Dear Mr Aninda Mazumdar,

We are grateful for the invitation to review our manuscript 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 both reviewers for their informative comments. It helped us a lot to improve the article. We have made the changes suggested by the two reviewers 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 of interest,

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 #1

Overall the data are of great interest to the scientific community. However, not all information is communicated for certain components of the study rendering it difficult for the reader to confirm unequivocally some of the important advances particularly those linked to the revised photosynthetic fractionation values for plants of 3.7 per mil. For example, one of the key variables to calculate 18epsilon photosynthesis is the oxygen isotope composition of leaf water (see eq 14). The authors explain that leaf samples were collected and IRMS measurements made to establish the d18O and d17O values but I could not find any reference to the values obtained or used in eq 14 or how this varied during the experiments and how stable the closed water irrigation values were during the experimental runs. It would be important that this information is provided where available in either Table 1 and/or Table 2.

The values of the leaf water measurements are now presented in supplementary Table S3 of the revised version. Unfortunately, because the experiments had to be carried in a closed chamber, we could not sample leaves during the experiment and only got a value at the end of each sequence. Still, we could compare the isotopic composition of the irrigation and soil water at the start and at the end of the experiment and the values were within the - 6 ‰ to 4 ‰ range, with respect to V-SMOW, with a tendency for higher values at the end of the sequence. If the leaf water isotopic composition follows this tendency, it means that the mean $\delta^{17}O_{lw}$ and $\delta^{18}O_{lw}$ are lower than measured during the experiments, which would then lead to an even higher fractionation factor for photosynthesis than the one presented in this manuscript. We added this text in the 2.3.3. “Photosynthesis and dark respiration experiment” section:

The values of the leaf water measurements are presented in supplementary Table S3. Because the experiments had to be carried in a closed chamber, we could not sample leaves during the experiment and only got a value at the end of each sequence. Nevertheless, we could compare the isotopic composition of the irrigation and soil water at the start and at the end of the experiment.

Here, the new table (Table S3) in the supplementary:

Table S3. Oxygen isotopic ratios for leaf water (lw), irrigation water (iw) and soil water (sw) at the beginning (t0) and end of the sequence (tf) of the photosynthesis and dark respiration experiment. The ^{17}R values are calculated here with a value of 12.03 ‰ (Luz and Barkan, 2011) for determination of the $\delta^{17}O$ of atmospheric O_2 vs $\delta^{17}O$ of VSMOW.

Sequence	$^{18}R_{lw}$	$^{17}R_{lw}$	$^{18}R_{iw,t0}$	$^{17}R_{iw,t0}$	$^{18}R_{sw,tf}$	$^{17}R_{sw,tf}$
1	0.9802	0.9899	0.9712	0.9852	0.9723	0.9858
2	0.9776	0.9885	0.9712	0.9852	0.9722	0.9857
3	0.9763	0.9878	0.9712	0.9852	0.9726	0.9859

In addition, there seems to be some inconsistencies in the development of the 18epsilon calculations.

Specifically, as written it is not clear how Eq 14 is simplified to Eq 18. Currently equation 18 has some issues with signs and a number of R's are missing. Thus, it is not possible for the reader to calculate and check the conclusions related to 18epsilon photosynthesis as valuable data and definitions are not provided. I am sure everything is fine but for the moment it is just not transparent and requires communication.

Thank you for pointing this inconsistency. In this revised version of the manuscript we addressed this issue by inverting "t" and "t+dt", which explains the issue with signs. On equation (18), the R's are actually not missing but an explanation is indeed missing. We can do the calculation at the beginning of the experiment, i.e. considering $R^{18}O_t=R^{18}O_{t0}=1$ and $n(O_2)_t = n(O_2)_{t0}$. We agree that this was impossible to understand implicitly. In the new version of the manuscript, we have explained as:

Thus, at each stage, dioxygen is both produced by photosynthesis and consumed by the aforementioned O_2 uptake processes (hereafter $total\_respi$) by the plant according to the mass conservation equation:

$$n(O_2)_{t+dt} = n(O_2)_t + dn_{total\_respi} + dn_{photosynthesis} \quad (14)$$

where dn_{total_respi} is the number of molecules of O_2 consumed by dark respiration, photorespiration and Mehler reaction between time t and t+dt, and $dn_{photosynthesis}$ is the number of molecules of O_2 produced by photosynthesis between t and t+dt.

The budget for ^{18}O of O_2 can be written as:

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

where $^{18}\alpha_{total\_respi}$ is the fractionation factors associated with each O_2 consuming process periods throughout the whole experiment.

We introduced the normalized fluxes of photosynthesis and total respiration as:

$$F_{photosynthesis} = \frac{dn_{photosynthesis}}{n(O_2)_{t0} \times dt} \quad (16)$$

$$F_{total\_respi} = \frac{dn_{total\_respi}}{n(O_2)_{t0} \times dt} \quad (17)$$

$$a^{18}R = \frac{d^{18}R}{dt} \quad (18)$$

This led to the following expression of ${}^{18}\alpha_{photosynthesis}$:

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

This equation can be simplified at t=0 for ${}^{18}R_t = {}^{18}R_{t0} = 1$ and $n(O_2)_t = n(O_2)_{t0}$

There are also certain parts of the introduction and discussion that assume a certain level of reader prior knowledge and if this paper is to appeal to a wider audience a little more work on briefly explaining the key processes involved (Mehler reactions, COX versus AOX, photorespiration) and some biological explanations could be appreciated

We propose to expand the introduction to explain the key processes:

For Mehler reaction: The Mehler reaction reduces oxygen to form a superoxide ion which is converted to hydrogen peroxide (H_2O_2) in photosystem I and then further converted to water (Mehler, 1951). Photorespiration is the result of the oxygenase activity of Rubisco (Sharkey, 1998). This enzyme can oxidize ribulose-1,5-bisphosphate with an oxygen molecule O_2. This reaction causes a loss of CO_2 incorporation, thus decreasing the photosynthetic yield (Bauwe et al., 2010). Guy et al. (1993) first found a photorespiratory discrimination of - 21.7 ‰ and a ${}^{18}O/{}^{16}O$ discrimination of - 15.3 ‰ for the Mehler reaction. Later, on a study performed on pea, Helman et al. (2005) found ${}^{18}O/{}^{16}O$ discriminations of - 21.3 ‰ and - 10.8 ‰ respectively for photorespiration and Mehler reaction.

