We thank the reviewer for his/her time and very valuable comments on the manuscript. This is very helpful. We propose to implement the following changes in a revised version

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

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 S2 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 $\delta^{18}O_{lw}$ and $\delta^{17}O_{lw}$ 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.

Table S2. 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	$^{18}R_{lw,tf}$	$^{17}R_{lw,tf}$	$^{18}R_{iw,t0}$	$^{17}R_{iw,t0}$	$^{18}R_{sw,tf}$	$^{17}R_{sw,tf}$
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 made it impossible to understand implicitly. In the new version of the manuscript it will be explained as:

"This led to the following expression of ${}^{18}\alpha_{photosynthesis}$ when we use the value of $R^{18}O_t=R^{18}O_{t0}=1$ and $n(O_2)_t = n(O_2)_{t0}$ (note that same numerical results are obtained if we directly apply equation 14 to our series of measurements)"

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 is the reaction that reduces oxygen to form a superoxide ion, which is in turn converted to hydrogen peroxide (H_2O_2) in photosystem I and then further converted to water Mehler, 1951)."

For COX/AOX: "The COX respiratory pathway (with the enzyme cytochrome oxidase) is present in the majority in plants. This enzyme catalyzes the oxygen reduction reaction. The plant respiratory chain also has a second, weakly expressed terminal oxidase: the alternative oxidase or AOX (cyanide insensitive). This alternative respiratory pathway directly couples the oxidation of ubiquinol molecules to the reduction of oxygen. Guy et al., 2005, show that the respiratory fractionation of the AOX pathway is much higher than for the COX pathway (e.g. for green tissues: 31‰ and 21‰, respectively). Similarly, Ribas-Carbo et al. 1995, found a higher respiratory fractionation 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)."

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 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 will add a text explaining this variability in the introduction. We will also present supplementary sensitivity tests 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.

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: "Angert and Luz, 2001, also shows using experiments on roots of Philodendron plants and wheat seedlings that the respiratory discrimination of a soil with roots is lower (about 12‰) than for the dark respiration (COX pathway) at 18‰. This is due to the low O_2 concentration in roots which have a slow diffusion. This shows the importance of not neglecting diffusion in soils with roots."

Discussion: "Soil respiration fractionations are lower than those found by Bender et al., 1994 (18‰) compared to dark respiration alone. This is due to the roles of root diffusion in the soil that decrease the respiratory fractionation (Angert and Luz, 2001)."

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 fractionation 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 by Dole and Jenks, 1959, who proposed a photosynthetic fractionation for plants and algae of 5‰. Vinogradov et al., 1959, challenge the result of Dole and Jenks, 1944, by explaining that their ^{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 this photosynthetic fractionation on spinach thylakoids and cyanobacteria (*Anacystis nidulans*) and Diatoms (*Phaeodactylum tricornutum*) and found only a slight fractionation of 0.3‰ which they considered negligible. Luz and Barkan, 2005, also corroborates this idea by studying photosynthetic fractionation on Philodendron and did not obtain an ^{18}O enrichment of the O_2 produced."

And: "More specifically, Eisenstadt et al., 2010, determined several photosynthetic fractionation values depending on the phytoplankton studied (*Phaeodactylum tricornutum* = 4.5‰, *Nannocloreopsis sp*. = 3 ‰, *Emiliania huxleyi* = 5.5 ‰ and *Chlamydomonas oreinhardtii* = 7‰)."

Conclusion: "More specifically, Eisenstadt et al., 2010, determined several photosynthetic fractionation 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., 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 this

conclusion. We should, however, note that we tested only one species. More experiments performed with different plants are needed to check if this fractionation factor should be applied for global Dole effect calculation."

And: "Vinogradov explains that the low photosynthetic fractionation 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 fractionation 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 fractionation."

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 fractionation value (see text above).

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 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 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 and then the $\delta^{18}O$ of O_2 produced by photosynthesis (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 or 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).

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 some text on the impact of roots on soil respiration.

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 S2 (cf general comment above): there is a slight but significant isotopic enrichment with time.

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
	On	05/09/19, 05:15	06/09/19, 08:30

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.

Ln 178 please provide info on the flow rate

The flow rate is equal to 1.6L/min.

Ln 198 define D17O

It is already defined in the introduction (Eq.1). We will therefore add a reference to this equation here.

Ln 217 please define dO2/Ar

We will add : "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.

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.

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

Indeed, "t" and "t+dt" should be reorganized (see comments above). This will be done in the next version of the manuscript.

Eq 10 R's should be deltas

We will add this new explanation:

"This led to the following expression of $^{18}\alpha_{photosynthesis}$ when we use the value of $R^{18}O_t$=$R^{18}O_{t0}$=1 and $n(O_2)_t = n(O_2)_{t0}$ (note that similar numerical results are obtained if we directly apply equation 14 to our series of measurements)"

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. This assumption will be explained in the next version of the manuscript as suggested by the reviewer.

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

See comment above. We will clarify this in the next version of the article.

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:

$$R^{18}O_{t+dt} \times \frac{n(O_2)_{t+dt}}{n(O_2)_{t0}} = R^{18}O_t \times \frac{n(O_2)_t}{n(O_2)_{t0}} + R^{18}O_t \times {}^{18}\alpha_{total\_respi} \times \frac{dn_{total\_respi}}{n(O_2)_{t0}} + R^{18}O_{lw} \times {}^{18}\alpha_{photosynthesis} \times \frac{dn_{photosynthesis}}{n(O_2)_{t0}}$$

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

See comments above for equation 18

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

We have only changed the title of the table to make it clearer: " List of variables used to quantify fractionations and their definitions."

We will adapt Table 1 with the requested information.

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

Yes, this will be specified.

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

Done.

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

See comments above, it will be added in a table.

Ln 403 provide mean value plus SD

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

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

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.

This will be accordingly removed

Please provide the leaf water information from the experiment.

Done, see comments above.

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 Table below.

Sequence	Period	$^{18}\alpha_{photosynthesis0}$	$^{18}\alpha_{photosynthesis1}$ Flux	$^{18}\alpha_{photosynthesis2}$ α_{soil_respi}	$^{18}\alpha_{photosynthesis3}$ Flux + α_{soil_respi}	$^{17}\alpha_{photosynthesis0}$	$^{17}\alpha_{photosynthesis1}$ Flux	$^{17}\alpha_{photosynthesis2}$ α_{soil_respi}	$^{17}\alpha_{photosynthesis3}$ Flux + α_{soil_respi}
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

Table. $\alpha_{photosynthesis}$ 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. 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.

In these sensitivity tests, we looked how the value of $\alpha_{photosynthesis}$ is affected when F_{dark_respi} changes from a maximum value (F_{dark_respi} during dark period) to a minimum value (F_{dark_soil} during dark period) and when α_{dark_respi} changes from a maximum value (α_{dark_respi} during dark period) to a minimum value (α_{dark_soil} during dark period) as well as when both $F_{dark\_respiration}$ and α_{dark_respi} are modified at the same time. The results from these sensitivity tests show variations in $\alpha_{photosynthesis}$ within a range which is smaller than the uncertainty range found for our determination of $\alpha_{photosynthesis}$, and therefore, our conclusions are not modified.

We propose to integrate these results and explanations in the next version.

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

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