^{18}CO$ as $CO_2$ diffuses through the boundary layer and stomata</td>
<td>$\frac{\theta_0 + R_N}{\theta_0 - \theta_r}$</td>
</tr>
<tr>
<td>$\delta^{18}C_5$</td>
<td>Weighted fractionation for $^{18}CO$ as $CO_2$ diffuses through the boundary layer and stomata</td>
<td>$\frac{\theta_0 + R_N}{\theta_0 - \theta_r}$</td>
</tr>
<tr>
<td>$\delta^{18}C_6$</td>
<td>Weighted fractionation for $^{18}CO$ as $CO_2$ diffuses through the boundary layer and stomata</td>
<td>$\frac{\theta_0 + R_N}{\theta_0 - \theta_r}$</td>
</tr>
<tr>
<td>$\delta^{18}C_7$</td>
<td>Weighted fractionation of $^{18}CO$ as it diffuses through the boundary layer, stomata and liquid phase in series</td>
<td>$\frac{\theta_0 + R_N}{\theta_0 - \theta_r}$</td>
</tr>
<tr>
<td>$\delta^{18}C_8$</td>
<td>Weighted fractionation of $^{18}CO$ as it diffuses through the boundary layer, stomata and liquid phase in series</td>
<td>$\frac{\theta_0 + R_N}{\theta_0 - \theta_r}$</td>
</tr>
<tr>
<td>$\delta^{18}C_9$</td>
<td>Fractionation of $^{18}CO$ as $CO_2$ diffuses through the boundary layer</td>
<td>$2.9%$</td>
</tr>
<tr>
<td>$\delta^{18}C_{10}$</td>
<td>Fractionation of $^{18}CO$ as $CO_2$ diffuses through the stomata</td>
<td>$4.4%$</td>
</tr>
<tr>
<td>$\delta^{18}C_{11}$</td>
<td>Fractionation factor for dissolution and diffusion through water</td>
<td>$1.8%$</td>
</tr>
<tr>
<td>$\delta^{18}C_{12}$</td>
<td>Fractionation factor for photorespiration</td>
<td>$10.4%$</td>
</tr>
<tr>
<td>$\delta^{18}C_{13}$</td>
<td>Fractionation factor for day respiration</td>
<td>$R_D + \delta^{18}C_{12}$</td>
</tr>
<tr>
<td>$\delta^{18}C_{14}$</td>
<td>Fractionation factor for uptake by Rubisco</td>
<td>$29.5%$</td>
</tr>
<tr>
<td>$\delta^{18}C_{15}$</td>
<td>Fractionation due to photorespiration (decarboxylation of glycine)</td>
<td>$1%$</td>
</tr>
<tr>
<td>$\delta^{18}C_{16}$</td>
<td>Fractionation due to day respiration</td>
<td>$1.7%$</td>
</tr>
<tr>
<td>$\delta^{18}C_{17}$</td>
<td>Fractionation due to uptake by Rubisco</td>
<td>$0.1%$</td>
</tr>
<tr>
<td>$\delta^{18}C_{18}$</td>
<td>Fractionation in $^{18}CO$ as $CO_2$ diffuses through the boundary layer</td>
<td>$2.9%$</td>
</tr>
<tr>
<td>$\delta^{18}C_{19}$</td>
<td>Fractionation in $^{18}CO$ as $CO_2$ diffuses through the stomata</td>
<td>$4.4%$</td>
</tr>
<tr>
<td>$\delta^{18}C_{20}$</td>
<td>Fractionation of $^{18}CO$ as $CO_2$ diffuses through the stomata</td>
<td>$12.9%$</td>
</tr>
<tr>
<td>$\delta^{18}C_{21}$</td>
<td>Fractionation in $^{18}CO$ as $CO_2$ diffuses through the boundary layer</td>
<td>$5.8%$</td>
</tr>
<tr>
<td>$\delta^{18}C_{22}$</td>
<td>Fractionation in $^{18}CO$ as $CO_2$ diffuses through the boundary layer</td>
<td>$5.8%$</td>
</tr>
<tr>
<td>$\delta^{18}C_{23}$</td>
<td>Fractionation in $^{18}CO$ as $CO_2$ diffuses through the boundary layer</td>
<td>$5.8%$</td>
</tr>
<tr>
<td>$\delta^{18}C_{24}$</td>
<td>Fractionation in $^{18}CO$ as $CO_2$ diffuses through the boundary layer</td>
<td>$5.8%$</td>
</tr>
<tr>
<td>$\delta^{18}C_{25}$</td>
<td>Fractionation in $^{18}CO$ as $CO_2$ diffuses through the boundary layer</td>
<td>$5.8%$</td>
</tr>
<tr>
<td>$\delta^{18}C_{26}$</td>
<td>Fractionation in $^{18}CO$ as $CO_2$ diffuses through the boundary layer</td>
<td>$5.8%$</td>
</tr>
<tr>
<td>$\delta^{18}C_{27}$</td>
<td>Fractionation in $^{18}CO$ as $CO_2$ diffuses through the boundary layer</td>
<td>$5.8%$</td>
</tr>
<tr>
<td>$\delta^{18}C_{28}$</td>
<td>Fractionation in $^{18}CO$ as $CO_2$ diffuses through the boundary layer</td>
<td>$5.8%$</td>
</tr>