For COX/AOX: It has been suggested that the strong discrimination observed for boreal and temperate soils is due to the involvement of the alternative oxidase pathway (AOX, Bendall and Bonner, 1971) in addition to the usual COX respiratory pathway. In the COX respiration pathway, present in the majority in plants, the cytochrome oxidase enzyme catalyzes the oxygen reduction reaction. In the AOX pathway, the oxidation of ubiquinol molecules is directly coupled to the reduction of oxygen. Guy et al. (2005) showed that, for green tissues, the respiratory discrimination of the AOX pathway is much higher (- 31 ‰) than the one of the COX pathway (- 21 ‰). Similarly, Ribas-Carbo et al. (1995) found a higher respiratory discrimination in phytoplankton that engage the AOX pathway (- 31 ‰) relative to bacteria that engage the COX pathway (- 24 ‰).

For photorespiration: Photorespiration is the result of the oxygenase activity of Rubisco (Sharkey, 1998). This enzyme can oxidize ribulose-1,5-bisphosphate with an oxygen molecule O_2. This reaction causes a loss of CO_2 incorporation, thus decreasing the photosynthetic yield (Bauwe et al., 2010). Guy et al. (1993) first found a photorespiratory discrimination of - 21.7 ‰ and a $^{18}O/^{16}O$ discrimination of - 15.3 ‰ for the Mehler reaction. Later, on a study performed on pea, Helman et al. (2005) found $^{18}O/^{16}O$ discriminations of - 21.3 ‰ and - 10.8 ‰ respectively for photorespiration and Mehler reaction.

As well as how they may vary in importance between environmental conditions for example dark respiration in the dark vs in the light

This is a good point which was overlooked in our initial manuscript. Indeed, we did not consider potential changes in respiration rates during the light and dark periods. Autotrophic (dark) respiration is actually inhibited by approximately 70% during light periods (Tcherkez et al. 2017 and Keenan et al., 2019). For heterotrophic (soil) respiration the flux is expected to be the same for different light conditions assuming that the other environmental drivers are constant (humidity, temperature, soil organic matter, etc.) (Davidson et al., 2016). As a consequence, we have added a text explaining this variability in the introduction:

Other studies had attempted to investigate the different respiratory discriminations in the light (dark respiration, Mehler reaction and photorespiration). As during the light period, dark respiration can be inhibited (70 % inhibition found by Tcherkez et al. (2017) and Keenan et al. (2019)), so that the other O_2 consuming processes are important to consider.

We will also present supplementary sensitivity tests (see supplementary text 1) for the determination of fractionation factors associated with photosynthesis considering this variation of respiration flux (see last comment of reviewer 1 for the results). The influence on the photosynthesis fractionation factors however, remains small compared to the propagated analytical uncertainties.

Supplementary text 1: Sensitivity tests to the flux of dark leaf respiration during the day

The rate of autotrophic respiration (dark leaf respiration) is expected to be lower during light periods than during dark periods (Tcherkez et al., 2017) which was not considered in the main text. These sensitivity tests hence aim at quantifying how the value of $\alpha_{photosynthesis}$ is affected when F_{dark_respi} changes from a maximum value (F_{dark_respi} during dark period) to an extreme minimum value (F_{soil_respi} during dark period, hence no dark leaf respiration during the light period) and when α_{dark_respi} changes from the global value α_{dark_respi} including leaf and soil respiration as during dark period to the value α_{soil_respi} measured during dark period. We test as well the combined effect of modification of both F_{dark_respi} and α_{dark_respi}. The results from these sensitivity tests (Table S4) show variations in $\alpha_{photosynthesis}$ within a range which is smaller than the analytical uncertainty range found for our initial determination of $\alpha_{photosynthesis}$. In particular, we found that when we modify both F_{dark_respi} and α_{dark_respi} to consider the extreme situation with only soil respiration, the mean value of $^{18}\alpha_{photosynthesis}$ is unchanged.

Table S4. $\alpha_{photosynthesis}$ values obtained from sensitivity tests with respect to different flux and fractionation factors associated with dark respiration during the day. Subscript 0: fractionation factor and flux for dark respiration during the day are the same as those determined during the night (case

described in the main text). Subscript 1: flux of dark respiration during the day is taken equal to the flux of soil respiration (no flux of dark leaf respiration), fractionation factor for dark respiration during the day is the same as during the night. Subscript 2: flux of dark respiration during the day is the same as during the night, fractionation factor for dark respiration during the day is equal to α_{soil_respi}. Subscript 3: flux of dark respiration during the day is taken equal to the flux of soil respiration, fractionation factor for dark respiration during the day is equal to α_{soil_respi}. μ is the average over all lines above of the different quantities and σ the associated standard deviation.

Sequence	Period	$^{18}\alpha_{photosynthesis,0}$	$^{18}\alpha_{photosynthesis,1}$	$^{18}\alpha_{photosynthesis,2}$	$^{18}\alpha_{photosynthesis,3}$	$^{17}\alpha_{photosynthesis,0}$	$^{17}\alpha_{photosynthesis,1}$	$^{17}\alpha_{photosynthesis,2}$	$^{17}\alpha_{photosynthesis,3}$
1	1	0.9947	0.9931	0.9948	0.9933	0.9972	0.9964	0.9972	0.9965
	2	1.0038	1.0038	1.0039	1.0038	1.0019	1.0019	1.0020	1.0019
	3	1.0037	1.0036	1.0038	1.0036	1.0016	1.0016	1.0017	1.0016
2	1	1.0023	1.0023	1.0033	1.0023	1.0024	1.0011	1.0017	1.0012
	2	1.0043	1.0046	1.0051	1.0046	1.0043	1.0020	1.0023	1.0021
3	1	1.0039	1.0032	1.0047	1.0039	1.0020	1.0017	1.0024	1.0018
	2	1.0024	1.0010	1.0033	1.0021	1.0014	1.0008	1.0019	1.0010
	3	1.0060	1.0059	1.0074	1.0068	1.0032	1.0031	1.0038	1.0034
μ		1.0026	1.0022	1.0033	1.0026	1.0018	1.0011	1.0016	1.0012
σ		0.0034	0.0039	0.0037	0.0040	0.0021	0.0020	0.0019	0.0020

and also, how dark respiration rates and isotope ratios may vary in soils with and without roots.

