Dear Mr Aninda Mazumdar,

We are grateful for the invitation to review our manuscript for a second time entitled "Determination of respiration and photosynthesis fractionation factors for atmospheric dioxygen inferred from a vegetation-soil-atmosphere analog of the terrestrial biosphere in closed chambers." We thank the reviewer because he/she helped us to improve the article. We have made the changes suggested by the reviewer in a version provided below. And, a detailed point-by-point response to the reviewers' comments is provided below.

We hope that you will find this revised manuscript suitable for publication,

On the behalf of all co-authors,

Clémence Paul

Point-to-point response

black = reviewer comment/ purple = answers/ blue = new text/ green = unchanged text

Reply to Referee

First, the formulations of Equation 8 and 14 is still confusing and counter-intuitive. I think it would be better to put a minus sign in front of dn(O2) and dn_total_respi, respectively:

n(O2)_t+dt = n(O2)_t - dn(O2)

n(O2)_t+dt = n(O2)_t - dn_total_respi + dn_photosynthesis

It preserves the intuitive sense that respiration removes O2, even though the value of those quantities is positive. The equations derived from these would then need to be modified accordingly. Equation 19 and aN in L567, for example, would need a negative sign in front of F_total_respi to preserve the same sense.

We have followed these suggestions and modified the equations in the text:

$$n(O_2)_{t+dt} = n(O_2)_t - dn(O_2) \qquad (8)$$

$$^{18}R_{t+dt} \times n(O_2)_{t+dt} = {}^{18}R_t \times n(O_2)_t - {}^{18}R_t \times {}^{18}\alpha_{soil\_respi} \times dn(O_2) \qquad (9)$$

$$^{18}R_{t+dt} \times \frac{n(O_2)_{t+dt}}{n(O_2)_{t0}} = {}^{18}R_t \times \frac{n(O_2)_t}{n(O_2)_{t0}} - {}^{18}R_t \times {}^{18}\alpha_{total\_respi} \times \frac{dn_{total\_respi}}{n(O_2)_{t0}} + {}^{18}R_{lw} \times {}^{18}\alpha_{photosynthesis} \times \frac{dn_{photosynthesis}}{n(O_2)_{t0}} \qquad (15)$$

$$^{18}\alpha_{photosynthesis} = \frac{n(O_2)_t \,/\, n(O_2)_{t0} \times a^{18}R + {}^{18}R_t \times \left(F_{photosynthesis} - F_{total_{respi}} + {}^{18}\alpha_{total_{respi}} \times F_{total_{respi}} \right)}{^{18}R_{lw} \times F_{photosynthesis}} \qquad (19)$$

$$ {}^{18}\alpha_{photosynthesis} = \frac{a^{18}R + aN + \langle {}^{18}\alpha_{total\_respi} \rangle \times \langle F_{total\_respi} \rangle}{{}^{18}R_{lw} \times F_{photosynthesis}} \tag{29} $$

We also calculated the net O_2 flux during light periods, $aN = F_{photosynthesis} - F_{total\_respi}$, as the linear regression, aN, of $\frac{n(O_2)_t}{n(O_2)_{t0}}$ with time. $a^{18}R$ is also obtained as a linear regression of ${}^{18}R$ with time over each light period.

Second, the estimation of fractionation factors in the light seems to underestimate systematic uncertainties. The fractions and fractionation factors for Mehler and photorespiration are assumed based on prior literature but it is not clear whether (or how) these assumptions were included in Monte Carlo uncertainty estimates. While some effort has been made to do some sensitivity tests in 3.2.2 and in the supplement, they could use with some elaboration and justification. How sensitive are the estimates to the two different values of 18e_Mehler?

We agree that we did not account for uncertainty associated with the values of ${}^{18}\varepsilon_{Mehler}$ since we assumed that the latest estimate given by Helman et al. (2005) was the one of reference. Indeed, this may not be true and we have now added a test (test 4 in the supplement and tables S8 and S9) in which we tested an older value for ${}^{18}\varepsilon_{Mehler}$: 15.3‰ (Guy et al., 1993). The resulting fractionation factors associated with photosynthesis is not significantly changed compared to the previous version of the manuscript when using this last value for Mehler discrimination (Table S8 and S9).