<tr>
<td>$\delta^{18}C_{29}$</td>
<td>Fractionation in $^{18}CO$ as $CO_2$ diffuses through the boundary layer</td>
<td>$5.8%$</td>
</tr>
<tr>
<td>$\delta^{18}C_{30}$</td>
<td>Fractionation in $^{18}CO$ as $CO_2$ diffuses through the boundary layer</td>
<td>$5.8%$</td>
</tr>
<tr>
<td>$\delta^{18}C_{31}$</td>
<td>Fractionation in $^{18}CO$ as $CO_2$ diffuses through the boundary layer</td>
<td>$5.8%$</td>
</tr>
<tr>
<td>$\delta^{18}C_{32}$</td>
<td>Fractionation in $^{18}CO$ as $CO_2$ diffuses through the boundary layer</td>
<td>$5.8%$</td>
</tr>
<tr>
<td>$\delta^{18}C_{33}$</td>
<td>Equilibrium fractionation of $CO$ and water for $^{18}CO$</td>
<td>$\frac{\theta_0}{\theta_r} - 17.93 \times 10^3$</td>
</tr>
<tr>
<td>$\delta^{18}C_{34}$</td>
<td>Kinetic fractionation of water vapor in air</td>
<td>$2 \times g_0 + 19 \times g_i$</td>
</tr>
<tr>
<td>$\delta^{18}C_{35}$</td>
<td>Equilibrium fractionation between liquid and gas phase water</td>
<td>$2.644 - 3.206 \left( \frac{\theta_0}{\theta_r} \right) + 1.534 \left( \frac{\theta_0}{\theta_r} \right)^2$</td>
</tr>
<tr>
<td>Equation</td>
<td>Description</td>
<td></td>
</tr>
<tr>
<td>----------</td>
<td>-------------</td>
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</tr>
<tr>
<td>$\delta^18O_{i}$</td>
<td>$\delta^18O$ of the assimilated CO$_2$</td>
<td></td>
</tr>
<tr>
<td>$\delta^18O_{a}$</td>
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<td></td>
</tr>
<tr>
<td>$\delta^18O_{r}$</td>
<td>$\delta^18O$ of CO$_2$ in the intercellular air space ignoring random correction</td>
<td></td>
</tr>
<tr>
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<tr>
<td>$\delta^18O_{r}$</td>
<td>$\delta^18O$ of transpirated water vapour</td>
<td></td>
</tr>
<tr>
<td>$\delta^18O_{w}$</td>
<td>$\delta^18O$ of water at the evaporation site</td>
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</tr>
<tr>
<td>$\delta^{13}C$</td>
<td>$\delta^{13}C$ of CO$_2$ at the site of CO$_2$-H$_2$O exchange</td>
<td></td>
</tr>
<tr>
<td>$\delta^{13}C_{\text{c}}$</td>
<td>$\delta^{13}C$ of CO$_2$ at the site of CO$_2$-H$_2$O exchange</td>
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<tr>
<td>$\delta^{13}C_{\text{c}}$</td>
<td>Isotopic composition ratio of substrate used for dark respiration</td>
<td></td>
</tr>
<tr>
<td>$\Delta^18O_{i}$</td>
<td>$\Delta^18O$-photosynthetic discrimination</td>
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<td>$\Delta^18O$-photosynthetic discrimination (Farquhar model)</td>
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<td>$\Delta^18O$-photosynthetic discrimination (assuming no mesophyll conductance, i.e. e=q)</td>
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<td>$\delta^{13}C_{\text{c}}$</td>
<td>$\delta^{13}C$ of CO$_2$ entering the cuvette</td>
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<tr>
<td>$\delta^{13}C_{\text{c}}$</td>
<td>$\delta^{13}C$ of CO$_2$ leaving the cuvette</td>
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</tr>
<tr>
<td>$\delta^{18}O_{\text{ev}}$</td>
<td>$\delta^{18}O$ of CO$_2$ equilibrated with the leaf water at the evaporating site relative to the CO$_2$ leaving the cuvette</td>
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<tr>
<td>$\delta^{18}O_{\text{w}}$</td>
<td>$\delta^{18}O$ of water vapour entering the cuvette</td>
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</tr>
<tr>
<td>$\delta^{18}O_{\text{w}}$</td>
<td>$\delta^{18}O$ of water vapour leaving the cuvette/leaf surrounding</td>
<td></td>
</tr>
</tbody>
</table>