We propose to add the following sentences in the introduction and discussion:

Introduction: Yet, results from studies conducted at a larger scale, e.g. at the soil scale by Angert et al. (2001) found a global terrestrial respiratory $^{18}O/^{16}O$ of O_2 discrimination for soil microorganisms varying between - 12 ‰ and - 15 ‰. This is lower than the - 18 ‰ discrimination classically used for respiration, with diffusion in soil playing a role in addition to the biological respiration isotopic discrimination. Angert and Luz (2001) also showed using experiments on roots of Philodendron plants and wheat seedlings that the respiratory discrimination of a soil with roots is lower (about - 12‰) than the - 18‰ discrimination associated with the dark respiration. This is due to the low O_2 concentration in roots whose presence favors a slower diffusion.

Discussion: The isotopic discrimination $^{18}\varepsilon_{soil\_respi}$ = - 12.3 ± 1.7‰ for the soil respiration experiments is comparable to the average terrestrial soil respiration isotopic discrimination found by Angert et al. (2001) of - 12‰. Still, among the diversity of soils studied by Angert et al. (2001), the soils showing the $^{18}\varepsilon$ values closest to our values are clay soil ($^{18}\varepsilon$ = - 13‰) and sandy soil ($^{18}\varepsilon$ = - 11‰). Soil respiration isotopic discriminations are less strong than isotopic discriminations due to dark respiration alone (- 18‰, Bender et al., 1994). These lower values for soil respiration isotopic discrimination are due to the roles of root diffusion in the soil (Angert and Luz, 2001). The soils studied by Angert and Luz (2001)

are however different from our soil which was enriched in organic matter. Further experiments are then needed to understand the variability in $^{18}\varepsilon$ associated with soil respiration.

The discussion could also benefit from summarizing the different phototrophs that have been measured in the past.

We propose to complete the introduction and the discussion as follow:

Introduction: First measurements have shown that the photosynthesis itself is not associated with a strong isotopic discrimination and produces oxygen with an isotopic composition which is close to the isotopic composition of the consumed water (Vinogradov et al., 1959; Stevens et al., 1975; Guy et al., 1993; Helman et al., 2005; Luz & Barkan, 2005). This is in contrast to the early results of Dole and Jenks (1959) who proposed a photosynthetic isotopic discrimination for plants and algae of 5‰. Vinogradov et al. (1959) challenged the results of Dole and Jenks (1944) by explaining that the ^{18}O enrichment of O_2 during their photosynthesis experiments is the result of contamination by atmospheric O_2 and respiration. Guy et al. (1993) studied the photosynthetic isotopic discrimination on spinach thylakoids, cyanobacteria (*Anacystis nidulans*) and diatoms (*Phaeodactylum tricornutum*) and found only a slight isotopic discrimination of 0.3‰ which they considered negligible. Luz and Barkan (2005) also corroborates this idea by studying photosynthetic isotopic discrimination on Philodendron and did not obtain a ^{18}O enrichment of the O_2 produced. This absence of isotopic discrimination can be theoretically explained by the process of O_2 generation within photosynthesis (photosystem II) involving water oxidation by the oxygen evolving complex (Tcherkez and Farquhar, 2007). For the oceanic biosphere, the isotopic composition of O_2 produced by photosynthesis is very close to the isotopic composition of the ocean.

And: More specifically, Eisenstadt et al. (2010) determined several photosynthetic isotopic discrimination values depending on the phytoplankton studied (*Phaeodactylum tricornutum* = 4.5 ‰, *Nannocloreopsis sp*. = 3 ‰, *Emiliania huxleyi* = 5.5 ‰ and *Chlamydomonas oreinhardtii* = 7‰). If marine and terrestrial Dole effects are similar, then the past variations of $\delta^{18}O_{atm}$ cannot be attributed to different proportions of terrestrial or marine Dole effects. They would better be related to low latitude water cycle influencing the leaf water $\delta^{18}O$ consumed by photosynthesis and then the $\delta^{18}O$ of O_2 produced by this process (with a larger flux in the low latitude vegetated regions).

Discussion: The average $^{18}\varepsilon_{photosynthesis}$ is + 3.7 ± 1.3‰ for *Festuca arundinacea* species which goes against the classical assumption that terrestrial photosynthesis does not fractionate (Vinogradov et al., 1959; Guy et al., 1993; Helman et al., 2005; Luz & Barkan, 2005). Vinogradov explains that the low photosynthetic isotopic discrimination that can occur is due to contamination by atmospheric O_2 or by respiration. Guy et al. (1993) corroborate this idea by finding a photosynthetic isotopic discrimination of 0.3‰ in cyanobacteria (Anacystis nidulans) and diatoms (Phaeodactylum tricornutum) that they consider negligible. Luz and Barkan (2005) in their study on *Philodendron*, consider that there is no photosynthetic isotopic discrimination. Our value proves that there is indeed a terrestrial photosynthetic isotopic discrimination and the value found for *Festuca arundinacea* is slightly smaller than the photosynthetic isotopic discrimination in marine environment $^{18}\varepsilon_{photosynthesis}$ = + 6 ‰ found by Eisenstadt et al. (2010). More specifically, Eisenstadt et al. (2010) determined several

photosynthetic isotopic discrimination values depending on the phytoplankton studied (*Phaeodactylum tricornutum* = 4.5‰, *Nannocloreopsis sp.* = 3 ‰, *Emiliania huxleyi* = 5.5 ‰ and *Chlamydomonas oreinhardtii* = 7‰). One of the conclusions given by Eisenstadt et al. (2010) is that eukaryotic organisms enrich their produced oxygen more in ^{18}O than prokaryotic organisms. Our conclusion based on experiments performed with *Festuca arundinacea* species is in agreement with these conclusions. We should however note that we tested only one species. Additional experiments with different plants are needed to check if this fractionation factor should be applied for global Dole effect calculation. Still, this positive ^{18}O discriminations during photosynthesis suggests that the terrestrial Dole effect may be higher than currently assumed and challenge the assumption that terrestrial and oceanic Dole effects have the same values (Luz and Barkan, 2011).

and how these vary and rather than stating that the new value is 3.7 perhaps the reality is that this value is somewhat variable across plant functional types and thus this parameter may require further investigation as hinted in the conclusion.

This is true. We now underline that we had this measurement for *Festusca arundinacea* and that it is not a general value because all organisms have their own isotopic discrimination value, see text above (discussion) and in the conclusion the mention of: More importantly, we document for the first time a significant ^{18}O discrimination during terrestrial photosynthesis with the *Festuca arundinacea* species (+ 3.7 ‰ ± 1.3‰). If confirmed by future studies, this can have a substantial impact on the calculation of the Dole effect, with important consequences for our estimates of the past global primary production.

