

From: Getachew Agmuas Adnew and co-authors

To: - Dear Dr. [Aninda Mazumdar](#)

5 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.

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Referee: 1

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

15 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
20 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 $\Delta^{17}\text{O}$, we need to know the *precise* effect of photosynthesis and respiration on $\Delta^{17}\text{O}$, in the

25 words of the referee, how does the $\Delta^{17}\text{O}$ signature actually look after being "erased" by exchange with the biosphere.

30 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.

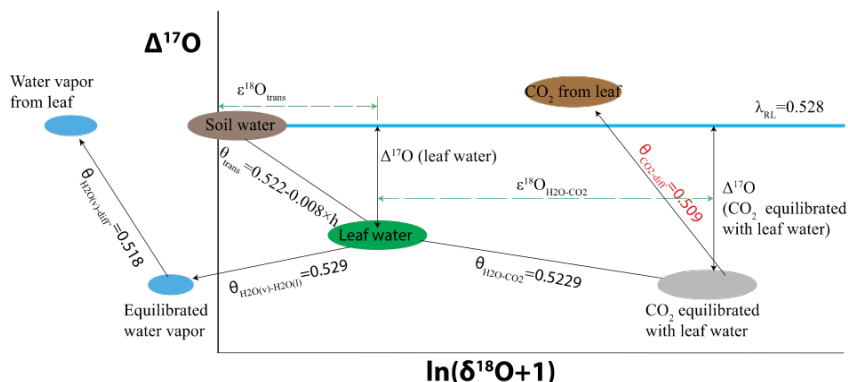
The referee is correct that in principle ^{18}O indeed cycles through the same biological system, and undergoes the same (bio)physical processes. However, we would like to nuance the idea that $\delta^{18}\text{O}$ can help us FULLY understand ALL processes of interest. This is because conventional $\delta^{18}\text{O}$ studies have a
35 number of distinct disadvantages. Notably, the $\delta^{18}\text{O}$ -signature of all water pools in the system must be known to use $\delta^{18}\text{O}$ as a carbon cycle tracer. In addition, significant changes in $\delta^{18}\text{O}$ can occur due to processes that are not of primary interest to understanding GPP, e.g., leaf evaporation, or soil equilibration.

40 $\Delta^{17}\text{O}$ 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 $\delta^{17}\text{O}$ and $\delta^{18}\text{O}$ follow a well-recognized isotope fractionation slope. In earlier studies many assumptions had to be made, because the effect on $\Delta^{17}\text{O}$ had never been quantified precisely. This is now accomplished through our study.

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

The reviewer correctly identifies that the small variations in λ values can impact the $\Delta^{17}\text{O}$ 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 $\Delta^{17}\text{O}$ signals.

50 In addition, we also include for the first-time experiments and model studies that involve artificially ^{17}O labeled CO_2 . We demonstrate how the resulting differences in $\Delta^{17}\text{O}$ between CO_2 and leaf water affect the results, and that experiments with ^{17}O labeled CO_2 actually increase the signal to (measurement) noise ratio.



60 Figure 1. Schematic representation of the processes that affect the $\Delta^{17}\text{O}$ of CO_2 and H_2O 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 CO_2 - H_2O exchange is $\theta_{\text{CO}_2\text{-H}_2\text{O}} = 0.5229$ (Barkan and Luz, 2012). The triple isotope slope for diffusion of CO_2 is $\theta_{\text{CO}_2\text{-diff}} = 0.509$ (Young et al., 2002) and for diffusion of water vapor $\theta_{\text{H}_2\text{O}(v)\text{-diff}} = 0.518$ (Barkan and Luz, 2007). The three isotope slope for equilibration between liquid (l) and gaseous (v) water is $\theta_{\text{H}_2\text{O}(v)\text{-H}_2\text{O}(l)} = 0.529$ (Barkan and Luz, 2005). $\epsilon^{18}\text{O}$ is the enrichment or depletion in ^{18}O due to the corresponding isotope fractionation process.

70 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 from ^{18}O studies and, in fact, much of the calculations here still depends on the ^{18}O measurements.

75 We appreciate that the referee acknowledges the considerable analytical effort that was made to produce our results. As mentioned above, we think that $\delta^{18}\text{O}$ 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 $\Delta^{17}\text{O}$ has been repeatedly suggested and already used as independent and potentially even superior tracer.

80 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 $\Delta^{17}\text{O}$ 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.

85 Specifically, the results communicated in our manuscript

- 1) demonstrate that the established theory is applicable to $\Delta^{17}\text{O}$ - CO_2 exchange at leaf-level.
- 2) experimentally quantify for the first time the effect of photosynthesis on $\Delta^{17}\text{O}$ of atmospheric CO_2
- 3) quantify the dependence of this effect on critical parameters
- 90 4) provide an independent bottom-up $\Delta^{17}\text{O}$ -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).

95 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 $\Delta^{17}\text{O}$ of atmospheric CO_2 . The relative difference between $\Delta^{17}\text{O}$ 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_m/C_a ratio”. Isn’t it that these ‘basic principles’ of leaf gas exchange are already fairly well known from previous CO_2 and the

100 ^{18}O studies?

We clearly acknowledge in our paper that the processes affecting $\delta^{18}\text{O}$ and $\Delta^{17}\text{O}$ 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 $\Delta^{17}\text{O}$ is actually demonstrated.

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It seems also that the notion of “discrimination against $\Delta^{17}\text{O}$ of atmospheric CO_2 ” is not clear. If this is confused with D in leaf photosynthesis as for D_{18} , then again ^{17}O is predictable and has no clear additional information (other than perhaps the reflection of the possible variations in the λ factor). The final estimate of global ^{17}O 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 $\delta^{17}\text{O}$ and $\Delta^{17}\text{O}$. What the referee calls “ Δ_A in leaf photosynthesis as for $\Delta_A^{18}\text{O}$ ” would be $\Delta_A^{17}\text{O}$. This was also shown in our original paper for consistency, but does indeed not provide additional information. Only the combination of $\Delta_A^{18}\text{O}$ and $\Delta_A^{17}\text{O}$ to $\Delta_A\Delta^{17}\text{O}$ provides the independent information. In the revised manuscript, we only present the results for $\Delta_A^{18}\text{O}$ and $\Delta_A\Delta^{17}\text{O}$. 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 $\delta^{17}\text{O}$ and $\delta^{18}\text{O}$ values, but only on $\Delta_A\Delta^{17}\text{O}$. 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 $\Delta^{17}\text{O}$ of CO_2 and leaf water from a recent 3D global $\Delta^{17}\text{O}$ 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 $\Delta^{17}\text{O}$ - CO_2 exchange at leaf-level
- 2) experimentally quantify for the first time the effect of photosynthesis on $\Delta^{17}\text{O}$ of atmospheric CO_2
- 3) study of the dependence of this effect on critical parameters
- 4) provide an independent bottom-up $\Delta^{17}\text{O}$ -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 CO_2 ($\Delta^{17}\text{OCO}_2$) had been regarded as spatiotemporally constant in the troposphere because of its short residence time (e.g., Luz et al., 2000). Recently, significant

145 seasonal and temporal variations of $\Delta^{17}\text{O}\text{CO}_2$ 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 CO_2 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 CO_2 budget. The next step, therefore, must be the process study involving oxygen
150 isotope fractionations in association with individual CO_2 fluxes.

This study by Adnew, Pons, Koren, Peters, Röckmann, aims to quantify the $\Delta^{17}\text{O}\text{CO}_2$ change during
photosynthetic CO_2 removal from the atmosphere, caused by tiny difference of 17O-18O relationship
between kinetic and equilibrium isotope fractionations inside the leaf.

155 To my knowledge, this is the first experimental study for $\Delta^{17}\text{O}\text{CO}_2$ 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
160 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
165 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
170 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.

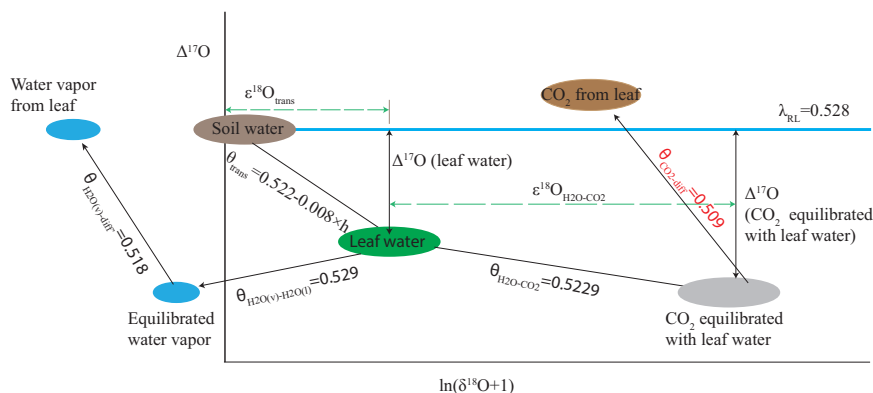
- 175 - The introduction, from line 91 to 106 was shortened and combined with the previous paragraph
- We shortened the materials and methods section and moved part of it to the supplementary material
- Section 2.1 was shortened based on the recommendation of the referee
- We shortened the theory part, 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

- 180 - 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
185 what the problem or limitation exists currently. Until this block (and perhaps in previous studies), you
mentioned the $\Delta^{17}\text{O}$ is free from any terrestrial MDF processes and made readers believe that $\Delta^{17}\text{O}$ be

190 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.

195 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.



200 Figure 1 Schematic of the process that affects the $\Delta^{17}\text{O}$ of CO₂ and H₂O during the photosynthetic gas exchange (not to scale). The three-isotope slopes θ for the individual isotope fractionation processes (both kinetic and equilibrium fractionation) are $\theta_{\text{trans}} = 0.522 - 0.008 \times h = 0.516$ for transpiration at $h = 75\%$ relative humidity (Landais et al., 2006), $\theta_{\text{CO}_2\text{-H}_2\text{O}} = 0.5229$ for isotope exchange between CO₂ and H₂O (Barkan and Luz, 2012), $\theta_{\text{CO}_2\text{-diff}} = 0.509$ for the diffusion of CO₂ (Young et al., 2002), $\theta_{\text{H}_2\text{O(v)-H}_2\text{O(l)}} = 0.529$ for the equilibrium between gas phase (v) and liquid (l) water, (Barkan and Luz, 2005) and $\theta_{\text{H}_2\text{O(v)-diff}}$ for the diffusion of water vapor (Barkan and Luz, 2007). $\epsilon^{18}\text{O}$, indicated along the x-axis, is the isotope fractionation in ¹⁸O due to the responding process.

210 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 $\Delta^{17}\text{O}$ 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.

215 # 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.

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

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

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

230 # 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.

235 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.

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

245 # The parameter c_m seems one of the most important numbers in this study. For obtaining this, only $\delta^{18}O$ and α^{18} values were used concerning isotope ratio, though. Is it possible to use $\Delta^{17}O$ and λ values to evaluate c_m instead? At least does it make sense to test its feasibility?

250 Yes, it is possible to calculate the mole fraction of CO_2 at the CO_2 - H_2O exchange site (c_m) using the $\Delta^{17}O$ 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 c_m using $\Delta^{17}O$ and λ values is under preparation.

As shown in Figure 5, the discrimination of $\Delta^{17}O$ of CO_2 during photosynthesis varies widely, and controlled by the magnitude of oxygen isotope equilibration at the CO_2 - H_2O site, that is to say, the

relative contribution of kinetic (diffusion) and equilibrium isotope fractionation. This conclusion is almost
255 identical to the knowledge using conventional $\delta^{18}\text{O}$ 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 $\delta^{18}\text{O}$. My impression after reading this manuscript is that the intra-MDF variation
dominate that of MIF signature on tropospheric CO_2 , which weakens the merit to study $\Delta^{17}\text{O}$ of CO_2 .
260 What is an advantage to use $\Delta^{17}\text{O}$ instead of $\delta^{18}\text{O}$? Please provide suggestions or implications to general
biogeochemists.

