

From: Getachew Agmuas Adnew and co-authors

Referee: 1

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

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

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

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

The referee is correct that in principle ^{18}O indeed cycles through the same biological system, and undergoes the same (bio)physical processes. However, we would like to nuance the idea that $\delta^{18}\text{O}$ can help us FULLY understand ALL processes of interest. This is because conventional $\delta^{18}\text{O}$ studies have a 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.

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

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.

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

CO₂ and leaf water affect the results, and that experiments with ¹⁷O labeled CO₂ actually increase the signal to (measurement) noise ratio.

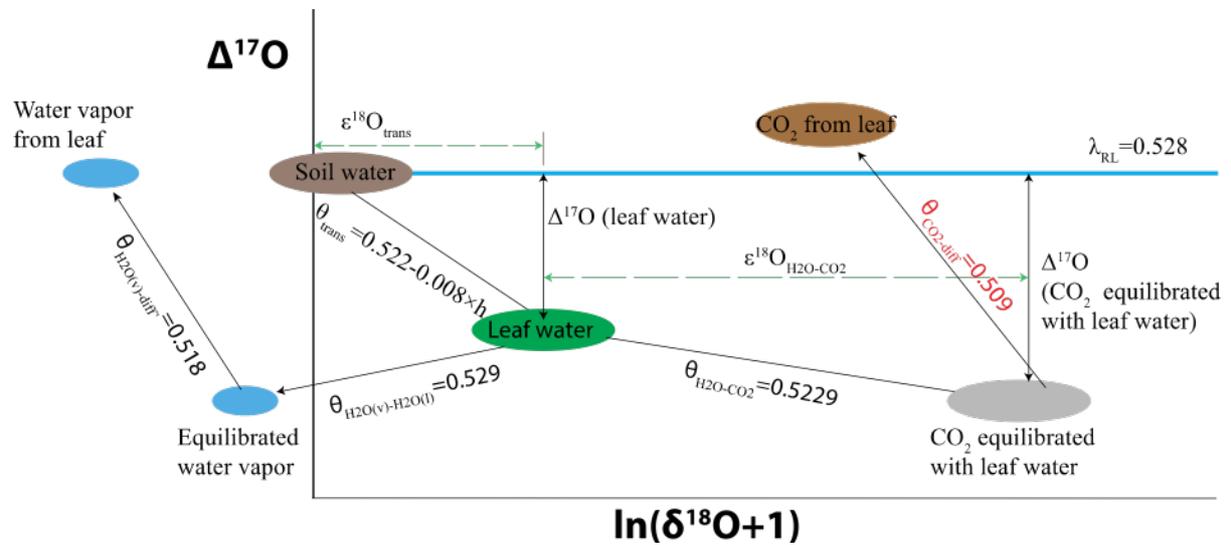


Figure 1. Schematic representation of the processes that affect the $\Delta^{17}\text{O}$ of CO₂ and H₂O during photosynthetic gas exchange (not to scale). The triple oxygen isotope slope for transpiration at $h=75\%$ relative humidity is $\theta_{\text{trans}}=0.522-0.008 \times h = 0.516$ (Landais et al., 2006). The triple isotope slope of CO₂-H₂O exchange is $\theta_{\text{CO}_2\text{-H}_2\text{O}} = 0.5229$ (Barkan and Luz, 2012). The triple isotope slope for diffusion of CO₂ is $\theta_{\text{CO}_2\text{-diff}} = 0.509$ (Young et al., 2002) and for diffusion of water vapor $\theta_{\text{H}_2\text{O(v)-diff}} = 0.518$ (Barkan and Luz, 2007). The three isotope slope for equilibration between liquid (l) and gaseous (v) water is $\theta_{\text{H}_2\text{O(v)-H}_2\text{O(l)}} = 0.529$ (Barkan and Luz, 2005). $\epsilon^{18}\text{O}$ is the enrichment or depletion in ¹⁸O due to the corresponding isotope fractionation process.

And so, while the present paper goes through an impressive exercise of gas exchange and isotopic measurements and calculations, I fail to see the purpose and merit of this exercise, beyond a test that verifies that indeed the ¹⁷O measurements are consistent with the ¹⁸O studies. **The omissions as much as I can see are already fairly well-known from ¹⁸O studies and, in fact, much of the calculations here still depends on the ¹⁸O measurements.**

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.

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.

Specifically, the results communicated in our manuscript

a) demonstrate that the established theory is applicable to $\Delta^{17}\text{O}$ -CO₂ exchange at leaf-level.

- b) experimentally quantify for the first time the effect of photosynthesis on $\Delta^{17}\text{O}$ of atmospheric CO_2
- c) quantify of the dependence of this effect on critical parameters
- d) 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).

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 ^{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.

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.

- a) demonstrate that the established theory is applicable to $\Delta^{17}\text{O}$ -CO₂ exchange at leaf-level
- b) experimentally quantify for the first time the effect of photosynthesis on $\Delta^{17}\text{O}$ of atmospheric CO₂
- c) study of the dependence of this effect on critical parameters
- d) provide an independent bottom-up $\Delta^{17}\text{O}$ -isoflux estimate based on these lab experiments.

Barkan, E., and Luz, B.: High precision measurements of $^{17}\text{O}/^{16}\text{O}$ and $^{18}\text{O}/^{16}\text{O}$ ratios in H₂O, Rapid Commun. Mass. Sp., 19, 3737-3742, 10.1002/rcm.2250, 2005.

Barkan, E., and Luz, B.: Diffusivity fractionations of H₂¹⁶O/H₂¹⁷O and H₂¹⁶O/H₂¹⁸O in air and their implications for isotope hydrology, Rapid Commun. Mass. Sp., 21, 6, 2007.

Barkan, E., and Luz, B.: High-precision measurements of $^{17}\text{O}/^{16}\text{O}$ and $^{18}\text{O}/^{16}\text{O}$ ratios in CO₂, Rapid Commun. Mass. Sp., 26, 2733-2738, 10.1002/rcm.6400, 2012.

Koren, G., Schneider, L., Velde, I. R. v. d., Schaik, E. v., Gromov, S. S., Adnew, G. A., D.J.Mrozek, Hofmann, M. E. D., Liang, M.-C., Mahata, S., Bergamaschi, P., Laan-Luijkx, I. T. v. d., Krol, M. C., Röckmann, T., and Peters, W.: Global 3-D Simulations of the Triple Oxygen Isotope Signature $\Delta^{17}\text{O}$ in Atmospheric CO₂ J. Geophys. Res-Atmos., 124, 28, 2019.

Landais, A., Barkan, E., Yakir, D., and Luz, B.: The triple isotopic composition of oxygen in leaf water, Geochim. Cosmochim. Ac., 70, 4105-4115, 10.1016/j.gca.2006.06.1545, 2006.

McManus, J. B., Nelson, D. D., Shorter, J. H., Jimenez, R., Herndon, S., Saleska, S., and Zahniser, M.: A high precision pulsed quantum cascade laser spectrometer for measurements of stable isotopes of carbon dioxide, J. Mod. Optic., 52, 12, 2005.

Young, E. D., Galy, A., and Nagahara, H.: Kinetic and equilibrium mass-dependent isotope fractionation laws in nature and their geochemical and cosmochemical significance, Geochim. Cosmochim. Ac., 66, 9, 2002.