Specific comments

Ln 52 First measurements, there were some measurements before Guy al., it less precise but still very provocative and it would be good to summarize which organisms were measured by Guy et al and others.

This has been done as explained in the general comments above.

Perhaps refer to the review of Tcherkez and Farquhar 2007 for a discussion on the theoretical aspects of the oxygen evolving complex. Ln 54 perhaps mention the process either as water photolysis, water-splitting or photosynthetic water oxidation and refer to its location in photosystem II of the chloroplast

Here is the new text that completes the information on Tcherkez and Farquhar 2007 and the photosystem II: "This absence of isotopic discrimination can be theoretically explained by the process of O_2 generation within photosynthesis (photosystem II) involving water oxidation by the oxygen evolving complex (Tcherkez and Farquhar, 2007)."

Ln68 it is not clear to the reader the logic that connects the +6 per mil enrichment to the low latitude water cycle. In fact, this latter part of the paragraph discussing past hydrology and d18O signals is not clearly presented and could benefit from being a separate paragraph after a clear explanation of the hydrological connections perhaps with the aid of a diagram explaining the budget fluxes, current understanding in the size and drivers and uncertainties.

We propose to add the following text: If marine and terrestrial Dole effects are similar, then the past variations of $\delta^{18}O_{atm}$ cannot be attributed to different proportions of terrestrial or marine Dole effects. They would better be related to low latitude water cycle influencing the leaf water $\delta^{18}O$ consumed by photosynthesis and then the $\delta^{18}O$ of O_2 produced by this process (with a larger flux in the low latitude vegetated regions).

Ln79 I would invert these processes and start with the MIF in the atmosphere the describe the MDF that is then followed logically by the definition for the MDF.

We propose this new text for the revised version of the manuscript: "Oxygen is fractionated in a mass-independent manner in the stratosphere producing approximately equal ^{17}O and ^{18}O enrichments (Luz et al., 1999). On the contrary, the biosphere fractionating processes are mass-dependent such that the ^{17}O enrichment is about half the ^{18}O enrichment relative to ^{16}O."

Ln 105 is the variability between COX and AOX the only possibility for soil fractionation? What about non-enzymatic weathering? Or decomposition of different substrates varying in oxidation level? Other enzymes linked to other biogeochemical cycles? Soil community composition? What about roots?

Few studies address these topics, i.e. the impact of soil community composition on isotopic fractionation or the impact of weathering, the impact of non-enzymatic decomposition of different substrates varying in oxidation level or the impact on fractionation of other enzymes related to other biogeochemical cycles. However, what we know from Guy et al. 1993 is that fractionation via the COX pathway is lower than via the AOX pathway (21‰ and 31‰ respectively) (see general comments above and associated explanation provided).

As for the roots, Angert and Luz, 2001, show that the photosynthetic fractionation of soils is lower (about 14‰) than for the dark respiration alone found by Bender et al. 1994 (18‰). This would be the result of diffusion preventing O_2 concentration in the roots and thus weakening its fractionation.

As mentioned in the answer of a general comment, we propose to add this text on the impact of roots on soil respiration:

Introduction: Yet, results from studies conducted at a larger scale, e.g. at the soil scale by Angert et al. (2001) found a global terrestrial respiratory $^{18}O/^{16}O$ of O_2 discrimination for soil microorganisms varying between - 12 ‰ and - 15 ‰. This is lower than the - 18 ‰ discrimination classically used for respiration, with diffusion in soil playing a role in addition to the biological respiration isotopic discrimination. Angert and Luz (2001) also showed using experiments on roots of Philodendron plants and wheat seedlings that the respiratory discrimination of a soil with roots is lower (about - 12‰) than the - 18‰ discrimination associated with the dark respiration. This is due to the low O_2 concentration in roots which have a slow diffusion.

Fig 1 No light sensor in the drawing.

The light sensor was placed inside the growth chamber hosting the closed chamber (but not inside the closed chamber). We choose not to represent it in the drawing as the light sensor was only used as an on/off check for light.

What is the impact on the d18O2 if it equilibrates with water vapour in the glass flask? Would it not be prudent to have a drier on the flask inlet? How did the irrigation water isotope composition vary between each experiment and during the experimental runs with and without plants?

There is no measurable effect of exchange between $\delta^{18}O$ of O_2 and $\delta^{18}O$ of water vapor. This has been tested extensively, in particular for the analyses of $\delta^{18}O$ of O_2 in air trapped in ice cores.

The isotopic composition of irrigation and soil water has been added on table S3 (cf general comment above): there is a slight but significant isotopic enrichment with time.

We have added this explanation in the section 2.3.3. "Photosynthesis and dark respiration experiment": The values of the leaf water measurements are presented in supplementary Table S3. Because the experiments had to be carried in a closed chamber, we could not sample leaves during the experiment and only got a value at the end of each sequence. Nevertheless, we could compare the isotopic composition of the irrigation and soil water at the start and at the end of the experiment.

Ln 135 change enlightenment to the explicit number hours in the dark and light expressed as a ratio/ Ln 257 provide day/night cycle in hrs here.

We propose to clarify this point by adding the following table S1 in the supplementary. There was not a constant ratio of day and night period durations because day and night period durations were function of O_2 change rate as our main objective was to achieve around 1% change in O_2 atmospheric concentration during day or night period. As a result, day and night periods were different from one experiment to the other.

Table S1. Summary of the illumination of the different sequences of the photosynthesis and dark respiration experiment.

Sequence	Light	Start date	End date
1	On	19/03/19, 08:00	25/03/19, 14:00
	Off	25/03/19, 14:00	28/03/19, 17:05
	On	28/03/19, 17:05	02/04/19, 08:00
	Off	02/04/19, 08:00	05/04/19, 06:50
	On	05/04/19, 06:50	16/04/19, 15:30
	Off	16/04/19, 15:30	19/04/19, 06:50
	On	19/04/19, 06:50	06/05/19, 14:00
	Off	06/05/19, 14:00	14/05/19, 14:20
	On	14/05/19, 14:20	15/05/19, 14:00
2	On	20/05/19,0 6:00	28/05/19, 13:00
	Off	28/05/19, 13:00	30/05/19, 20:35
	On	30/05/19, 20:35	10/06/19, 11:00
	Off	10/06/19, 11:00	14/06/19, 15:25
	On	14/06/19, 15:25	23/06/19, 14:30
	Off	23/06/19, 14:30	27/06/19, 05:25
	On	27/06/19, 05:25	28/06/19, 08:35
3	On	29/07/19, 07:00	05/08/19, 14:00
	Off	05/08/19, 14:00	08/08/19, 05:20
	On	08/08/19, 05:20	19/08/19, 13:00
	Off	19/08/19, 13:00	22/08/19, 05:25
	On	22/08/19, 05:25	02/09/19, 13:00
	Off	02/09/19, 13:00	05/09/19, 05:15

Ln 151 how was the Oxy1-SMA O2 concentration calibrated?

