Comment on bg-2021-324
Anonymous Referee #1

Referee comment on "Determination of respiration and photosynthesis fractionation factors for atmospheric dioxygen inferred from a vegetation-soil-atmosphere analog of the terrestrial biosphere in closed chambers" by Clémence Paul et al., Biogeosciences Discuss., https://doi.org/10.5194/bg-2021-324-RC1, 2022

Article title: Determination of respiration and photosynthesis fractionation coefficients for atmospheric dioxygen inferred from a vegetation-soil-atmosphere analog of the terrestrial biosphere in closed chambers

The marine and terrestrial biosphere regulate the concentration of O$_2$ in the atmosphere through the processes of photosynthesis and respiration (both photorespiratory and mitochondrial). These processes also modify the oxygen isotope composition of O$_2$ ($d^{17}$O and $d^{18}$O) differently providing a promising tool to investigate variations in biosphere function in response to changes in climate. However, to use these tracers to resolve the global mass balance and interpret ice core data, it is necessary to understand how the various biosphere processes fractionate isotopically and how variable these isotope effects are for different components of the biosphere (e.g. marine vs terrestrial organisms). This need has been hampered somewhat, as measuring $d^{17}$O and $d^{18}$O variations represents a technical challenge and only a few labs in the world measure routinely the $^{17}$D of O$_2$.

Up until quite recently the scientific community assumed that during photosynthesis, there was no isotopic fractionation of the O$_2$ evolved based on some of the first studies by Stevens et al., (1975) and Guy et al., (1993) on enzymes, intact cells and spinach thylakoids. These experiments found that the $d^{18}$O of O$_2$ was identical in isotope composition to the substrate water. Since then, further studies have been made using far more precise IRMS techniques on phytoplankton cultures/communities (Helman et al., 2005; Eisenstadt et al., 2010). Collectively, these studies and data from other ocean studies now indicate that during photosynthesis, marine organisms produce O$_2$ significantly enriched in $^{18}$O with respect to the seawater bathing the phytoplankton. Furthermore, the extent of $^{18}$O enrichment may vary depending on whether the photosynthetic organisms are eukaryotic or prokaryotic, with the former producing O$_2$ relatively more $^{18}$O-enriched than the latter. Although a number of datasets now exist for
marine phototrophs, there are very few data for terrestrial plants. The first measurements on plants by Dole and Jenks, (1944) concluded that plants and algae caused an $^{18}$O enrichment of $O_2$, however, these results were challenged repeatedly by several later studies (Vinogradov et al., 1959; Guy et al., 1993; Helman et al., 2005; Luz & Barkan, 2005). The present study of Paul et al., under review suggests that grass plants (*Festuca arundinacea*) may actually produce $^{18}$O-enriched $O_2$ during photosynthesis. Given that the global flux of $O_2$ produced by land plants is significant, a photosynthetic fractionation of 3.7+/- 1.3 per mil would impact the overall value of the terrestrial Dole Effect and our estimates of terrestrial biosphere productivity during past glacial and interglacial periods using ice cores.

A further novelty of the present study is the independent measurements of $^{17}$D of $O_2$ consumed by soils in the dark and the $^{17}$D of $O_2$ produced and consumed by plants and soils exposed to day/night light cycles. These experiments demonstrated that soils incubated without plants did not exhibit any overall trend in $^{17}$D values over extended incubation periods of 1 to 2 months. In contrast, replicated incubations with both plants and soils exhibited an overall increase in $^{17}$D of ~80 ppm over the same timeframe demonstrating that the biosphere and especially the metabolism of plants has a strong impact on the $^{17}$D of the atmosphere in contrast to other abiotic processes that occur in the stratosphere that tend to decrease $^{17}$D signals. Collectively these experimental data may be useful to help parameterise models that are used to interpret variations in biosphere productivity from ice core records during glacial and inter-glacial periods.

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 $^{18}$epsilon 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 d$^{18}$O and d$^{17}$O 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. In addition, there seems to be some inconsistencies in the development of the $^{18}$epsilon 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 $^{18}$epsilon 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.
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 as well as how they may vary in importance between environmental conditions for example dark respiration in the dark vs in the light and also how dark respiration rates and isotope ratios may vary in soils with and without roots. The discussion could also benefit from summarizing the different phototrophs that have been measured in the past 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.

Specific comments

Please do a search in the manuscript to correct “leave“ to “leaves“ when referring to plant foliage.

Line 34 change to “one of the most important biogeochemical cycles on Earth as oxygen...”

Line 39 change to “variations of the low latitude”

Line 45 change to “knowledge of the various fractionation..”

Line 46 change to “interpreting the relationship between d18Oatm variations in ice core air with the low latitude water cycle”

Line 48 change to “multiple processes involved”

Ln 48 change to “had shown that d18Oatm”
Ln 51 change to “several fractionations caused by biosphere processes”

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

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

Line 56 change to “in the terrestrial biosphere the d18O of water split during photosynthesis...”

Ln 58 for higher latitudes/elevations please cite Dansgaard, 1964 and during leaf evaporation please cite Dongmann et al., 1974

Ln 59 change to “The mean d18O enrichment of leaf water isotopic composition has been estimated between...”

Ln 60 change to “composition of mean global ocean water”

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.

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.
Ln 88 change to “increases”

Ln 103 change to “to the biological”

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?

Ln 109 change to “a simplified vegetation-soil-atmosphere analog”

Ln 110 change to “in a closed chamber of 120 dm3”

Fig 1 No light sensor in the drawing. 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?

Ln 123 dm3 is the preferred SI unit

Ln 125 change to “Microcosm”

Ln 135 change enlightenment to the explicit number hours in the dark and light expressed as a ratio.

Ln 138 change to approximately

Ln 139 invert to read mature fescue

Ln 143 change “one “ to “smaller chamber”

Ln 151 how was the Oxy1-SMA O2 concentration calibrated?
Ln 155 invert to “closed water”

Ln 156 change to “was condensed”

Ln 162 provide model ref for the foil bags

Ln 164 change to “inflated or deflated”

Ln 164 change to “variations caused either by”

Ln 166 place a space before Almeno

Ln 178 please provide info on the flow rate

Ln 186 change to “immersed”

Ln 187 change to “pressure was reached”

Ln 189 change to “vial for the water was then immersed”

Ln 189 remove the brackets and the text therein and replace “and” with “whilst”

Ln 190 change to “transferred to a water bath”

Ln 196 “an IRMS”

Ln 198 define D17O
Ln 201 “the isotopic composition”

Ln 217 please define dO2/Ar

Ln 220 “the isotopic”

Ln 228 “leaf dark respiration”

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

Ln 236 maybe “characterise the” experimental repeatability

Ln 246 why no light dark cycle?

Ln 254 change during to for

Ln 255 controlled to ~ 400umol mol⁻¹ by a...

Ln 256 change to composition as this

Ln 257 change “enlightenment” to “light cycle” and provide day/night cycle in hrs here.

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

Ln 283 “breathed”? overall the notation throughout is difficult to follow and not intuitive

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
Eq 8 definition sign not intuitive

Eq 10 R’s should be deltas

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.

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

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

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.

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

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

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

Ln 403 provide mean value plus SD

Ln 411 respiration not significantly different? Test

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

Fig 4 legend not consistent with the axis purple is O2 not CO2
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. Please provide the leaf water information from the experiment.

Is this caused by a technical problem?

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

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

Ln 558 change to “photorespire”