We added this text in the supplementary text 2:

From uncertainties on these values of 0.5 ‰ and 0.3 ‰ for ${}^{18}\varepsilon_{photo\_respi}$ and ${}^{17}\varepsilon_{photo\_respi}$ (Helman et al., 2005), we used alternative values of -20.8 ‰ and -11.04 ‰ for ${}^{18}\varepsilon_{photo\_respi}$ and ${}^{17}\varepsilon_{photo\_respi}$. For the Mehler reaction we also chose the value proposed by Helman et al. (2005): ${}^{18}\varepsilon_{Mehler} =$ - 10.8 ‰ and ${}^{17}\varepsilon_{Mehler} =$ - 5.7 ‰. We also tested the uncertainty in the fractionation factors of the Mehler reaction (test 4) as given by Guy et al. (1993): ${}^{18}\varepsilon_{Mehler} =$ - 15.3 ‰ and ${}^{17}\varepsilon_{Mehler} =$ - 7.98 ‰.

Table S7. Details of input parameters for sensitivity tests. White columns detail the tests on the fractions of oxygen consumption; grey column details the test on the values of isotopic discrimination.

	Test 0	Test 1	Test 2	Test 3	Test 4
f_{dark_respi}	1	0.9	0.4	0.6	0.6
f_{photo_respi}	0	0	0.5	0.3	0.3
f_{Mehler}	0	0.1	0.1	0.1	0.1
${}^{18}\varepsilon_{photo\_respi}$	-21.3	-21.3	-21.3	-20.8	-20.8
${}^{17}\varepsilon_{photo\_respi}$	-11.07	-11.07	-11.07	-11.04	-11.04
${}^{18}\varepsilon_{Mehler}$	-10.8	-10.8	-10.8	-10.8	-15.3
${}^{17}\varepsilon_{Mehler}$	-5.7	-5.7	-5.7	-5.7	-7.9

Table S8. α_{total_respi} values obtained from the sensitivity tests: results of sensitivity tests on dioxygen consumption fractions (white columns) and on uncertainties in fractionation factor values

(grey column). µ is the average over all lines above of the different quantities and σ the associated standard deviation. The missing data are due to a problem during measurements.

Sequence	Period	$^{18}\alpha_{total\_res}$ Test 0	$^{18}\alpha_{total\_res}$ Test 1	$^{18}\alpha_{total\_res}$ Test 2	$^{18}\alpha_{total\_res}$ Test 3	$^{18}\alpha_{total\_resp}$ Test 4	$^{17}\alpha_{total\_res}$ Test 0	$^{17}\alpha_{total\_res}$ Test 1	$^{17}\alpha_{total\_res}$ Test 2	$^{17}\alpha_{total\_res}$ Test 3	$^{17}\alpha_{total\_resp}$ Test 4
1	1	0.9868	0.9870	0.9830	0.9847	0.9841	0.9933	0.9934	0.9912	0.9921	0.9932
	2	0.9865	0.9868	0.9829	0.9846	0.9840	0.9933	0.9934	0.9912	0.9921	0.9932
	3	0.9871	0.9873	0.9831	0.9849	0.9843	0.9935	0.9936	0.9913	09922	0.9934
	4	0.9859	0.9862	0.9826	0.9842	0.9836	0.9926	0.9927	0.9909	0.9917	0.9925
2	1	0.9828	0.9835	0.9814	0.9824	0.9818	0.9913	0.9916	0.9904	0.9909	0.9906
	2	0.9781	0.9792	0.9795	0.9795	0.9789					
	3	0.9834	0.9839	0.9816	0.9827	0.9820	0.9913	0.9916	0.9904	0.9909	0.9907
3	1	0.9827	0.9833	0.9813	0.9822	0.9908	0.9916	0.9919	0.9908	0.9911	1.0020
	2	0.9797	0.9806	0.9801	0.9805	0.9894	0.9892	0.9897	0.9898	0.9897	1.0014
	3	0.9879	0.9880	0.9834	0.9854	0.9922	0.9938	0.9939	0.9917	0.9924	1.0031
µ		0.9838	0.9843	0.9818	0.9829	0.9861	0.9922	0.9924	0.9909	0.9914	0.9918
σ		0.0031	0.0028	0.0013	0.0019	0.0061	0.0014	0.0013	0.00055	0.0008	0.0014

Table S9. $\alpha_{photosynthesis}$ values obtained from the sensitivity tests: results of sensitivity tests on dioxygen consumption fractions (white columns) and on uncertainties in fractionation factor values (grey column). µ is the average over all lines above of the different quantities and σ the associated standard deviation.