Measures from the Oxy1-SMA O2 are not calibrated. Before each experiment the values measured by the sensor during a few hours where considered to be the baseline reference with the atmospheric O_2 concentration assumed to be 20.9%. This value was then used as a reference and the offset observed from the assumed theoretical value used to correct all following measurements assuming a linear offset. We have added this explanation:

Because precise O_2 concentration are determined in our samples by mass spectrometry (see next section), the measurements of the Oxy1-SMA were only used as a control during the experiment. The measured O_2 value for atmospheric air was adjusted to 20.9% before each sequence of experiments and the same adjustment (offset) was then applied to the O_2 record during the following sequence.

Ln 178 please provide info on the flow rate

The flow rate was equal to 1.6 L/min.

Ln 198 define D17O

It is already defined in the introduction (Eq.1). We have added a reference to this equation here.

Ln 217 please define dO2/Ar

We have added: The uncertainty associated with each measurement was obtained from the standard deviation of the three runs and from the repeated peak jumping measurement for $\delta O_2/Ar$ which was defined by $\left[\frac{\left(\frac{n(O_2)}{n(Ar)}\right)sample}{\left(\frac{n(O_2)}{n(Ar)}\right)standard} - 1\right] * 1000$, and $n(O_2)$ is the number of moles of O_2 and $n(Ar)$ the number of moles of Ar.

Ln 233 I would rearrange this sentence so that 2 weeks is before 23 days.

This was a mistake, it should be 3 days instead of 23. We have corrected this mistake.

Ln 246 why no light dark cycle?

We decided not to apply any diurnal cycles during dark respiration experimentations for two reasons. First, we wanted to prevent the development of algae, mosses or any photosynthetic organisms in the chamber. Secondly, it was easier to optimize temperature control as the light radiation could increase the temperature inside the closed chamber. We have added this text:

To conduct the soil respiration experiment, 2.6 kg of soil (*Terreau universel, Botanic*) were placed in 12 different pots. The light was turned off during this experimental run (Table S1). We decided not to apply any diurnal cycles during dark respiration experimentations for two reasons. First, we wanted to prevent the development of algae, mosses or any photosynthetic organisms in the chamber. Secondly, it was easier to optimize temperature control as the light radiation could increase the temperature

inside the closed chamber. During this dark period, CO_2 from soil respiration accumulates in the biological closed chamber.

Ln256 change to composition as this

"This was done to ensure that the CO_2 in the chamber did not reach levels too far from the atmospheric composition as this could have affected the physiology of the plant."

Ln 275 change subscripts to alphas not epsilon to be consistent with the equation that follows Done

Ln 283 "breathed"? overall the notation throughout is difficult to follow and not intuitive Replaced by "respired".

Ln 287 remove the phrase "evolution of the" if you really want to define n(O2) as evolution implies something that changes i.e. would require the definition of a flux

Done

Eq 8 definition sign not intuitive

We have corrected the equation: $n(O_2)_{t+dt} = n(O_2)_t + dn(O_2)$ Eq 8

R's should be deltas

Done

Eq 12 perhaps worth pointing out which leaf water pool is likely most important but an assumption is made that it can represented by bulk leaf water signal.

Indeed, we study here the link between the bulk leaf water isotopic composition and the isotopic composition of oxygen produced by photosynthesis which is relevant when doing the global budget of the Dole effect as discussed here. Still, the reviewer is right that the important water pool is the water where chloroplasts are found, i.e. in the mesophyll layers of the leaf. For our study of *Festuca arundinacea* we consider that the water in the mesophyll layer can be represented by bulk leaf water. We have added this explanation after this equation in the section 2.4.3.:

$$^{18}\alpha_{photosynthesis} = \frac{^{18}R_{produced\ O_2}}{^{18}R_{lw}} \quad (13)$$

For our study of *Festuca arundinacea* we consider that the water in the mesophyll layer can be represented by bulk leaf water.

Eq 322 maybe also important to note how the differences in dark respiration in the light and dark may differ.

See comment above.

Eq 18 this equation needs to be revised it is incorrect in its current form and is not consistent with the previous eq 14

Corrected equation:

Thus, at each stage, dioxygen is both produced by photosynthesis and consumed by the aforementioned O_2 uptake processes (hereafter $total\_respi$) by the plant according to the mass conservation equation:

$$n(O_2)_{t+dt} = n(O_2)_t + dn_{total\_respi} + dn_{photosynthesis} \quad (14)$$

where dn_{total_respi} is the number of molecules of O_2 consumed by dark respiration, photorespiration and Mehler reaction between time t and t+dt, and $dn_{photosynthesis}$ is the number of molecules of O_2 produced by photosynthesis between t and t+dt.

The budget for ^{18}O of O_2 can be written as:

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

where $^{18}\alpha_{total\_respi}$ is the fractionation factors associated with each O_2 consuming process periods throughout the whole experiment.

We introduced the normalized fluxes of photosynthesis and total respiration as:

$$F_{photosynthesis} = \frac{dn_{photosynthesis}}{n(O_2)_{t0} \times dt} \quad (16)$$

$$F_{total\_respi} = \frac{dn_{total\_respi}}{n(O_2)_{t0} \times dt} \quad (17)$$

$$a^{18}R = \frac{d^{18}R}{dt} \quad (18)$$

This led to the following expression of $^{18}\alpha_{photosynthesis}$:

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

This equation can be simplified at t=0 for $^{18}R_t$=$^{18}R_{t0}$=1 and $n(O_2)_t$= $n(O_2)_{t0}$

387 Eq 28 same as Eq18 and has problems with missing R's

388 See comments above for equation 18

389 $$^{18}\alpha_{photosynthesis} = \frac{a^{18}R + aN - \langle {}^{18}\alpha_{total\_respi}\rangle \times \langle F_{total\_respi}\rangle}{^{18}R_{lw} \times F_{photosynthesis}} \quad (29)$$

390

391 Table 1 Strongly suggest a third column that provides information about all the values used or if they
392 are variable and what the units are.