The referee is correct that the processes that affect $\delta^{18}\text{O}$ are the same that affect $\Delta^{17}\text{O}$. Nevertheless, the
quantitative evaluation of $\Delta^{17}\text{O}$ is largely independent of $\delta^{18}\text{O}$. The limitation of using $\delta^{18}\text{O}$ of
265 atmospheric CO_2 as a tracer is its dependency on the $\delta^{18}\text{O}$ 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 $\delta^{18}\text{O}$, $\Delta^{17}\text{O}$ variation is much smaller and is better defined
(Miller, 2018). This is because conventional bio-geo-chemical processes that modify $\delta^{17}\text{O}$ and $\delta^{18}\text{O}$
follow well-defined three-isotope fractionation slope. Consequently, the formulation of the CO_2 budget
270 using $\Delta^{17}\text{O}$ is a lot simplified, compared to using $\delta^{18}\text{O}$. Furthermore, unlike δ 's, λ is insensitive to
temperature (Cao and Liu, 2011; Bao et al., 2016; Hofmann et al., 2012; Dauphas and Schauble,
2016; Miller, 2018).

275 **Specific comments**

L41: "replaced using..." What this means? Be more specific.

280 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"

285 In the revised manuscript we used "see equation (1)"

L51: "the latter term" I guess it should be "the former term," which means photosynthetic CO_2 uptake.

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

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

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

L56: Delete "the isotopically exchanged"

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Deleted

L45-57: 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.

300

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}O and ^{16}O (1.0042 amu) is approximately half as large as the mass difference between ^{18}O and ^{16}O (2.0042 amu)." at the end of the paragraph.

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

310

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.

315

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

320

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)"

325

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

330

"In nature, it was believed all process that modify the oxygen isotope distribution are mass dependent isotope fractionation until the discovery of the 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

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equation 3). The $\Delta^{17}\text{O}$ of ozone can be transferred to other oxygen bearing molecules via direct chemical reaction with ozone or via $\text{O}(^1\text{D})$.”

and rearranged the whole paragraph

340

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

345 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.

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

L78: "The $\Delta^{17}\text{O}$ of CO_2 " instead of "The ^{17}O -excess of CO_2 ($\Delta^{17}\text{O}$) (equation 4)"

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

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

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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.

365

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

370

“The mole fraction of CO_2 at the $\text{CO}_2\text{-H}_2\text{O}$ exchange site (c_m) 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 $\text{CO}_2\text{-H}_2\text{O}$ exchange can occur anywhere between the plasma membrane and the

375

chloroplast. For C_4 plants, CA is mainly found in the cytosol, the CO_2 - H_2O 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: " $\Delta^{17}O$ " instead of "triple oxygen isotopic composition"

Changed accordingly

390 Equations 1 and 2: Should be merged such as, $\delta n O = n R_{\text{sample}} / n R_{\text{VSMOW}} - 1$, n refers 17 or 18 or simpler, $\delta = R_{\text{sample}} / R_{\text{VSMOW}} - 1$.

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

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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 $\Delta^{17}O = \delta^{17}O - \lambda \times \delta^{18}O$ (Miller, 2002), but this approximation causes an error that increases with $\delta^{18}O$.” for more clarity.

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L139-140: I recommend “Note that $\Delta^{17}O$ changes not only by MIF processes, but also MDF processes with a different λ value from the definition,”

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Changed accordingly

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

Changed accordingly

L147-148: Delete "Note that ... $\delta^{18}\text{O}$."

425 Changed accordingly

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

430 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

435

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

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

Changed accordingly

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

445

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.

450 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.

455

Changed accordingly

Section 2.3: I recommend moving this section to the Discussion.

460 Changed accordingly

L217: Delete “which is a net sink,”

465 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.

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L241-259: Here detail but still insufficient description was made only for δm , on the other hand, no description for c_i 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.

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

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

480

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

L262-265: Could it be shorter?

485 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.

490 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 “

495 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

500 Section 3.3: Could this section be shorter to several sentences? The description for δD 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.

505 L349: Water was converted to O₂

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

510 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 ‰

515 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.

520 In the revised manuscript, we excluded the sentence.

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

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

530 L414-415: Delete this sentence

Changed accordingly

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

535 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.

540
In the revised manuscript we incorporated the reference triple oxygen isotope fractionation slope (λ), also in the caption. Sorry for the confusion we did not mention it clearly. When we described $\Delta^{17}\text{O}$ in the theory section, we clearly mentioned which lambda value we used ($\lambda=0.528$).

545
Section 5.2: Avoid using “ ^{17}O -excess” for uniformity
Changed accordingly

Figure 3: Add individual flow direction.
550 Changed accordingly

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

Figure 5: Move Panel a to Figure 4 as above
560 Changed accordingly

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

565 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.

570 **Typographic errors**

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

L42: Welp et al. (2011)
575 Corrected

L45: The concept of the latter study..
Corrected

580 L60: equation 4))

Corrected, now it is “see equation 2”

L207: Figure 2

Now Figure S1

585

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).

590

“
This section has also been moved to the supplementary material.

L267: Maize

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

L279, L297: Need grammatical correctness.

Line 279

600 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

605 **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).

“

610

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

615 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.

620

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.

625

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

630 **Corrected**

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

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Leaf-scale quantification of the effect of photosynthetic gas exchange on $\Delta^{17}\text{O}$ of atmospheric CO_2

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Abstract

740 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 $\Delta^{17}\text{O}$. We directly quantify for the first time the effect of photosynthesis on $\Delta^{17}\text{O}$ of atmospheric CO_2 . Our results demonstrate the established theory for $\delta^{18}\text{O}$ is applicable to $\Delta^{17}\text{O}$ - CO_2 at leaf-level and we confirm the two key factors determine the effect of photosynthetic gas exchange on the $\Delta^{17}\text{O}$ of atmospheric CO_2 . 745 The relative difference between $\Delta^{17}\text{O}$ 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_m/c_a ratio where c_a is the CO_2 mole fraction in the surrounding air and c_m the one at the site of oxygen

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isotope exchange between CO₂ and H₂O. At low c_m/c_a ratio the discrimination is governed mainly by diffusion into the leaf, and at high c_m/c_a ratio by back-diffusion of CO₂ that has equilibrated with the leaf water. Plants with a higher c_m/c_a ratio modify the $\Delta^{17}\text{O}$ of atmospheric CO₂ more strongly than plants with a lower c_m/c_a ratio. Based on the leaf cuvette experiments, the global value for discrimination against $\Delta^{17}\text{O}$ of atmospheric CO₂ during photosynthetic gas exchange is estimated to be $-0.57 \pm 0.14 \text{‰}$ using c_m/c_a values of 0.3 and 0.7 for C₄ and C₃ plants, respectively. The main uncertainties in this global estimate arise from variation in c_m/c_a ratios among plants and growth conditions.

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1. Introduction

Stable isotope measurements of CO₂ provide important information on the magnitude of the CO₂ fluxes between atmosphere and biosphere, which are the largest components of the global carbon cycle (Farquhar et al., 1989; 1993; 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 CO₂ 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 CO₂ 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 $\delta^{18}\text{O}$ of atmospheric CO₂ after the 1997/98 El Nino event; see equation 1 for definition of the δ value.

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The concept behind the latter study was that atmospheric CO₂ exchanges oxygen isotopes with leaf and soil water, and this isotope exchange mostly determines the observed variations in $\delta^{18}\text{O}$ of CO₂ (Francey and Tans, 1987; Yakir, 1998). Following the 97/98 ENSO event, the anomalous $\delta^{18}\text{O}$ signature imposed on tropical leaf and soil waters was transferred to atmospheric CO₂, before slowly disappearing as a function of the lifetime of atmospheric CO₂. This in turn is governed by the land vegetation uptake of CO₂ during photosynthesis, as well as soil invasion of CO₂ (Miller et al., 1999; Wingate et al., 2009). For the photosynthesis term, the equilibration of CO₂ with water is an uncertain parameter in this calculation, partly because the $\delta^{18}\text{O}$ of water at the site of isotope exchange in the leaf is not well defined. Importantly,

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a significant $\delta^{18}\text{O}$ variation can occur in leaves due to the preferential evaporation of H_2^{16}O relative to H_2^{18}O (Gan et al., 2002; Farquhar and Gan, 2003; Gan et al., 2003; Cernusak et al., 2016), which induces a considerable uncertainty in estimating $\delta^{18}\text{O}$ of CO_2 . Similar considerations for the transfer of the $\delta^{18}\text{O}$ 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 $^{17}\text{O}/^{16}\text{O}$ ratio changes by approximately half of the corresponding change in $^{18}\text{O}/^{16}\text{O}$ (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 ($\Delta^{17}\text{O}$, equation 3). In the stratosphere, the $\Delta^{17}\text{O}$ of O_3 is transferred to CO_2 via isotope exchange of CO_2 with $\text{O}(^1\text{D})$ produced from O_3 photolysis (Shaheen et al., 2007; Yung et al., 1991; Yung et al., 1997), which results a large $\Delta^{17}\text{O}$ in stratospheric CO_2 (Wiegel et al., 2013; Thiemens et al., 1991; 1995; Lyons, 2001; Lämmerzahl et al., 2002; Thiemens, 2006; Kawagucci et al., 2008).

Once $\Delta^{17}\text{O}$ 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_2O to HCO_3^- and H^+ and vice versa (Francey 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; Francey and Tans, 1987).

$\Delta^{17}\text{O}$ of CO_2 has been suggested as 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, $\Delta^{17}\text{O}$ at the $\text{CO}_2\text{-H}_2\text{O}$ exchange site in the leaf will vary much less than $\delta^{18}\text{O}$. 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 $\Delta^{17}\text{O}$ of the H_2O 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; Meijer 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, $\Delta^{17}\text{O}$ of leaf water gets modified following a humidity dependent three-isotope slope $\theta_{\text{trans}}=0.522-0.008 \times h$ (Landais et al., 2006). Exchange of oxygen isotopes between leaf water and CO_2 follows $\lambda_{\text{CO}_2\text{-H}_2\text{O}}=0.5229$ (Barkan and Luz, 2012) which determines the $\Delta^{17}\text{O}$ of CO_2

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inside the leaf at the CO₂-H₂O exchange site. Finally, the $\Delta^{17}\text{O}$ of the CO₂ is modified when CO₂ diffuses into and out of the leaf with $\lambda_{diff} = 0.509$ (Young et al., 2002).

In the first box model study of Hoag et al. (2005), the small deviations in $\Delta^{17}\text{O}$ of CO₂ due to differences in three-isotope slopes were neglected and exchange with water was assumed to reset $\Delta^{17}\text{O}$ 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 $\Delta^{17}\text{O}$ of CO₂ in a 3D atmospheric model and investigated the spatiotemporal variability of $\Delta^{17}\text{O}$ and its use as tracer for GPP. Using these and other similar models, numerous measurements of $\Delta^{17}\text{O}$ in atmospheric CO₂ 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 results in a realistic overall modification of $\Delta^{17}\text{O}$ of CO₂ 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 $\Delta^{17}\text{O}$ of CO₂ in the surrounding air at the leaf scale. We measured $\Delta^{17}\text{O}$ of CO₂ 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 C₃ 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 C₃ species *Hedera hybernica* (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 C₄ species with a high A_N and a low g_s , typical for savanna type vegetation (Weijde et al., 2013). The mole fraction of CO₂ at the CO₂-H₂O exchange site (c_m) is an important parameter to determine the effect of photosynthesis on $\Delta^{17}\text{O}$ of CO₂. In C₃ plants, the CO₂-H₂O 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 C₄ plants, CA is mainly found in the cytosol and the CO₂-H₂O exchange occurs there (Badger and Price, 1994). In our experiments, sunflower and ivy are used to cover the wide c_m/c_a ratio range among C₃ plants and maize represents the c_m/c_a ratio for the C₄ plants. Using our results from the leaf scale experiments, we estimated the effect of terrestrial vegetation on $\Delta^{17}\text{O}$ of CO₂ in the global atmosphere.