Sequence	Period	$^{18}\alpha_{photosynthe}$ Test 0	$^{18}\alpha_{photosynthe}$ Test 1	$^{18}\alpha_{photosynthe}$ Test 2	$^{18}\alpha_{photosynthe}$ Test 3	$^{18}\alpha_{photosynthesi}$ Test 4	$^{17}\alpha_{photosynthe}$ Test 0	$^{17}\alpha_{photosynth}$ Test 1	$^{17}\alpha_{photosynth}$ Test 2	$^{17}\alpha_{photosynth}$ Test 3	$^{17}\alpha_{photosynthesis4}$ Test 4
1	1	0.9941	0.9944	0.9951	0.9969	0.9974	0.9969	0.9970	0.9974	0.9972	0.9974
	2	1.0040	1.0040	1.0036	1.0038	1.0021	1.0020	1.0020	1.0018	1.0019	1.0020
	3	1.0039	1.0039	1.0034	1.0037	1.0018	1.0017	1.0017	1.0015	1.0016	1.0018
2	1	1.0021	1.0023	1.0023	1.0024	1.0022	1.0013	1.0014	1.0013	1.0013	1.0022
	2	1.0043	1.0044	1.0040	1.0043	1.0042	1.0021	1.0021	1.0019	1.0020	1.0042
3	1	1.0037	1.0039	1.0039	1.0040	1.0038	1.0096	1.0021	1.0021	1.0021	1.0020
	2	1.0019	1.0022	1.0026	1.0024	1.0022	1.0013	1.0014	1.0016	1.0014	1.0014
	3	1.0063	1.0066	1.0056	1.0062	1.0059	1.0034	1.0035	1.0030	1.0035	1.0031
μ		1.0026	1.0027	1.0026	1.0030	1.0025	1.0023	1.0014	1.0013	1.0013	1.0014
σ		0.0039	0.0034	0.0030	0.0025	0.0034	0.0033	0.0018	0.0016	0.0017	0.0017

The requested Monte-Carlo calculation has also been included as explained below in the answer to comment of L584-599.

Finally, is there a working rationale for why terrestrial plant photosynthesis would enrich O2 in 18O if chloroplasts do not? The result is unexplained (similar to that of Eisenstadt) and therefore a bit unsatisfying. Can one rule out remnants of some oxygen-consuming process like light-dependent oxygen consumption at a terminal oxidase (Mehler/PTOX etc.)?

The reviewer is right that we cannot exclude the occurrence of oxygen-consuming processes not considered in our approach to explain the observed fractionation that we attribute to photosynthesis. Our approach is a macroscopic one so we do not study the processes at the scale of the chloroplasts. This approach is pertinent for our purpose of understanding the global $\delta^{18}O$ of O_2 in the atmosphere and we assume that if a remnant oxygen-consuming process exists in our closed biological chambers, it should also exist at global scale so that it has to be taken into account.

We thus propose to keep the value of $^{18}\varepsilon_{photosynthesis}$ that we determined at the macroscopic scale because it is appropriate to our general aim of understanding the global $\delta^{18}O$ of O_2 but explain that it may also include fractionation induced by another light dependent oxygen consuming process.

We added this text in the 4.2. section:

Our experiments were performed at the scale of the plants which is different to previous studies performed at the scale of the chloroplast (e.g. Guy et al., 1993) where no evidence of oxygen fractionation has been found. We can thus not exclude that this fractionation attributed here to photosynthesis is due to oxygen consuming processes not taken into account in our approach. Our main goal however is to interpret the global $\delta^{18}O$ of atmospheric O_2 using the fractionation observed at the scale of the plants. As a consequence, we believe that if there is a light-dependent oxygen fractionation process that we did not identify in our approach, it will also be present at the global scale. It should thus be taken into account in our future interpretation of the Dole effect. We thus keep our estimate of the photosynthesis ^{18}O discrimination described above but name it as an *effective* photosynthesis ^{18}O discrimination at the scale of the plants because the details of the processes at play is not fully elucidated.

Specific comments:

L48-50: Dole's precise measurement of the 18O/16O ratio dates back to 1935 (10.1021/ja01315a511) or 1936 (10.1063/1.1749834) when he reported the relative atomic weight of oxygen in air and Lake Michigan water. His result translates somewhere in the ballpark of +21 per mil, not the 23.88 value implied in this sentence. Incidentally, the 23.88 per mil value from Barkan and Luz is perhaps overly precise given the more recent measurements from Pack (24.15; 10.1038/ncomms15702) and Wostbrock (24.05; already cited). I suggest rephrasing the latter part of this sentence to be more clear and representative of the literature and using "~24 per mil" instead.