393 We have changed the title of the table to make it clearer: " List of variables used to quantify
394 fractionations and their definitions. * means either oxygen 17 or oxygen 18." And we have added this
395 column: "Origin of the value", which allows to know now if they are variable (depending on if we got
396 them from the literature or if we determined them ourselves). We have not added a column with
397 information about the values used since it depends if it is for oxygen 18 or 17. As far as units are
398 concerned, most of the values do not have any or this is defined in the definition column.

Symbol	Definition	Origin of the value
$^{*}\alpha$	Fractionation factor	
$^{*}\alpha_{dark\_respi}$	Fractionation factor of soil and plant respiration during night periods	Determined by our study
$^{*}\alpha_{dark\_leaf\_respi}$	Fractionation factor of leaf respiration during night periods	Determined by our study
$^{*}\alpha_{Mehler}$	Fractionation factor associated with Mehler respiration	Value from Helman et al. (2005)
$^{*}\alpha_{photorespi}$	Fractionation factor associated with photorespiration	Value from Helman et al. (2005)
$^{*}\alpha_{photosynthesis}$	Fractionation factor associated with photosynthesis	Determined by our study
$^{*}\alpha_{soil\_respi}$	Fractionation factor associated with soil respiration	Determined by our study
$^{*}\alpha_{total\_respi}$	Fractionation factor associated with total respiration during light period	Determined by our study
$^{*}\varepsilon$	Isotopic discrimination	

${}^{*}\varepsilon_{dark\_respi}$	Isotopic discrimination of soil and plant respiration during night periods	Determined by our study
${}^{*}\varepsilon_{dark\_leaf\_respi}$	Isotopic discrimination of leaf respiration during night periods	Determined by our study
${}^{*}\varepsilon_{photosynthesis}$	Isotopic discrimination associated with photosynthesis	Determined by our study
${}^{*}\varepsilon_{soil\_respi}$	Isotopic discrimination of soil respiration associated with soil respiration experiment	Determined by our study
θ	Ratio of $ln({}^{17}\alpha)\ to\ ln({}^{18}\alpha)$	
θ_{dark_respi}	Ratio of $ln({}^{17}\alpha_{dark\_respi})\ to\ ln({}^{18}\alpha_{dark\_respi})$	Determined by our study
$\theta_{dark\_leaf\_respi}$	Ratio of $ln({}^{17}\alpha_{dark\_leaf\_respi})\ to\ ln({}^{18}\alpha_{dark\_leaf\_resp}$	Determined by our study
$\theta_{photosynthesis}$	Ratio of $ln({}^{17}\alpha_{photosynthesis})\ to\ ln({}^{18}\alpha_{photosynthesis})$	Determined by our study
θ_{soil_respi}	Ratio of $ln({}^{17}\alpha_{soil\_respi})\ to\ ln({}^{18}\alpha_{soil\_respi})$	Determined by our study
aN	Linear regression coefficient of the evolution of $n(O_2)$ as a function of time	Determined by our study
$a^{*}R$	Linear regression coefficient of the evolution of $R^{*}O$ as a function of time	Determined by our study
$dn_{photosynthesis}$	Number of moles of O_2 produced by photosynthesis between t and t+dt	Determined by our study
dn_{total_respi}	Number of moles of O_2 consumed by total respiration during light periods between time t and t+dt	Determined by our study
F_{dark_respi}	Dark respiration flux (normalized vs number of moles of O_2 at the start of the experiment)	Determined by our study
F_{Mehler}	Mehler flux (normalized vs number of moles of O_2 at the start of the experiment)	Determined by our study and Landais et al. (2007)
$F_{photorespi}$	Photorespiration O_2 flux (normalized vs number of moles of O_2 at the start of the experiment)	Determined by our study and Landais et al. (2007)
$F_{photosynthesis}$	Photosynthesis O_2 flux (normalized vs number of moles of O_2 at the start of the experiment)	Determined by our study

F_{total_respi}	Total respiration O_2 flux during light period (normalized vs number of moles of O_2 at the start of the experiment)	Determined by our study
f_{dark_respi}	Fraction of the dioxygen flux corresponding to dark respiration process	Value from Landais et al. (2007)
f_{Mehler}	Fraction of the dioxygen flux corresponding to Mehler process	Value from Landais et al. (2007)
$f_{photorespi}$	Fraction of the dioxygen flux corresponding to photorespiration process	Value from Landais et al. (2007)
$n(O_2)$	Number of moles of O_2	Determined by our study
${}^{*}R$	Ratio of heavy (^{18}O or ^{17}O) isotope to light isotope (^{16}O) of O_2 in air	Determined by our study
${}^{*}R_{lw}$	${}^{*}R$ of leaf water	Determined by our study

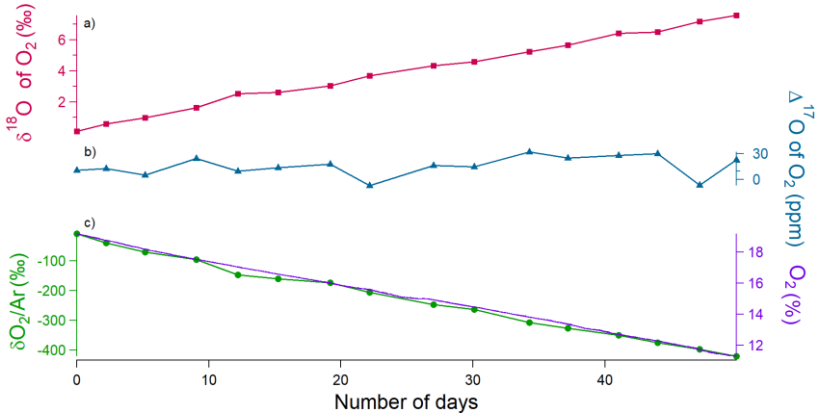
399

400 R*O in the table 1 of O2 in air?

401 Yes, this was specified in the text.

402 Fig 2 x axis would be easier to follow if the Day # was provided instead of Date

403 Done.



404

405 Fig 2 would also be useful to indicate the variation of the soil water d18O over time.

406 See comments above (Table S3).

407 Ln 403 provide mean value plus SD

408 The p-value for sequence 1 were equal to 0.40, sequence 2 = 0.08, sequence 3 = 0.58, sequence 4 =
409 0.47.

410 Ln 411 respiration not significantly different? Test

We consider that given that we only have a low number of sequences (which are the equivalent of temporal replicates of the same treatment), it's statistically inappropriate to assess whether the individual sequences are statistically different. Instead we now add more information on the variation among the sequences as follows:

It could be observed that despite differences in respiratory fluxes for the different sequences (the standard deviation is equal to 50% of the average flux across sequences; see Table S3), the relationship between $\delta^{18}O$ of O_2 and O_2 concentration (or $\delta O_2/Ar$), and hence the calculated fractionation factor associated with respiration, is not much affected."