1. Theory

1.1. Notation and definition of δ values

Isotopic composition is expressed as the deviation of the heavy to light isotope ratio in a sample relative to a reference ratio, and it is denoted as δ , expressed in per mill (‰). In the case of oxygen isotopes, the

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980 isotope ratios are $^{18}\text{R} = [^{18}\text{O}]/[^{16}\text{O}]$ and $^{17}\text{R} = [^{17}\text{O}]/[^{16}\text{O}]$ and the reference material is Vienna Standard Mean Ocean Water (VSMOW):

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

985 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).

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

990 The mass-dependent isotope fractionation factor λ 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). $\Delta^{17}\text{O}$ is used to quantify the degree of deviation from equation (2) (see equation 3). Note that $\Delta^{17}\text{O}$ changes not only by mass-independent isotope fractionation processes, but also by mass-dependent isotope fractionation processes, with a different λ value from the one used in the definition of $\Delta^{17}\text{O}$ (Barkan and Luz, 2005; Landais et al., 2006; 2008; Luz and Barkan, 2010; Barkan and Luz, 2011; Pack and Herwartz, 2014).

$$\Delta^{17}\text{O} = \ln(\delta^{17}\text{O} + 1) - \lambda \times \ln(\delta^{18}\text{O} + 1) \quad (3)$$

995 The choice of λ 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; Brand et al., 2010; Luz and Barkan, 2010; Barkan and Luz, 2012; Sharp et al., 2018). Equation 3 can be linearized to (Miller, 2002) $\Delta^{17}\text{O} = \delta^{17}\text{O} - \lambda \times \delta^{18}\text{O}$, but this approximation causes an error that increases with $\delta^{18}\text{O}$ (Miller, 2002; Bao et al., 2016).

1.2. Discrimination against $\Delta^{17}\text{O}$ of CO_2

1000 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 Δ_A for discrimination due to assimilation in this manuscript since the commonly used Δ is already used for the definition of $\Delta^{17}\text{O}$ (see equation 3). Δ_A 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; Barbour et al., 2016) as:

$$\Delta_A^n\text{O}_{\text{obs}} = \frac{n\text{R}_A}{n\text{R}_A} - 1 = \frac{\delta^n\text{O}_a - \delta^n\text{O}_A}{1 + \delta^n\text{O}_A} = \frac{\zeta \times (\delta^n\text{O}_a - \delta^n\text{O}_e)}{1 + \delta^n\text{O}_a - \zeta \times (\delta^n\text{O}_a - \delta^n\text{O}_e)} \quad (4)$$

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- Deleted: . This relation is generally referred to as mass dependent fractionation and can also be expressed as $\left[\frac{^{17}\text{R}}{^{17}\text{R}_{\text{ref}}}\right] = \left(\frac{^{18}\text{R}}{^{18}\text{R}_{\text{ref}}}\right)^\lambda$, or $\alpha^{17} = (\alpha^{18})^\lambda$ where α^{17} and α^{18} are the fractionations of ^{17}O and ^{18}O relative to a reference material, respectively. For small δ^{17} and δ^{18} values, equation (3) can be linearized to $\delta^{17}\text{O} = \lambda \times \delta^{18}\text{O}$.
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where the indices e , a and A refer to CO_2 entering and leaving the cuvette and being assimilated, respectively. $\zeta = \frac{c_e}{c_e - c_a}$, where c_e and c_a are the mole fractions of CO_2 entering and leaving the cuvette.

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

It is important to note that when the logarithmic definition of $\Delta^{17}\text{O}$ or $\Delta_A \Delta^{17}\text{O}$ is used, values are not additive (Kaiser et al., 2004). In linear calculations, the error gets larger when the relative difference in $\delta^{18}\text{O}$ between the two CO_2 gases increases regardless of the $\Delta^{17}\text{O}$ of the individual CO_2 gases (Figure S1). Therefore, $\Delta_A \Delta^{17}\text{O}$ values have to be calculated from the individual $\Delta_A^{17}\text{O}$ and $\Delta_A^{18}\text{O}$ values, and not by linear combinations of the $\Delta^{17}\text{O}$ 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 hybernica* 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 CO_2 mole fraction of about 400 ppm. The photosynthetic photon flux density (PPFD) was about 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ 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 cm^3 (l x w x h) 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).

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- Moved down [3]: F_{LA} and F_{AL} are total CO_2 fluxes from leaf to
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210 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).

220 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). 225 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(ClO₄)₂ dryer before entering the sampling flask.

230 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

235 The WVIA was calibrated using five water standards provided by IAEA (Wassenaar et al., 2018) for both δ¹⁸O and δD (Figure S2). We did not calibrate the WVIA for δ¹⁷O, so the δ¹⁷O 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 δD and δ¹⁸O, respectively. The detailed characterization and calibration of the water WVIA is provided in the supplementary material (Figure S2 to S4).

240 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). Details are provided in the supplementary material. The δ¹⁷O and δ¹⁸O 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).

2.4. Carbon dioxide extraction and isotope analysis

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- Deleted: The results are shown in supplementary material Figure S1. The isotopic composition of the water standards ranged from -50.93 to 3.64‰ and -396.98 to 25.44‰ for δD and δ¹⁸O, respectively. Based on the calibration using the five standards, a working standard was prepared to correct for short-term variability and to determine the non-linearity (dependency of δD and δ¹⁸O on the water vapor mole fraction). Each day the LGR was calibrated with 3 standards that cover the isotopic composition of the samples measured (δ¹⁸O value of -24.777‰, -8.640‰ and 0.11‰, provided by IAEA (Wassenaar et al., 2018)). Supplementary Figure S2, shows the results of the non-linearity tests. All three isotope signatures of water vapor showed relatively different dependence on the mole fraction of water vapor measured. The δ¹⁸O is independent of the mole fraction above 11000 ppm but decreases at lower mole fraction until 4000 ppm, and then increases again. δ¹⁷O is relatively stable for mole fractions higher than 17000 ppm, but increases strongly and in a non-linear manner below. Similarly, δD is independent of the mole fraction of water vapor [22]
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330 CO₂ was extracted from the air samples in a system made from electropolished stainless steel
(Supplementary Figure 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 CO₂. The flow rate during
extraction was 55 mL min⁻¹, controlled by a mass flow controller (Brooks Instruments, Holland). The
335 reproducibility of the extraction system was 0.030 ‰ for δ¹⁸O and 0.007 ‰ for δ¹³C determined on 14
extractions (1σ standard deviation, Supplementary Table S1).

The Δ¹⁷O of CO₂ was determined using the CO₂-O₂ exchange method (Mahata et al., 2013; Barkan et al.,
2015; Adnew et al., 2019). The CO₂-O₂ exchange system used at Utrecht University is described in
340 (Adnew et al., 2019). In short, equal amounts of CO₂ and O₂ were mixed in a quartz reactor containing a
platinum sponge catalyst and heated at 750°C for 2hrs. After isotope equilibration, the CO₂ was trapped
at liquid nitrogen temperature, while the O₂ was collected with 1 pellet of 5Å molecular sieve (1.6 mm,
Sigma Aldrich, USA) at liquid nitrogen temperature. The isotopic composition of the isotopically
equilibrated O₂ was measured with a Delta^{plus}XL isotope ratio mass spectrometer in dual inlet mode with
345 reference to a pure O₂ calibration gas that has been assigned values of δ¹⁷O = 9.254 ‰ and δ¹⁸O = 18.542
‰ by E. Barkan at the Hebrew University of Jerusalem. The reproducibility of the Δ¹⁷O measurement
was better than 0.01 ‰ (Supplementary Table S1).

2.5. Leaf cuvette model

350 We used a simple leaf cuvette model to evaluate the dependence of Δ_AΔ¹⁷O 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 drawdown of CO₂, similar to the
leaf exchange experiments, i.e., the CO₂ mole fraction decreases from 500 ppm in the entering air (c_e) to
400 ppm in the outgoing air (c_o), which is identical to the air surrounding the leaf (c_a) as a result of
355 thorough mixing in the cuvette. The assimilation rate is set to 20.0 μmol m⁻²s⁻¹. The leaf area and flowrate
of air are set to 30 cm² and 0.7 L min⁻¹, respectively. The isotope composition of leaf water at the site
where the H₂O-CO₂ exchange occurs is δ¹⁷O = 5.39 ‰ and δ¹⁸O = 10.648 ‰, which is the mean of the
measured δ¹⁷O and δ¹⁸O 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 δ¹⁸O of the CO₂ entering the cuvette is set to 30.47
360 ‰ for all the simulations, as in the normal CO₂ experiments, but the assigned Δ¹⁷O values ranges from -
0.5 ‰ to 0.5 ‰ which encompasses both the stratospheric intrusion and combustion components. The
corresponding δ¹⁷O of the CO₂ entering the cuvette is calculated from the assigned δ¹⁸O value (30.47 ‰)
and Δ¹⁷O 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
365 detailed code and description is available at https://git.wur.nl/leaf_model/D17O.

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3. Results

3.1. Gas exchange parameters

Table 1 summarizes the isotopic composition and mole fraction of the CO₂ used in this study for sunflower, ivy and maize. The Δ¹⁷O of CO₂ used in this study varies from -0.215 ‰ to 0.44 ‰ while the δ¹⁸O value is close to 30 ‰ for all the experiments. For all the experiments, the mole fraction of CO₂ entering the leaf (c_a) is 400 ppm whereas the mole fraction of the CO₂ in the intercellular air space (c_i), at the CO₂-H₂O 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_m 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 ¹⁸O of CO₂

Figure 3a shows discrimination against ¹⁸O associated with photosynthesis (Δ_A¹⁸O) for sunflower, ivy, and maize as a function of the c_m/c_a ratio. Δ_A¹⁸O varies with c_m/c_a, as found in previous studies (Gillon and Yakir, 2000a; Barbour et al., 2016). For sunflower, we observe Δ_A¹⁸O values between 29 ‰ and 64 ‰ for c_m/c_a between 0.54 and 0.86. Ivy shows a relatively little variation of Δ_A¹⁸O around a mean of 22 ‰ for c_m/c_a between 0.48 and 0.58. For maize, Δ_A¹⁸O is lower than for the C₃ plants measured in this study, with values between 10 ‰ and 20 ‰ for c_m/c_a between 0.15 and 0.37.

For sunflower changing the irradiance from 300 μmol m⁻²s⁻¹ (low light, hereafter LL) to 1200 μmol m⁻²s⁻¹ (high light, hereafter HL) leads to a clear decrease in Δ_A¹⁸O (average 22 ‰). For maize, the Δ_A¹⁸O change is only 4.4 ‰ on average. For ivy, changing the light intensity does not significantly change the observed Δ_A¹⁸O. The solid lines in Figure 3a show results of leaf cuvette model calculations, where the dependence of Δ_A¹⁸O on c_m/c_a 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 ¹⁷O of CO₂

The discrimination of photosynthesis against ¹⁷O of CO₂ (Δ_AΔ¹⁷O) is shown in Figure 3b. Δ_AΔ¹⁷O is negative for all experiments and it depends strongly on the c_m/c_a ratio and |Δ_AΔ¹⁷O| increases with c_m/c_a ratio. For instance, for Δ¹⁷O of CO₂ entering the cuvette of -0.215 ‰, Δ_AΔ¹⁷O is -0.25 ‰ for maize with c_m/c_a ratio of 0.3, -0.3 ‰ for ivy with c_m/c_a ratio of 0.5 ‰ and -0.5 ‰ for sunflower with c_m/c_a ratio of 0.7 (Figure 3b). For sunflower and ivy, Δ_AΔ¹⁷O is also strongly dependent on the Δ¹⁷O of CO₂ supplied to the cuvette, whereas no significant dependence is found for maize. For an increase in Δ¹⁷O of CO₂ entering the cuvette from -0.215 ‰ to 0.435 ‰, Δ_AΔ¹⁷O increases from -0.3 ‰ to -0.9 ‰ at c_m/c_a ratio of 0.5 for ivy. For sunflower, an increase in Δ¹⁷O of CO₂ entering the cuvette from -0.215 ‰ to 0.31 ‰ increases Δ_AΔ¹⁷O from -0.8 ‰ to -1.7 ‰ at c_m/c_a ratio of 0.8. The leaf cuvette model results illustrate

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475 the shape of the dependence on the c_m/c_a ratio and agree well with the experiments. For the leaf cuvette model, the $\Delta^{17}\text{O}$ value of the water is assigned a constant value of -0.122‰ (average $\Delta^{17}\text{O}$ value for the bulk leaf water).