We added the information on Pack et al. 2007 and Wostbrock and Sharp (2019), while mentioning that a given average value for the overall Dole Effect would be 24‰.

We added this text:

First, interpreting the relationship between $\delta^{18}O$ of O_2 (or $\delta^{18}O_{atm}$) variations in ice core air and the low latitude water cycle (e.g. Severinghaus et al., 2009; Landais et al., 2010; Seltzer et al., 2017) is still debated because of the multiple processes involved. Dole (1936) reported the relative atomic weight of oxygen in the air and water of Lake Michigan and gave as a measure of the ^{18}O /^{16}O ratio a value of about 21 ‰. Barkan and Luz (2005) showed that $\delta^{18}O_{atm}$ is enriched compared to the $\delta^{18}O$ of water of the global ocean (taken here as the Vienna Standard Mean Ocean Water, VSMOW) with a value of 23.88 ‰. With the more recent values of Pack et al. (2017) of 24.15 ‰ and Wostbrock and Sharp (2021) of 24.05 ‰, we can envisage an enrichment of $\delta^{18}O_{atm}$ of about ~ 24 ‰.

L61: The isotopic discrimination found by Guy et al. varied a bit depending on origin (cyano/diatom/chloroplast). This sentence should at least acknowledge that what is being reported is an average of the three results.

We mentioned that the given value is an average of these three organism.

L82/83, 663/664: Nannocloreopsis --> Nannochloropsis & oreinhardtii --> reinhardtii

Corrected.

L85: better be --> be better

Corrected.

L88: delete "the" before Southeast Asia

Corrected.

L122: delete "the" before dark respiration

Corrected.

L584-599: Please include more information about the sensitivity tests in the main text. I don't know from here what the numerical range of each variable was, how it was chosen, nor how multivariate problems were addressed (e.g., photorespiration and Mehler). The supplement only shows a couple tests -- I imagine the authors could do a Monte Carlo estimate quite easily and derive a more accurate uncertainty range from that.

As explained in the previous comment, we added a test with another Mehler value given by Guy et al., 1993. This new test did not lead to significant changes of our previous conclusions.
We also added a Monte Carlo test to see the global effect of all sensitivity tests on the value of photosynthetic fractionation. This allowed us to find a standard deviation of 0.3 ‰ for $^{18}\varepsilon_{photosynthesis}$ and 0.15 ‰ for $^{17}\varepsilon_{photosynthesis}$.

Here is the text we added at the end of the 3.2.2. section:

Finally, we evaluated by a Monte Carlo calculation how the different uncertainties listed in the 3 sensitivity tests described above influence the final uncertainty on the photosynthesis isotopic discrimination. We found a final standard deviations (1σ) equal to 0.3 ‰ for $^{18}\varepsilon_{photosynthesis}$ and 0.15 ‰ for $^{17}\varepsilon_{photosynthesis}$.

L607: DARK respiration

We have no reason to add "dark" since we were discuss soil respiration.

L608-610: Theta, the slope of ln(1+d17O) vs. ln(1+d18O), and gamma are not comparable, as they are defined differently. Generally speaking, if theta = 0.516, then the observed triple-isotope slope and gamma in experiments would be larger. This section seems to suggest that they are equivalent.

Thanks for the comment, we have given the θ value associated with the γ value of (Helman et al., 2005).

We added this text:

This result is in good agreement and within the uncertainties given by Helman et al. (2005) with the γ value of 0.5174 (equivalent to a θ of 0.515 ± 0.0003) obtained with respiration experiments on several micro-organisms.

L614-624: I think it would be more appropriate to say that the increase in D17O in these experiments is due to light-dependent processes rather than photosynthesis specifically, given that it was not possible to separate them.

Actually, we would like to keep photosynthesis since with photosynthesis, we can explain the evolution of $\Delta^{17}O$ of O_2. However, we do not exclude the possibility that other light dependent processes occur during this period as mentioned in the second part of the discussion section.

L647-650: It is worth noting that Guy et al. (1989) (10.1007/BF00392616) found similar fractionation factors in P. triconutum and land plants.

We have added it, here is the added text:

Still, even if it was obtained on different organism and experimental set-up, this value is in agreement with the values for isotopic discrimination for dark respiration determined by Helman et al. (2005) on bacteria from the Lake Kinneret ($^{18}\varepsilon$= -17.1 ‰) and Synechocystis ($^{18}\varepsilon$= - 19.4 ‰ and - 19.5 ‰) and Guy et al. (1989) on Phaeodactylum tricornutum and on terrestrial plants (-17 to -19 ‰ for COX respiration).