Ln 412 you cannot explain only speculate you did not measure this. Furthermore, this should be in the discussion.

We have chosen to delete this discussion from this article because it does not help in understanding the fractionations.

Fig 4 legend not consistent with the axis purple is O2 not CO2

Done:

Fig.4. Example of the evolution of the different concentrations and isotopic ratios in the sequence 1 of photosynthesis and dark respiration experiment in the closed chamber over 31 days (day 0 is the beginning of the sequence). Grey rectangles correspond to night periods and white rectangles to light periods. (a) $\delta^{18}O$ of O_2 (red) variations. (b) $\Delta^{17}O$ of O_2 variations (blue). (c) Dioxygen concentration (purple) from the optical sensor and $\delta O_2/Ar$ variations (green) measured by IRMS.

Ln 437-440 Again this is a bit of discussion not really results unless you actually compare with the leaf water data from the experiment that is not presented in the paper.

We have chosen to delete this discussion from this article because it does not help in understanding the fractionations.

Please provide the leaf water information from the experiment.

Done, see comments above (Table S3).

Ln 444 Is this caused by a technical problem?

No technical problem occurred during this experiment.

Ln 453 assuming that respiration rates or fractionation during the dark and light do not vary

Indeed, the rate of autotrophic respiration is lower in light periods (Tcherkez et al. 2017) which was not considered in the first version of the manuscript. We therefore propose to add sensitivity tests with no autotrophic (i.e. dark leaf) respiration during the day. The results of the sensitivity tests are included in the supplementary text 1. See comments above.

We have added in the section 3.2.2. “Fractionation factors”, “Photosynthesis”, a text explaining that we have done several sensitivity tests:

We performed different sensitivity tests (supplementary texts 1 and 2). Sensitivity test 1 (Table S4) quantifies the influence of vanishing flux of dark leaf respiration during the day. This test shows that the assumption of similar flux of dark leaf respiration during the night and light periods did not influence much the values of photosynthesis fractionation factors. It results in an additional uncertainty of 0.0006 and 0.0005 for the values of $^{18}\alpha_{photosynthesis}$ and $^{17}\alpha_{photosynthesis}$.

Sensitivity tests 2 (Tables S7, S8 and S9) were performed on values of the O_2 flux and associated fractionation factors for photorespiration and Mehler reaction. They resulted in additional uncertainties of 0.0007 and 0.0005 for the values of $^{18}\alpha_{photosynthesis}$ and $^{17}\alpha_{photosynthesis}$ (Table S10).

Sensitivity tests 3 concerned the possible evolution of the isotopic composition of leaf water on the course of an experiment. The comparison of the $\delta^{18}O$ of irrigation water and soil water at the end of the experiment shows a possible increase up to 2 ‰ (Table S3). We thus estimate that our values of leaf water $\delta^{18}O$ measured at the end of the experiment may be overestimated by 1 ‰ compared to the mean value of leaf water $\delta^{18}O$ during the course of the experiment. Taking this possible effect into account would lead to a fractionation factor for photosynthesis higher by 1 ‰ compared to the presented one of 3.7 ± 1.3 ‰, hence a higher isotopic discrimination associated with photosynthesis.

Finally, note that we have corrected all grammar and spelling comments and added the requested author citations.

Referee #2

Paul et al. describe a novel environmental chamber apparatus, as well as its first results, focused on obtaining isotopic fractionation factors associated with respiration and photosynthesis in terrestrial analogue systems. In this case, the authors report results from a study of a commercial potting soil and a grass (tall fescue). It is a difficult system to control and to study, and the authors have done perhaps the best job of controlling the environment compared to all the terrarium studies done over the past two decades in this vein (i.e., those led by Luz, Angert, and Yeung), namely by maintaining carbon dioxide concentrations and a closed water cycle with constant relative humidity below saturation. In this sense the study is quite welcome and I look forward to seeing more studies come of this apparatus. However, I have technical concerns about a couple elements of the manuscript, which are listed below:

1. When describing the mass balance equations for the experiment, the sign of dn sometimes does not make sense relative to the direction of the oxygen flux. For example, in 2.4.3, equation 13 describes d$n_{photosynthesis}$ as “the number of molecules of O2 produced by

photosynthesis," yet to have the correct sign I believe it needs to have a negative value in the equation (i.e., it is of opposite sign to dn_{total_respi}, which is a consumption term and has a positive value like in equation 8). I am not sure whether this confusion is an error in words only or if it propagates into the mass balance equations, but the authors should check.

Indeed, the equations were not written in the most logical way (inversion of "t" and "t+dt") and this was the reason why "absolute" values were introduced after, especially to have dnphotosynthesis of opposite value than $dn_{total\_respiration}$ as mentioned by the reviewer). We have therefore corrected this way:

In the section 2.4.1. "soil respiration":

The number of molecules of dioxygen in the air of the closed chamber, $n(O_2)$, between time t and time t+dt can be written as:

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

with $dn(O_2)$ the number of dioxygen molecules respired during the time period dt. A similar equation can be written for the number of dioxygen molecules containing ^{18}O remaining in the air of the chamber:

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

The evolution of the isotopic ratio of oxygen, ^{18}R, between time t and time t+dt can be written as:

$$^{18}R_{t+dt} = {}^{18}R_t + dR \quad (10)$$

In the section 2.4.3. "photosynthesis": (we added an equation (Eq.10), so from this equation, all the numbers of the equations mentioned in the referees' questions will be shifted to the higher number)

Thus, at each stage, dioxygen is both produced by photosynthesis and consumed by the aforementioned O_2 uptake processes (hereafter $total\_respi$) by the plant according to the mass conservation equation:

$$n(O_2)_{t+dt} = n(O_2)_t + dn_{total\_respi} + dn_{photosynthesis} \quad (14)$$

where dn_{total_respi} is the number of molecules of O_2 consumed by dark respiration, photorespiration and Mehler reaction between time t and t+dt, and $dn_{photosynthesis}$ is the number of molecules of O_2 produced by photosynthesis between t and t+dt.