480 Figure 4b shows the same values of $\Delta_A\Delta^{17}\text{O}$ as a function of the difference between $\Delta^{17}\text{O}$ of CO_2 entering the leaf and the calculated $\Delta^{17}\text{O}$ of leaf water at the evaporation site where $\text{CO}_2\text{-H}_2\text{O}$ exchange takes place ($\Delta^{17}\text{O}_a - \Delta^{17}\text{O}_{\text{wes}}$), for different c_m/c_a ratios. The leaf cuvette model results (solid lines in Figure 4b) suggest a linear dependence between $\Delta_A\Delta^{17}\text{O}$ and ($\Delta^{17}\text{O}_a - \Delta^{17}\text{O}_{\text{wes}}$). The experimental results agree with the hypothesis that $\Delta_A\Delta^{17}\text{O}$ is linearly dependent on $\Delta^{17}\text{O}_a - \Delta^{17}\text{O}_{\text{wes}}$ at a certain c_m/c_a ratio. Figure 4a shows the corresponding relation where $\Delta_A\Delta^{17}\text{O}$ is divided by $\Delta^{17}\text{O}_a - \Delta^{17}\text{O}_m$. All the values follow the same relationship as function of the c_m/c_a ratio, which can be approximated quite well by an exponential function (equation 5). This function quantifies the dependence of $\Delta_A\Delta^{17}\text{O}$ on c_m/c_a , and thus the effect of the diffusion of isotopically exchanged CO_2 back to the atmosphere, which increases with increasing c_m/c_a ratio.

$$\frac{\Delta_A\Delta^{17}\text{O}}{\Delta^{17}\text{O}_a - \Delta^{17}\text{O}_m} = -0.150 \times \exp(3.707 \times c_m/c_a) + 0.028 \quad (5)$$

490 Figure 5 a and c show results from the leaf cuvette model that illustrates in more detail how $\Delta^{17}\text{O}_e$ and $\Delta^{17}\text{O}_{\text{wes}}$ affect $\Delta^{17}\text{O}_a$ and $\Delta_A\Delta^{17}\text{O}$ and their dependence on c_m/c_a . At lower c_m/c_a , only a very small fraction of CO_2 that has undergone isotopic equilibration in the mesophyll diffuses back to the atmosphere, and therefore $\Delta^{17}\text{O}_a$ stays close to the incoming $\Delta^{17}\text{O}_e$, modified by the fractionation during CO_2 diffusion through the stomata (Figure 5a). Figure 5c confirms that indeed at low c_m/c_a , $\Delta_A\Delta^{17}\text{O}$ 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 $\Delta_A\Delta^{17}\text{O}$ 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 $\Delta^{17}\text{O}$, because now the effect on $\Delta^{17}\text{O}$ 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.

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

510 Figure 6 shows how $\delta^{18}\text{O}$ and $\Delta^{17}\text{O}$ vary in key compartments of the leaf cuvette system that determine the oxygen isotope effects associated with photosynthesis, based on the previously established three-

Deleted: ‰ (average $\Delta^{17}\text{O}$ value for the bulk leaf water). Results from the Farquhar model (equation 7 for $\Delta_A^{18}\text{O}_{\text{EM}}$ and analogous equation for $\Delta_A^{17}\text{O}_{\text{EM}}$) fit well with the measurements ($R^2 = 0.959$, RMSE = 0.1‰) (Figure 5a, Figure S7 (supplementary material)). The RMSE is lower than the measurement error for the $\Delta_A\Delta^{17}\text{O}$ in our experimental setup. Based on our measurement, the error introduced in $\Delta_A\Delta^{17}\text{O}$ for the individual experiment is 0.25‰ (SD) calculated from the individual errors of $\Delta_A^{17}\text{O}$ and $\Delta_A^{18}\text{O}$.

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isotope slopes of the various processes (Figure 1). The irrigation water has a $\Delta^{17}\text{O}$ value of 0.017. The measured bulk leaf water is 6-16 ‰ enriched in ^{18}O and its $\Delta^{17}\text{O}$ value is lower by -0.075 to -0.200 ‰ (mean value -0.121 ‰) than the irrigation water, calculated using a three-isotope slope of $\lambda_{trans} = 0.516$ at 80 % humidity (Landais et al., 2006, Figure 1). $\Delta^{17}\text{O}$ of leaf water at the evaporation site, calculated from the transpired water, has slightly lower $\Delta^{17}\text{O}$, 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, $\Delta^{17}\text{O}$ 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 $\Delta^{17}\text{O}$ 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 $\lambda_{\text{CO}_2\text{-H}_2\text{O}} = 0.5229$ (Barkan and Luz, 2012, Figure 1), leading to the lower $\Delta^{17}\text{O}$ values of the equilibrated CO_2 . In our experiments, the $\Delta^{17}\text{O}$ value of CO_2 in equilibrium with leaf water is lower than the $\Delta^{17}\text{O}$ value of CO_2 entering the leaf. The $\Delta^{17}\text{O}$ of the CO_2 in the intercellular air space is a mixture between two end members, the $\Delta^{17}\text{O}$ of the CO_2 entering the leaf and $\Delta^{17}\text{O}$ of the CO_2 in equilibrium with leaf water. This explains why the observed values of $\Delta_A \Delta^{17}\text{O}$ are negative for the experiments performed in this study.

4. Discussion

4.1. Discrimination against ^{18}O of CO_2

The higher $\Delta_A^{18}\text{O}_{\text{obs}}$ values for sunflower compared to maize and ivy (Figure 3a) are mainly due to a higher back-diffusion flux ($c_m/(c_a - c_m)$). The back-diffusion flux is higher for the C_3 plants sunflower and ivy than for the C_4 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_m/c_a ratio and thus leads to a lower back-diffusion flux, which explains the decreases of $\Delta_A^{18}\text{O}_{\text{obs}}$ for maize and most clearly for sunflower. A similar trend of increase in $\Delta_A^{18}\text{O}_{\text{obs}}$ with an increase in c_m/c_a ratio has been reported in previous studies (Gillon and Yakir, 2000b, a; Osborn et al., 2017). For ivy, $\Delta_A^{18}\text{O}_{\text{obs}}$ and $\Delta_A^{17}\text{O}_{\text{obs}}$ do not decrease with an increase in irradiance, because the change in assimilation rate with irradiance is small. Thus, c_m/c_a will not decrease strongly and the effect on the back diffusion is smaller than the variability in $\Delta_A^{18}\text{O}_{\text{obs}}$ of different leaves of the same plant.

In our experiments, photosynthesis causes enrichment $\delta^{18}\text{O}$ of atmospheric CO_2 for both C_3 and C_4 plants, i.e. positive values $\Delta_A^{18}\text{O}$. In principle, $\Delta_A^{18}\text{O}$ can also be negative if the $\delta^{18}\text{O}_m$ are depleted relative to the ambient CO_2 . This is in contrast to $\Delta_A^{13}\text{C}$, which will always be positive since it is determined by the fractionation due to the PEPC and RuBisCO enzyme activity (Figure S8 and S9, supplementary material). In general, in our experiments, the $\Delta_A^{18}\text{O}_{\text{obs}}$ values are about five times larger than $\delta^{18}\text{O}_a - \delta^{18}\text{O}_e$, the $\delta^{18}\text{O}$ difference between CO_2 entering and leaving the cuvette (Figure S10 to S12, supplementary

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material). This is easy to understand from the definition of Δ_A . Taking $\Delta_A^{18}\text{O}$ as an example, $\Delta_A^{18}\text{O}_{\text{obs}} = \frac{\zeta(\delta^{18}\text{O}_a - \delta^{18}\text{O}_e)}{1 + \delta^{18}\text{O}_a - \zeta(\delta^{18}\text{O}_a - \delta^{18}\text{O}_e)} \approx \zeta(\delta^{18}\text{O}_a - \delta^{18}\text{O}_e)$ and in our experiments, $\zeta = c_e / (c_e - c_a) \approx 500 / (500 - 400) = 5$.

4.2. Discrimination against the $\Delta^{17}\text{O}$ 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 $\Delta_A^{18}\text{O}_{\text{obs}}$ but has now been confirmed for $\Delta_A \Delta^{17}\text{O}$.

Unlike ivy and sunflower, maize does not show a significant change in $\Delta_A \Delta^{17}\text{O}$ when CO_2 gases with different $\Delta^{17}\text{O}$ 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_m/c_a ratio. At low c_m/c_a ratios, $\Delta_A \Delta^{17}\text{O}$ is expected to be close to the weighted fractionation due to diffusion through boundary layer and stomata. In general, the effect of diffusion on $\Delta^{17}\text{O}$ of atmospheric CO_2 can be expressed as follows:

$$\Delta^{17}\text{O}_{\text{Modified}} = \Delta^{17}\text{O}_a + (\lambda_{\text{RL}} - \lambda_{\text{diffusion}}) \times \ln \alpha_{\text{diffusion}} \quad (6)$$

where $\Delta^{17}\text{O}_a$ is the $\Delta^{17}\text{O}$ of the CO_2 surrounding the leaf, $\Delta^{17}\text{O}_{\text{modified}}$ is the $\Delta^{17}\text{O}$ of the CO_2 modified due to diffusional fractionation and $\lambda_{\text{diffusion}}$, λ_{RL} and $\alpha_{\text{diffusion}}$ are the oxygen three-isotope relationships during diffusion from the CO_2 - H_2O 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 $\lambda_{\text{RL}} = 0.528$, $\lambda_{\text{diffusion}} = 0.509$ (Young et al., 2002, Figure 1) and $\alpha_{\text{diffusion}} = 0.9912$ (Farquhar and Lloyd, 1993), the effect of diffusional fractionation on the $\Delta^{17}\text{O}$ 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_m/c_a ratio (Figure 5c and d, inset).

At a high c_m/c_a ratio, $\Delta^{17}\text{O}_a$ is dominated by the back-diffusion flux of CO_2 that has equilibrated with water. As a consequence, $\Delta^{17}\text{O}_a$ 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 $\Delta^{17}\text{O}$ of CO_2 in equilibrium with leaf water, $\Delta^{17}\text{O}_m$. In fact, when $\Delta^{17}\text{O}_a = \Delta^{17}\text{O}_m$, $\Delta^{17}\text{O}_a$ does not change with c_m/c_a , indicating that in this case the $\Delta^{17}\text{O}$ of the CO_2 diffusing back from the leaf is the same as the $\Delta^{17}\text{O}(\text{CO}_2)$ entering the leaf.

$\bar{\alpha}_{18}$ is the overall discrimination occurring during the diffusion of $^{12}\text{C}^{18}\text{O}^{16}\text{O}$ from the ambient air surrounding the leaf to the CO_2 - H_2O exchange site (see Table 3 for the list of variables). In our study $\bar{\alpha}_{18}$ ranges from 5‰ to 7.2‰, lower than the literature estimate of 7.4‰ (Farquhar et al., 1993). $\bar{\alpha}_{18}$ 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_{m18}) the lower the $\bar{\alpha}_{18}$ (Table S2, supplementary material). The difference in $\bar{\alpha}_{18}$ of 2.4‰ between

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the literature value of 7.4 ‰ and the lowest \bar{a}_{18} estimate in this study will introduce an error of only 0.046 ‰ in the $\Delta^{17}\text{O}$ value (see equation 6). The uncertainty \bar{a}_{18} has lower influence on the $\Delta_A\Delta^{17}\text{O}$ of C_3 plants compared to C_4 plants since the diffusional fractionation is less important at the higher c_m/c_a ratio where C_3 plants operate.

4.3. Global average value of $\Delta_A\Delta^{17}\text{O}$ and $\Delta^{17}\text{O}$ isoflux

We can use the established relationship between $\Delta_A\Delta^{17}\text{O}$ and $\Delta^{17}\text{O}_a - \Delta^{17}\text{O}_{\text{wes}}$ for a certain c_m/c_a ratio to provide a bottom-up estimate for the global effect of photosynthesis on $\Delta^{17}\text{O}$ 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 ($\Delta^{17}\text{O}(\text{CO}_2) = -0.168\text{‰}$, $\Delta^{17}\text{O}(\text{H}_2\text{O}_{\text{leaf}}) = -0.067\text{‰}$; (Koren et al., 2019); Figure S13 and 14, supplementary material). The $\Delta^{17}\text{O}(\text{CO}_2)$ values agree well with the limited amount of available measurements (Table 1).

To extrapolate $\Delta_A\Delta^{17}\text{O}$ determined in the leaf scale experiments to the global scale, global average c_m/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_m/c_a values with a standard deviation of 0.12 and 0.17 for C_4 and C_3 plants respectively (Figure S15, supplementary material) (Schaefer et al., 2008; Koren et al., 2019). We use this variability as upper limit of the error estimate for c_m/c_a as shown in the light orange and light pink shaded areas in Figure 4b. This error is converted to an error in $\Delta_A\Delta^{17}\text{O}$ using the relation with c_m/c_a . Based on the linear dependency of $\Delta_A\Delta^{17}\text{O}$ and $\Delta^{17}\text{O}_a - \Delta^{17}\text{O}_{\text{wes}}$, we estimate the $\Delta_A\Delta^{17}\text{O}$ for tropospheric CO_2 based on the $\Delta^{17}\text{O}$ of leaf water and c_m/c_a ratio. In Figure 4b, the dashed black vertical line indicates $\Delta^{17}\text{O}_a - \Delta^{17}\text{O}_{\text{wes}}$ obtained from the 3D global model (Koren et al., 2019). The results of the global estimate and parameters used for the extrapolation of leaf scale study to the global scale are summarized in Table 1.

The $\delta^{17}\text{O}$ value of atmospheric CO_2 (21.53 ‰) is calculated from the global $\delta^{18}\text{O}$ and $\Delta^{17}\text{O}$ values (41.5 ‰ and -0.168 ‰, respectively) (Koren et al., 2019). The $\delta^{17}\text{O}$ and $\delta^{18}\text{O}$ values of global mean leaf water are calculated from the soil water. A global mean $\delta^{18}\text{O}$ value of soil water is -8.4 ‰ assuming soil water to be similar to precipitation (Bowen and Revenaugh, 2003; Koren et al., 2019). The $\delta^{17}\text{O}$ value of soil water is -4.4 ‰, calculated using equation 7 (Luz and Barkan, 2010).

$$\ln(\delta^{17}\text{O}_{\text{soil}} + 1) = 0.528 \times \ln(\delta^{18}\text{O}_{\text{soil}} + 1) + 0.033 \quad (7)$$

$\delta^{17}\text{O}$ and $\delta^{18}\text{O}$ of leaf water are calculated from $\delta^{17}\text{O}$ and $\delta^{18}\text{O}$ 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 $\delta^{17}\text{O}$ is calculated using $\alpha^{17} = (\alpha^{18})^{\lambda_{\text{trans}}}$ with $\lambda_{\text{trans}} = 0.516$, assuming relative humidity to be 75% (Landais et al., 2006). The $\delta^{17}\text{O}$ and $\delta^{18}\text{O}$ values of global mean leaf water are then -0.136 ‰ and -0.131 ‰, respectively. Thus, the difference between global atmospheric CO_2 and leaf water is $\delta^{17}\text{O}_{\text{CO}_2 - \text{water}} = 21.666\text{‰}$ and $\delta^{18}\text{O}_{\text{CO}_2 - \text{water}} = 41.631\text{‰}$. This yields $\Delta^{17}\text{O}_{\text{CO}_2 - \text{water}} = -0.101\text{‰}$, 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

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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_m/c_a ratios of C₃ and C₄ plants **corresponds** to the $\Delta_A\Delta^{17}\text{O}$ value of C₃ and C₄ plants. For C₄ plants ($c_m/c_a = 0.3$) this yields $\Delta_A\Delta^{17}\text{O} = -0.3\text{‰}$ (gray dashed line in **figure 4b**) and for C₃ plants ($c_m/c_a = 0.7$), $\Delta_A\Delta^{17}\text{O} = -0.65\text{‰}$ (black dashed line in **Figure 4b**).

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Three main factors contribute to the uncertainty of the extrapolated $\Delta_A\Delta^{17}\text{O}$ 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 $\Delta^{17}\text{O}$ of atmospheric CO₂ and leaf water, **and we use results from the global model to estimate an error. For $\Delta^{17}\text{O}$ 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 $\Delta^{17}\text{O}$ 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 ($\Delta^{17}\text{O}_a - \Delta^{17}\text{O}_{\text{ves}}$) to be 0.043‰.** The third uncertainty in the extrapolation of $\Delta^{17}\text{O}$ comes from the uncertainty in the c_m/c_a ratio. For C₃ and C₄ plants, these errors are indicated by the light orange and light blue shadings in **Figure 4b**.

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Taking these uncertainties into account leads to a mean value of $\Delta_A\Delta^{17}\text{O} = -0.3 \pm 0.18\text{‰}$ for C₄ plants and $\Delta_A\Delta^{17}\text{O} = -0.65 \pm 0.18\text{‰}$ for C₃ plants. **The leaf scale discrimination against $\Delta^{17}\text{O}$ is then extrapolated to global vegetation using these representative values of $\Delta_A\Delta^{17}\text{O}$ and the relative fractions of photosynthesis by C₄ and C₃ plants, respectively as:**

$$\Delta_A\Delta^{17}\text{O}_{\text{global}} = f_{C4} \times \Delta_A\Delta^{17}\text{O}_{C4} + f_{C3} \times \Delta_A\Delta^{17}\text{O}_{C3} \quad (8)$$

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where f_{C4} and f_{C3} are the photosynthesis weighted global coverage of C₄ and C₃ vegetation. $\Delta_A\Delta^{17}\text{O}_{C4}$ and $\Delta_A\Delta^{17}\text{O}_{C3}$ quantify the discrimination against $\Delta^{17}\text{O}$ by C₄ and C₃ plants, which are calculated using estimated values of c_m/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 $\Delta_A\Delta^{17}\text{O}$ obtained from equation 8 is -0.57 ± 0.14‰.

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

$$F_A \times \Delta_A\Delta^{17}\text{O} = A \times (f_{C4} \times \Delta_A\Delta^{17}\text{O}_{C4} + f_{C3} \times \Delta_A\Delta^{17}\text{O}_{C3}) \quad (9)$$

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805 where, $A=0.88 \times \text{GPP}$ is the terrestrial assimilation rate. The factor 0.88 accounts for the fraction of CO_2 released due to autotrophic respiration (Ciais et al., 1997a). The $\Delta_A \Delta^{17}\text{O}$ isoflux due to photosynthesis is calculated using a GPP value of 120 PgCyr^{-1} (Beer et al., 2010) and $A=0.88 \times \text{GPP}$, resulting in an isoflux of $-60 \pm 15 \text{ ‰ PgCyr}^{-1}$ globally. This is the first global estimate of $\Delta_A \Delta^{17}\text{O}$ 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_m/c_a ratio of 0.7 for both C_4 and C_3 plants and $\Delta^{17}\text{O}$ of -0.147 ‰ for atmospheric CO_2 . A model-estimated value from (Hoag et al., 2005) is -47 ‰ PgCyr^{-1} (converted to our reference slope of $\lambda=0.528$), derived with a more simple model and using $\Delta^{17}\text{O}$ of -0.146 ‰ with c_m/c_a ratio of 0.33 and 0.66 for C_4 and C_3 plants, respectively.

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815 The main uncertainty in the extrapolation of $\Delta_A \Delta^{17}\text{O}$ from the leaf experiments to the global scale is the uncertainty in the c_m/c_a ratio. The error from the uncertainty in c_m/c_a ratio increases when the relative difference in $\Delta^{17}\text{O}$ between CO_2 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, CO_2 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 c_m/c_a both in the laboratory and under field conditions. This could lead to a better understanding of variations in the c_m/c_a ratio among plant species and, temporally, spatially and environmentally.

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5. Conclusions

825 In order to directly quantify the effect of photosynthetic gas exchange on the $\Delta^{17}\text{O}$ 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 ^{18}O agree with results reported in the literature previously. Our results for $\Delta^{17}\text{O}$ confirm that the formalism developed by Farquhar and others for $\delta^{18}\text{O}$ is also applicable to the evaluation of $\Delta^{17}\text{O}$. In particular, our experiments confirm that two parameters determine the effect of photosynthesis on CO_2 : 1) the $\Delta^{17}\text{O}$ difference between the incoming CO_2 and CO_2 in equilibrium with leaf water and 2) the c_m/c_a ratio, which determines the degree of back-flux of isotopically exchanged CO_2 from the mesophyll to the atmosphere. At low c_m/c_a ratios, $\Delta_A \Delta^{17}\text{O}$ is mainly influenced by the diffusional fractionation. Under our experimental conditions, the isotopic effect increased with c_m/c_a , e.g. $\Delta_A \Delta^{17}\text{O}$ was -0.3 ‰ and -0.65 ‰ for maize and sunflower with c_m/c_a ratios of 0.3 and 0.7, respectively. However, experiments with mass independently fractionated CO_2 demonstrate that the results depend strongly on the $\Delta^{17}\text{O}$ difference between the incoming CO_2 and CO_2 in equilibrium with leaf water. This is supported by calculations with a leaf cuvette model.

840 $\delta^{18}\text{O}$ is largely affected by kinetic and equilibrium processes between CO_2 and leaf water, and also leaf water isotopic inhomogeneity and dynamics. The $\Delta^{17}\text{O}$ variation is much smaller compared to $\delta^{18}\text{O}$ and is better defined since conventional bio-geo-chemical processes that modify $\delta^{17}\text{O}$ and $\delta^{18}\text{O}$ follow a well-

defined three-isotope fractionation slope. Results from the leaf exchange experiments were upscaled to the global atmosphere using modeled values for $\Delta^{17}\text{O}$ of leaf water and CO_2 , which results in $\Delta_A\Delta^{17}\text{O} = -0.57 \pm 0.14 \text{‰}$ and a value for the $\Delta^{17}\text{O}$ isoflux of $-60 \pm 15 \text{‰ PgCyr}^{-1}$. This is the first study that provides such an estimate based on direct leaf chamber measurements, and the results agree with previous $\Delta^{17}\text{O}$ calculations. The largest contribution to the uncertainty originates from uncertainty in the c_m/c_a 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. $\Delta_A\Delta^{17}\text{O}$ is less sensitive to c_m/c_a ratios at lower values of c_m/c_a , for instance for C_4 plants, maize.

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$\Delta^{17}\text{O}$ 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 $\Delta^{17}\text{O}$ atmospheric CO_2 we recommend measuring the effects of foliage respiration and soil invasion both in the laboratory and at the ecosystem scale.

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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.