The budget for ^{18}O of O_2 can be written as:

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

where ${}^{18}\alpha_{total\_respi}$ is the fractionation factors associated with each O_2 consuming process periods throughout the whole experiment.

We introduced the normalized fluxes of photosynthesis and total respiration as:

$$F_{photosynthesis} = \frac{dn_{photosynthesis}}{n(O_2)_{t0} \times dt} \quad (16)$$

$$F_{total\_respi} = \frac{dn_{total\_respi}}{n(O_2)_{t0} \times dt} \quad (17)$$

$$a^{18}R = \frac{d^{18}R}{dt} \quad (18)$$

This led to the following expression of ${}^{18}\alpha_{photosynthesis}$:

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

This equation can be simplified at t=0 for ${}^{18}R_t = {}^{18}R_{t0} = 1$ and $n(O_2)_t = n(O_2)_{t0}$

2. Accurate isotopic scaling between VSMOW and air is taken as a matter of fact when there is a known discrepancy of order 0.1 per mil in both $\delta^{18}O$ and $\delta^{17}O$ differences between labs that measure the O_2 analyte together with Ar (e.g., Hebrew U., Princeton, the present study) and those who measure it as pure O_2 (U. New Mexico, Gottingen, Open University, UCLA, Rice U.). It may seem like a minor point, but Yeung et al. RCMS (2018) showed that inconsistent/assumed scaling can lead to spurious disagreements in discrimination factors and triple-isotope slopes in the range of 0.1 per mil and 0.005, respectively. It poses a problem for the soil-respiration γ value because this type of uncertainty is systematic and thus would not be included implicitly in the random errors; the reported uncertainty range is too small. Indeed, Stolper et al. GCA (2018) and Ash et al. ACS Earth Space Sci. (2020) report evidence

   -- from two independent labs -- that dark respiration might not be characterized by the "canonical" 0.516 value. Many of the other reported uncertainty ranges are significantly larger than the level of these disagreements, but the photosynthetic endmember does depend strongly on the assumed value of VSMOW, which Wostbrock and others have shown are far from in agreement. I suggest the authors (1) acknowledge that this disagreement in the field exists, citing the relevant literature, and (2) make note of the possibility that the fractionation factors may need to be revised in the future once everyone gets on the same reference frame. I don't necessarily believe that the reported values need revision per se, but the field would do well to acknowledge outstanding issues in papers rather than continue to ignore them.

Thank you for this comment. In the initial manuscript, we have discussed the uncertainty linked to the scaling between VSMOW and air for the $\delta^{17}O$ in Table 2 and have quoted the paper of Sharp and Wostbrock (2021) quoting the Yeung et al. (2018) paper in section 3.2.2 for a related issue. Now, we have added a discussion the scaling uncertainty for $\delta^{18}O$ between VSMOW and air and the fact that it has possible influence on the the determination of $\alpha_{photosynthesis}$ and on the 17O vs 18O slope. Still, we explain the now in the manuscript the reason for our choice but we follow the suggestion of the reviewer stating that the fractionation factors may need to be revised in the future once everyone gets on the same reference frame.

We have therefore corrected this way:

In the section 2.2.1. "Water extraction from leaf and isotopic analysis":

For analysis of $\delta^{17}O$ and $\delta^{18}O$ of water, leaf water was converted to O_2 using a fluorination line for reaction of H_2O with CoF_3 heated to 370°C at LSCE. The isotopic composition of the dioxygen was measured an IRMS equipped with dual inlet (Thermo Scientific MAT253 mass spectrometer). The standard that was chosen was an O_2 standard calibrated against VSMOW. The precision was 0.015 ‰ for $\delta^{17}O$, 0.010 ‰ for $\delta^{18}O$ and 6 ppm for $\Delta^{17}O$ (Eq. (1)), for more details, refer to Landais et al. (2006).

The values of $\delta^{18}O$ and $\delta^{17}O$ of leaf water measured with respect to VSMOW are then expressed with respect to the isotopic composition of dioxygen in atmospheric air (classical standard for $\delta^{18}O$ and $\delta^{17}O$ of O_2 measurements). No consensus has been reached for the values of $\delta^{18}O$ and $\delta^{17}O$ of O_2 in atmospheric air with respect to $\delta^{17}O$ and $\delta^{18}O$ of H_2O of VSMOW. These differences are most probably to be attributed to the different analytical techniques used for preparing and measuring the samples (Yeung et al., 2018; Wostbrock et al., 2021). In our case, because we use a similar set-up with the one developed by Barkan and Luz (2003) for the analyses of the triple isotopic composition of O_2 in air (cf next section), we have chosen to base our calculation on their estimates. In this study, we have thus chosen the value of 23.88 ‰ for $\delta^{18}O$ of O_2 values with respect to VSMOW following (Barkan and Luz, 2005). As for the $\delta^{17}O$ of O_2 value with respect to VSMOW value, we use two different possible estimates from these authors, either 12.03 ‰ (Luz and Barkan, 2011) or 12.08 ‰ (Barkan and Luz, 2005). We acknowledge that because of the absence of consensus, slightly different values could be obtained for the fractionation factors determined in this study if a different choice is made for the reference values of $\delta^{18}O$ and $\delta^{17}O$ of O_2 in atmospheric air with respect to $\delta^{17}O$ and $\delta^{18}O$ of H_2O of VSMOW.

Minor comments

1. There is some nonstandard notation: the use of $R^{18}O$ instead of the more common ^{18}R when describing $^{18}O/^{16}O$ ratios; the use of γ without mention of its equivalence to the symbol θ used elsewhere in the triple-isotope literature; the use of "fractionation coefficient" rather than the more common term "fractionation factor" for α.

We followed this suggestion and exchange the notations ($R^{18}O$ and γ) to (^{18}R and θ). We now use the term “fractionation factor”

2. “Since” refers to a time in the past (e.g., since 1980) and “because” refers to a cause (“because Ar is an inert gas”). In most instances of “since” in the manuscript I think the authors should be using “because” instead.

Indeed, we changed "since" to "because" in the appropriate formulations.

3. In the abstract, Table 1, L531, and 546: “respiration of leave” --> “leaf respiration” Done.

4. L451, 453, 458, and 460 : “leave” --> “leaf”

Done.

References added

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