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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_2 extraction and $\text{CO}_2\text{-H}_2\text{O}$ 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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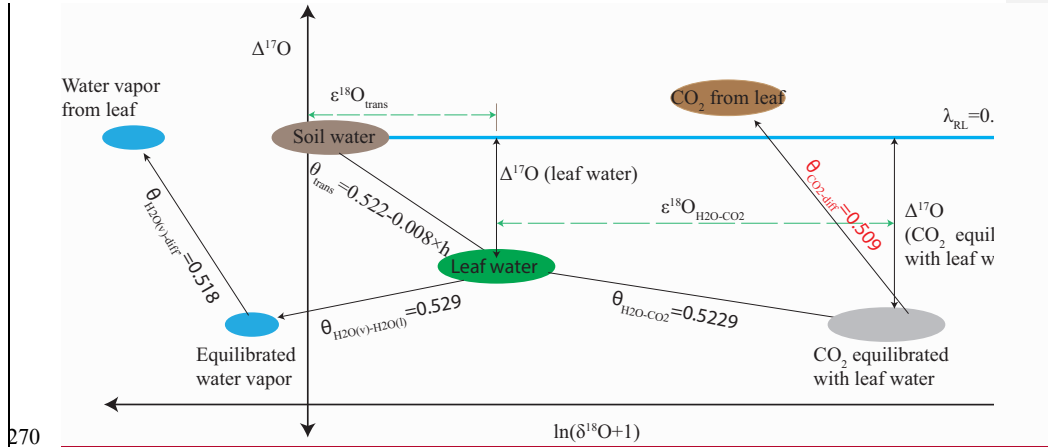
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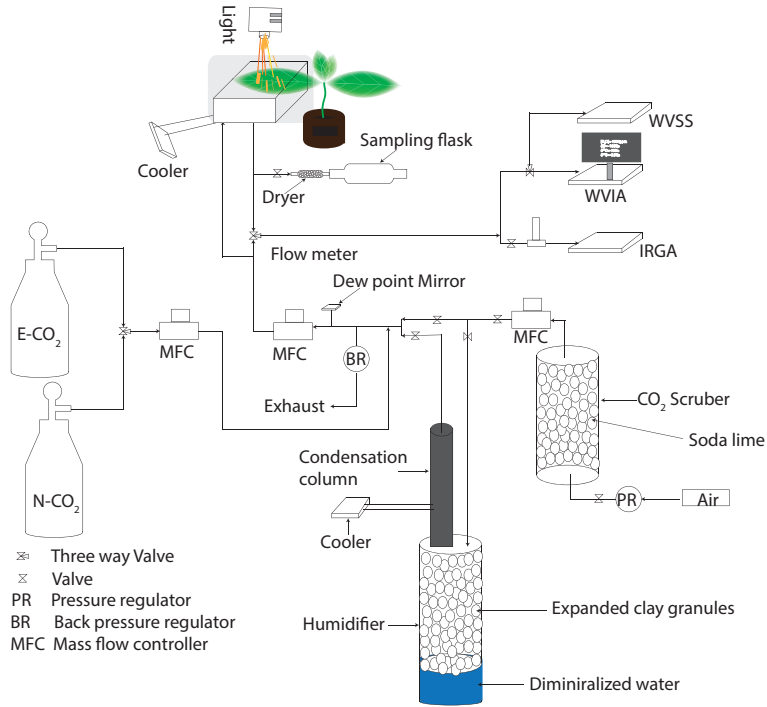
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Leaf exchange parameters are calculated following (Caemmerer and Farquhar, 1981). The transpiration rate (E) is calculated from the air flowrate, leaf area and concentration of water vapor entering and leaving the cuvette as: ... [31]

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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-CO₂ is normal CO₂, E-CO₂ is ¹⁷O-enriched CO₂.

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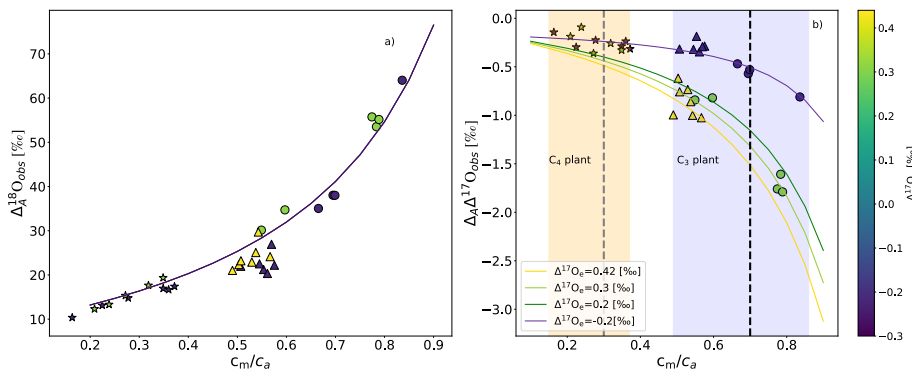
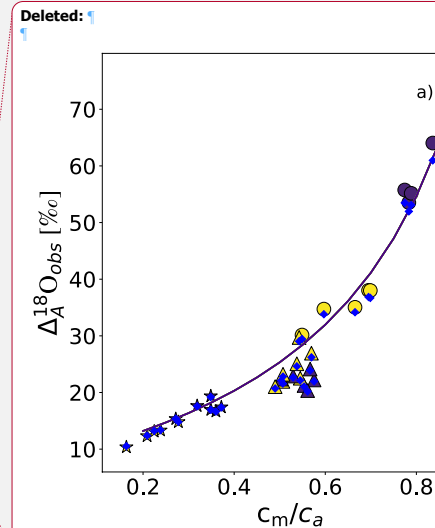
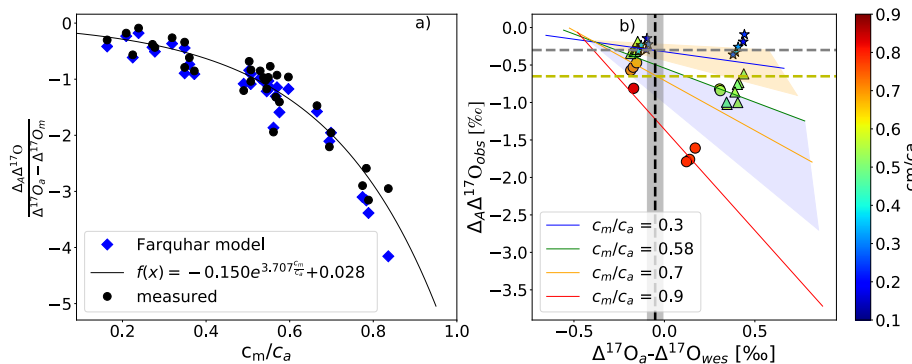


Figure 3 a) $\Delta_A^{18}O_{obs}$ during photosynthesis for two C₃ plants, sunflower (circles) and ivy (triangles) and C₄ plant maize (stars) as a function of c_m/c_a . The solid lines show results from the leaf cuvette model, where $\delta^{18}O$ of the CO₂ entering the cuvette is 30.47‰. b) $\Delta_A \Delta^{17}O$ of CO₂ as a function of c_m/c_a for isotopically different CO₂ gases entering the cuvette (color bar shows $\Delta^{17}O_e$) for sunflower (circles), ivy (triangles) and maize (stars). $\Delta_A \Delta^{17}O$ 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 areas indicate the c_m/c_a ranges for C₄ and C₃ 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 line are leaf cuvette model results for the corresponding c_m/c_a ratio.**



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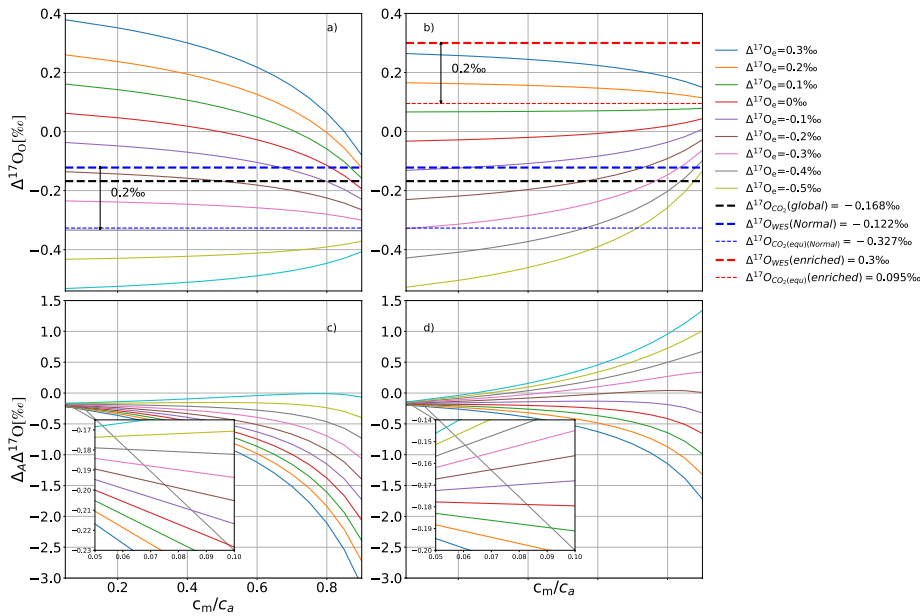
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Figure 4 a) Dependency of $\Delta_A \Delta^{17}\text{O}$ on the relative difference on the $\Delta^{17}\text{O}$ CO_2 entering the leaf and the $\Delta^{17}\text{O}$ of CO_2 in equilibrium with leaf water against c_m/c_a ratio. b) dependency of $\Delta_A \Delta^{17}\text{O}$ on the difference between the $\Delta^{17}\text{O}$ of CO_2 entering the cuvette and the $\Delta^{17}\text{O}$ of leaf water at the evaporation site color coded for different c_m/c_a ratios. The solid lines are results of the leaf cuvette model for different c_m/c_a ratios stated in the legend. The dashed vertical black line indicates the difference between the global average $\Delta^{17}\text{O}$ value for CO_2 (-0.168 ‰) and leaf water (-0.067 ‰) (Koren et al., 2019). The gray and yellow horizontal dashed lines indicate $\Delta_A \Delta^{17}\text{O}$ of C_4 and C_3 plants for c_m/c_a ratio of 0.3 and 0.7, respectively globally.

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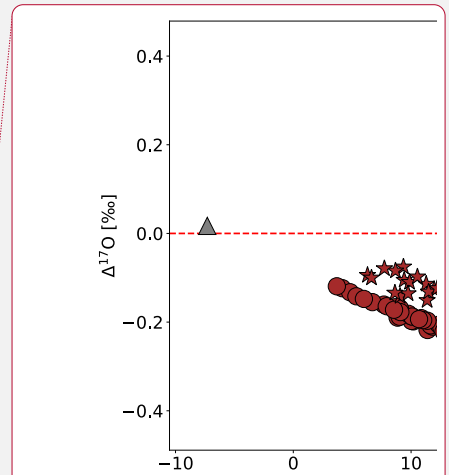
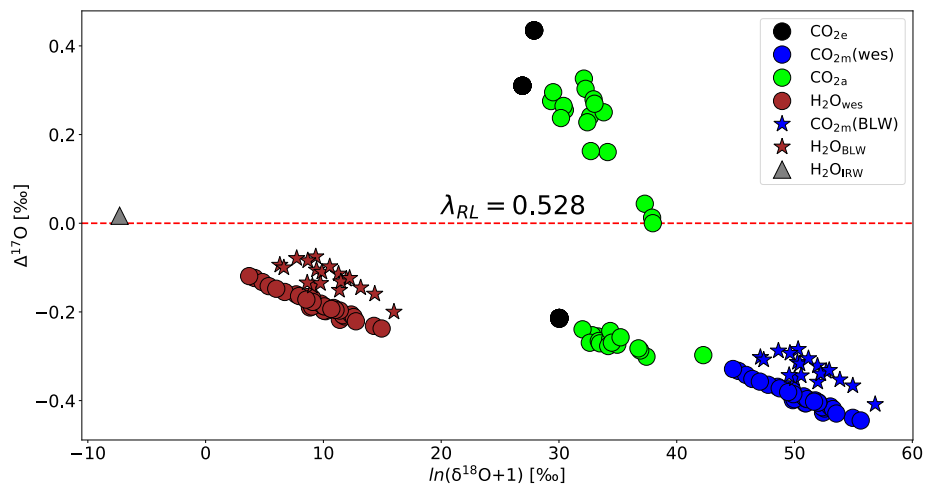
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Figure 5 a) and b) $\Delta^{17}\text{O}_a$ as a function of c_m/c_a for various values of $\Delta^{17}\text{O}_e$ (see legend) for $\Delta^{17}\text{O}_{\text{wes}} = -0.122$ ‰ in a) and $\Delta^{17}\text{O}_{\text{wes}} = 0.300$ ‰ in b). c) and d) show the corresponding values for $\Delta_A \Delta^{17}\text{O}$. $\Delta^{17}\text{O}_{\text{global}}$ is the global average $\Delta^{17}\text{O}$ value for atmospheric CO_2 (Koren et al., 2019). When $\Delta^{17}\text{O}$ of CO_2 entering the cuvette is approximately 0.2‰ lower than the $\Delta^{17}\text{O}$ of leaf water at the CO_2 - H_2O exchange site, $\Delta^{17}\text{O}$ of the CO_2 leaving the cuvette does not change when c_m/c_a vary.

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415 Figure 6 Isotopic composition of various relevant oxygen reservoirs that affect the $\Delta^{17}\text{O}$ 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). $\Delta^{17}\text{O}$ is calculated with $\lambda=0.528$.

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 CO₂-H₂O exchange site. The water at the CO₂-H₂O 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 σ).

Parameter		Sunflower	Ivy	Maize	Irradiance ($\mu\text{mol m}^{-2} \text{s}^{-1}$)
A_n	$\mu\text{mol mol}^{-1} \text{m}^{-2} \text{s}^{-1}$	18(0.7)	12(0.7)	17(2)	300
		29(2)	15(2)	32(2)	1200
g_s	$\text{mol m}^{-2} \text{s}^{-1}$	0.45(0.14)	0.11(0.02)	0.08(0.01)	300
		0.40(0.04)	0.15(0.03)	0.16(0.02)	1200
$\delta^{18}\text{O}_e$	‰	27.26 to 31.80	28.28 to 30.48	27.26 to 30.48	
$\Delta^{17}\text{O}_e$	‰	-0.227 to 0.409	-0.215 to 0.435	-0.215 to 0.310	
$\delta^{18}\text{O}_a$	‰	33.25 to 43.87	32.64 to 35.86	34.04 to 29.764	
$\Delta^{17}\text{O}_a$	‰	-0.333 to 0.163	-0.276 to 0.327	-0.270 to 0.296	
$\Delta^{18}\text{O}_{\text{obs}}$	‰	57.12(4.70)	22.20(1.32)	17.23(1.32)	300
		34.48(3.25)	24.35(3.09)	12.78(0.83)	1200
$\Delta\Delta^{17}\text{O}_{\text{obs}}$	‰	-2.61 to -0.43	-1.03 to -0.19	-0.36 to -0.09	
$\delta^{18}\text{O}_m$	‰	52.02(1.24)	47.17(1.17)	52.62(0.52)	300
		52.62(1.42)	51.09(1.76)	55.15(1.55)	1200
$\Delta^{17}\text{O}_m$	‰	-0.41(0.001)	-0.35(0.001)	-0.40(0.01)	300
		-0.41(0.01)	-0.38(0.02)	-0.42(0.02)	1200
c_a	ppm	402 (3)	403 (3)	403 (3)	
c_i	ppm	357(10)	284(0.1)	194(20)	300
		323(10)	301(13)	194(15)	1200
c_c	ppm	277(15)	188(30)		300
		201(42)	163(21)		1200
c_m	ppm	320(10)	220(10)	134(15)	300
		252(27)	214(12)	88 (17)	1200

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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 $\Delta^{17}\text{O}$ measurements.**

Parameters and values used for global estimation		
Parameter	Value	ref
GPP	120 PgCyr ⁻¹	(Beer et al., 2010)
f_{C4}	23 %	(Still et al., 2003)
f_{C3}	77 %	(Still et al., 2003)
c_m/c_a (C ₃)	0.7	(Hoag et al., 2005)
c_m/c_a (C ₄)	0.3	(Hoag et al., 2005)
$\Delta^{17}\text{O}$ leaf water (global mean, modelled)	-0.067±0.04 ‰	(Koren et al., 2019)
$\Delta^{17}\text{O}$ CO ₂ (global mean, modelled)	-0.168±0.013 ‰	(Koren et al., 2019)
$\Delta\Delta^{17}\text{O}$ (global mean for C ₄)	-0.3±0.18 ‰	(Figure 5b, for c_m/c_a ratio of 0.3)
$\Delta\Delta^{17}\text{O}$ (global mean for C ₃)	-0.65±0.18 ‰	(Figure 5b, for c_m/c_a ratio of 0.7)
$\Delta\Delta^{17}\text{O}$ (global mean for whole vegetation)	-0.57±0.14 ‰	(Equation 13)
$\Delta\Delta^{17}\text{O}$ -isoflux (global mean for C ₄)	-7.3±4 ‰PgCyr ⁻¹	(Equation 14, only for C ₄)
$\Delta\Delta^{17}\text{O}$ -isoflux (global mean for C ₃)	-53±15 ‰PgCyr ⁻¹	(Equation 14, only for C ₃)
$\Delta\Delta^{17}\text{O}$ -isoflux (global mean for whole vegetation)	-60±15 ‰PgCyr ⁻¹	(equation 14)
$\Delta\Delta^{17}\text{O}$ -isoflux (global mean for whole vegetation)	-47 ‰PgCyr ⁻¹	(Hoag et al., 2005)
$\Delta\Delta^{17}\text{O}$ -isoflux (global mean for whole vegetation)	-42 to -92 ‰PgCyr ⁻¹	(Hofmann et al., 2017)
$\Delta^{17}\text{O}$ value of tropospheric CO ₂		
$\Delta^{17}\text{O}(\text{CO}_2)$ for CO ₂ samples collected in La Jolla-UCSD (California, USA) (1990 to 2000)	-0.173±0.046 ‰	(Thiemens et al., 2014)
$\Delta^{17}\text{O}(\text{CO}_2)$ for CO ₂ samples collected in Israel	0.034±0.010 ‰	(Barkan and Luz, 2012)
$\Delta^{17}\text{O}(\text{CO}_2)$ for CO ₂ samples collected in South china sea (2013-2014)	-0.159±0.084 ‰	(Liang et al., 2017a;Liang et al., 2017b)
$\Delta^{17}\text{O}(\text{CO}_2)$ for CO ₂ samples collected in Taiwan (2012-2015)	-0.150±0.080 ‰	(Liang et al., 2017a;Liang et al., 2017b)
$\Delta^{17}\text{O}(\text{CO}_2)$ for CO ₂ samples collected in California (USA) (2015)	-0.177±0.029 ‰	(Liang et al., 2017a;Liang et al., 2017b)
$\Delta^{17}\text{O}(\text{CO}_2)$ for CO ₂ samples collected in Göttingen (Germany) (2010-2012)	-0.122±0.065 ‰	(Hofmann et al., 2017)

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Table 3 List of symbols and variables

Symbol	description	Unit/calculation/value
Gas exchange		
A_n	Rate of CO ₂ assimilation	$\frac{u_a}{s} \left(c_e - c_a \frac{1-w_e}{1-w_a} \right)$ mol m ⁻² s ⁻²
E	Transpiration rate	$\frac{u_a}{s} \left(\frac{w_a - w_e}{1-w_a} \right)$ mol m ⁻² s ⁻²
w_i	Mole fraction of water vapour inside leaf	$\frac{613.65 \times e^{(17.502 \times T_{leaf})}}{(243.97 + T_{leaf})} \times 10^{-5}$ mol mol ⁻¹
w_a	Mole fraction of water vapour leaving the cuvette /leaf surrounding	WVIA / IRGA, mol mol ⁻¹
w_e	Mole fraction of water vapour entering the cuvette	WVIA/IRGA, mol mol ⁻¹
c_e	Mole fraction of CO ₂ entering the cuvette	IRGA, mol mol ⁻¹
c_a	Mole fraction of CO ₂ in the leaf surrounding/ leaving the cuvette	IRGA, mol mol ⁻¹
u_a	Flow rate of air entering the cuvette	mol s ⁻¹
s	Surface area of the leaf inside the cuvette	m ²
P	Atmospheric pressure	bar
T_{leaf}	Leaf temperature	°C
g_{w/H_2O}	Stomatal conductance for water vapour	$\frac{g_{H_2O} \times g_{B/H_2O}}{g_{B/H_2O} + g_{H_2O}}$
g_{B/H_2O}	Boundary layer conductance for water vapour	Calibrated for the cuvette we used
$g_{H_2O}^s$	Conductance for water vapor through the boundary layer and stomata	$E \left(\frac{1 - (w_i - w_a)}{w_i - w_a} \right)$ mol m ⁻² s ⁻¹
g_s	Stomatal conductance for CO ₂	$\frac{g_{H_2O}^s}{1.6}$
g_b	Boundary conductance for CO ₂	$\frac{g_{B/H_2O}}{1.37}$
$g_{CO_2}^s$	Conductance for CO ₂ through the boundary layer and stomata	$\frac{g_s \times g_b}{g_s + g_b}$
Γ^*	CO ₂ compensation point	45 μmol m ⁻² s ⁻¹
g_{m13}	CO ₂ conductance from intercellular air space to the site of carboxylation calculated using Δ _A ¹³ C (for C ₃ plants only)	mol m ⁻² s ⁻¹ bar ⁻¹
g_{m14}	CO ₂ conductance from intercellular air space to CO ₂ -H ₂ O exchange site calculated using Δ _A ¹⁸ O	mol m ⁻² s ⁻¹ bar ⁻¹
g_{m12}	CO ₂ conductance from intercellular air space to CO ₂ -H ₂ O exchange site calculated using Δ _A ¹⁷ O	mol m ⁻² s ⁻¹ bar ⁻¹
g_{m11}	CO ₂ conductance from intercellular air space to CO ₂ -H ₂ O exchange site calculated using Δ _A ¹⁷ O	mol m ⁻² s ⁻¹ bar ⁻¹
c_i	Mole fraction of CO ₂ in the intercellular air space	$\frac{(g_{CO_2}^s - \Gamma^*)c_a - A_n}{(g_{CO_2}^s + \Gamma^*)}$ mol mol ⁻¹
c_s	Mole fraction of CO ₂ at the leaf surface	$c_a - \frac{A_n}{g_b}$
c_m	Mole fraction of CO ₂ at the site of CO ₂ -H ₂ O exchange	mol mol ⁻¹
c_c	Mesophyll conductance to the chloroplast (for C ₃ plants)	$c_i - \frac{A_n}{g_{m13}}$ mol mol ⁻¹
f^{13}	Ternary correction for ¹³ CO ₂	$\frac{(1 + \alpha_{1382})E}{2g_{CO_2}^s}$
f^{18}	Ternary correction for ¹⁸ CO ₂	$\frac{(1 + \alpha_{1882})E}{2g_{CO_2}^s}$
f^{17}	Ternary correction for ¹⁷ CO ₂	$\frac{(1 + \alpha_{1782})E}{2g_{CO_2}^s}$

R_D	Dark respiration rate	$0.8 \mu\text{mol m}^{-2}\text{s}^{-1}$
R_L	Day respiration rate	$0.5 \times R_D \mu\text{mol m}^{-2}\text{s}^{-1}$
Oxygen and carbon isotope effects		
ϵ^{18}_k	Kinetic fractionation of water vapour in air	$\frac{28g_b + 19g_s}{g_b + g_s} \text{‰}$
$\epsilon^{18}_{\text{eq}}$	Equilibrium fractionation between liquid and gas phase of water vapor	$2.644 - 3.206 \left(\frac{10^3}{T_{\text{leaf}}}\right) + 1.534 \left(\frac{10^6}{T_{\text{leaf}}}\right) \text{‰}$
a_{13b}	Weighted fractionation for $^{13}\text{CO}_2$ as CO_2 diffuses through the boundary layer and stomata	$\frac{(c_s - c_l)a_{13s} + (c_a - c_s)a_{13b}}{c_a - c_l} \text{‰}$
a_{17b}	Weighted fractionation for C^{17}O as CO_2 diffuses through the boundary layer and stomata	$\frac{(c_s - c_l)a_{17s} + (c_a - c_s)a_{17b}}{c_a - c_l} \text{‰}$
a_{18b}	Weighted fractionation for C^{18}O as CO_2 diffuses through the boundary layer and stomata	$\frac{(c_s - c_l)a_{18s} + (c_a - c_s)a_{18b}}{c_a - c_l} \text{‰}$
a_{13l}	Weighted fractionation for $^{13}\text{CO}_2$ as CO_2 diffuses through the boundary layer and stomata	$\frac{(c_s - c_l)a_{13s} + (c_a - c_s)a_{13b}}{c_a - c_l} \text{‰}$
a_{18l}	Weighted fractionation for C^{18}O as CO_2 diffuses through the boundary layer and stomata	$\frac{(c_s - c_l)a_{18s} + (c_a - c_s)a_{18b}}{c_a - c_l} \text{‰}$
a_{17l}	Weighted fractionation for C^{17}O as CO_2 diffuses through the boundary layer and stomata	$\frac{(c_s - c_l)a_{17s} + (c_a - c_s)a_{17b}}{c_a - c_l} \text{‰}$
\bar{a}_{17}	Weighted fractionation of C^{17}O as it diffuses through the boundary layer, stomata and liquid phase in series	$\frac{(c_l - c_m)a_{17w} + (c_s - c_l)a_{17b} + (c_a - c_s)a_{17b}}{c_a - c_m} \text{‰}$
\bar{a}_{18}	Weighted fractionation of C^{18}O as it diffuses through the boundary layer, stomata and liquid phase in series	$\frac{(c_l - c_m)a_{18w} + (c_s - c_l)a_{18b} + (c_a - c_s)a_{18b}}{c_a - c_m} \text{‰}$
a_{13}	Fractionation in $^{13}\text{CO}_2$ as CO_2 diffuses through the boundary layer	2.9‰
a_{13s}	Fractionation in $^{13}\text{CO}_2$ as CO_2 diffuses through the stomata	4.4‰
a_w	Fractionation factor for dissolution and diffusion through water	1.8‰
f	Fractionation factor for photorespiration (decarboxylation of glycine)	16‰
e	Fractionation factor for day respiration	$R_D + e^* \text{‰}$
e^*	Apparent fractionation for day respiration	$\delta^{13}C_R - \Delta_A^{13}C - \delta^{13}C_{\text{substrate}} \text{‰}$
b	Fractionation factor for uptake by RubisCO	29‰
g	Fractionation due to photorespiration (decarboxylation of glycine)	$1+f$
g_e	Fractionation due to day respiration	$1+e$
g_b	Fractionation due to uptake by RubisCO	$1+b$
a_{17a}	Fractionation of C^{17}O as CO_2 diffuses through the boundary layer	2.9‰
a_{17s}	Fractionation in C^{17}O as CO_2 diffuses through stomata	4.4‰
a_{18a}	Fractionation of C^{18}O as CO_2 diffuses through the boundary layer	5.8‰
a_{18s}	Fractionation in C^{18}O as CO_2 diffuses through stomata	8.8‰
a_{17w}	Fractionation in C^{17}O due to diffusion and dissolution in water	0.382‰
a_{18w}	Fractionation in C^{18}O due to diffusion and dissolution in water	0.8‰
ϵ^{18}_R	Equilibrium fractionation of CO_2 and water for C^{18}O	$\frac{17604}{T_{\text{leaf}}} - 17.93 \text{‰}$
ϵ^{18}_k	kinetic fractionation of water vapor in air	$\frac{28 \times g_b + 19 \times g_s}{g_b + g_s}$
$\epsilon^{18}_{\text{eq}}$	equilibrium fractionation between liquid and gas phase water	$2.644 - 3.206 \times \left(\frac{10^3}{T}\right) + 1.534 \times \left(\frac{10^6}{T}\right)$

Isotopic composition		
$\delta^{17}O_A$	$\delta^{17}O$ of the assimilated CO_2	$\frac{\delta^{17}O_A - \Delta A^{17}O}{\Delta A^{17}O + 1} = \delta^{17}O_A - \frac{\epsilon_e}{\epsilon_e - \epsilon_A} (\delta^{17}O_A - \delta^{17}O_e)$
$\delta^{18}O_A$	$\delta^{18}O$ of the assimilated CO_2	$\frac{\delta^{18}O_A - \Delta A^{18}O}{\Delta A^{18}O + 1} = \delta^{18}O_A - \frac{\epsilon_e}{\epsilon_e - \epsilon_A} (\delta^{18}O_A - \delta^{18}O_e)$
$\delta^{17}O_{int}$	$\delta^{17}O$ of CO_2 in the intercellular air space ignoring ternary correction	$\delta^{17}O_A \left(1 - \frac{\epsilon_A}{c_i}\right) (1 + a17bs) + \frac{\epsilon_A}{c_i} (\delta^{17}O_A - a17bs) + a17bs \text{ ‰}$
$\delta^{18}O_{int}$	$\delta^{18}O$ of CO_2 in the intercellular air space ignoring ternary correction	$\delta^{18}O_A \left(1 - \frac{\epsilon_A}{c_i}\right) (1 + a18bs) + \frac{\epsilon_A}{c_i} (\delta^{18}O_A - a18bs) + a18bs \text{ ‰}$
$\delta^{17}O_c$	$\delta^{17}O$ of CO_2 in the intercellular air space	$\frac{\delta^{17}O_{i0} + t^{17} (\delta^{17}O_A (\frac{\epsilon_A + 1}{c_i}) - \delta^{17}O_e \frac{\epsilon_A}{c_i})}{1 + t^{17}} \text{ ‰}$
$\delta^{18}O_c$	$\delta^{18}O$ of CO_2 in the intercellular air space	$\frac{\delta^{18}O_{i0} + t^{18} (\delta^{18}O_A (\frac{\epsilon_A + 1}{c_i}) - \delta^{18}O_e \frac{\epsilon_A}{c_i})}{1 + t^{18}} \text{ ‰}$
$\delta^{18}O_{wms}$	$\delta^{18}O$ of transpired water vapour	$\left(\frac{w_a}{w_a - w_e}\right) (\delta^{18}O_{wa} - \delta^{18}O_{we}) + \delta^{18}O_{we} \text{ ‰}$
$\delta^{18}O_{wes}$	$\delta^{18}O$ of water at the evaporation site	$\delta^{18}O_{wes} = \delta^{18}O_{trans} + \epsilon^{18}k + \epsilon^{18} \text{ equ} + \frac{w_a}{w_i} \times (\delta^{18}O_{wa} - \epsilon^{18}k + \delta^{18}O_{trans})$
$\delta^{17}O_m$	$\delta^{17}O$ of CO_2 at the site of CO_2 - H_2O exchange	$(\delta^{17}O_{wes} + 1) \times (1 + \epsilon_w^{17}) - 1 \text{ ‰}$
$\delta^{18}O_m$	$\delta^{18}O$ of CO_2 at the site of CO_2 - H_2O exchange	$(\delta^{18}O_{wes} + 1) \times (1 + \epsilon_w^{18}) - 1 \text{ ‰}$
$\delta^{13}C_{substrate}$	Isotope (^{13}C) ratio of substrate used for dark respiration	$\frac{\delta^{13}C_s - \Delta A^{13}C}{\Delta A^{13}C + 1} \text{ ‰}$
$\Delta A^{13}C$	^{13}C -photosynthetic discrimination	$\frac{c_i (\delta^{13}C_s - \delta^{13}C_e)}{1 + \delta^{13}C_s - c_i (\delta^{13}C_s - \delta^{13}C_e)} \text{ ‰}$
$\Delta A^{13}C_{obs}$	^{13}C -photosynthetic discrimination (Farquhar model)	$\left(\frac{1}{1-t}\right) \left[a_{13bs} \frac{c_a - c_i}{c_a} + \left(\frac{1+t}{1-t}\right) \left[a_m \frac{c_i - c_c}{c_a} + b \frac{c_c}{c_a} - \frac{\alpha_b}{\alpha_e} e \frac{R_D}{R_D + A_n} - \frac{c_c - \Gamma^*}{c_a} - \frac{\alpha_b}{\alpha_f} \frac{\Gamma^*}{c_a} \right] \right]$
$\Delta A^{13}C_i$	^{13}C -photosynthetic discrimination (assuming no mesophyll conductance, i.e. $c_i = c_c$)	$\left(\frac{1}{1-t}\right) \left[a \frac{c_a - c_i}{c_a} \right] + \left(\frac{1+t}{1-t}\right) \left[b \frac{c_i}{c_a} - \frac{\alpha_b}{\alpha_e} e \frac{R_D}{R_D + A_n} - \frac{c_i - \Gamma^*}{c_a} - \frac{\alpha_b}{\alpha_f} \frac{\Gamma^*}{c_a} \right]$
$\Delta A^{18}O$	^{18}O -photosynthetic discrimination	Equation 3, ‰
$\Delta A^{17}O$	^{17}O -photosynthetic discrimination	$\frac{c_i (\delta^{17}O_s - \delta^{17}O_e)}{1 + \delta^{17}O_s - c_i (\delta^{17}O_s - \delta^{17}O_e)} \text{ ‰}$
$\Delta A^{17}O_{FM}$	Farquhar model for ^{17}O -photosynthetic discrimination	$\frac{\bar{a}_{17} + \frac{c_m}{c_a - c_m} \delta^{17}O_{ma}}{1 - \frac{c_m}{c_a} \delta^{17}O_{ma}} \text{ ‰}$
$\Delta A^{18}O_{FM}$	Farquhar model for ^{18}O -photosynthetic discrimination	$\frac{\bar{a}_{18} + \frac{c_m}{c_a - c_m} \delta^{18}O_{ma}}{1 - \frac{c_m}{c_a} \delta^{18}O_{ma}} \text{ ‰}$
$\delta^{17}O_e$	$\delta^{17}O$ of CO_2 entering the cuvette	‰
$\delta^{17}O_e$	$\delta^{17}O$ of CO_2 leaving the cuvette	‰
$\delta^{18}O_e$	$\delta^{18}O$ of CO_2 entering the cuvette	‰
$\delta^{18}O_e$	$\delta^{18}O$ of CO_2 leaving the cuvette	‰
$\delta^{17}O_{int}$	$\delta^{17}O$ of CO_2 equilibrated with the leaf water at the evaporating site relative to the CO_2 leaving the cuvette	$\frac{\delta^{17}O_m - \delta^{17}O_e}{1 - \delta^{17}O_e} \text{ ‰}$
$\delta^{18}O_{int}$	$\delta^{18}O$ of CO_2 equilibrated with the leaf water at the evaporating site relative to the CO_2 leaving the cuvette	$\frac{\delta^{18}O_m - \delta^{18}O_e}{1 - \delta^{18}O_e} \text{ ‰}$
$\delta^{18}O_{we}$	$\delta^{18}O$ of water vapour entering the cuvette	WVIA, ‰
$\delta^{18}O_{wa}$	$\delta^{18}O$ of water vapour leaving the cuvette/leaf surrounding	WVIA, ‰

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