Reviewer 1

Many thanks for your detailed review and your feedback.
> All our responses or comments are written in color through the text.

The reviewed manuscript presents measurements of an important parameter: The discrimination against 18O of O2 during respiration. The authors here choose to use a mesocosm approach. The average values reported here are at the top of the range of previously reported values. While these results are interesting and important, I feel a lot is lacking.

A large part of the previous work they compare to is based on experimental work in the ocean, which should reflect discrimination in natural ecosystems. As the authors note, the (dark respiration) discrimination in their mesocosm experiment is at least partly a function of the temperature, nutrient status, and experiment temperature. Thus, it is unclear how the results obtained in the experiments here could be extrapolated to Earth's oceans.
The authors acknowledge this limitation and suggest that further experiments should test for the effects of the temperature, species, etc. To me, the advantage of a controlled lab experiment is that one can set up these parameters and understand how they control isotopic discrimination. Moreover, in such lab experiments, the question is whether this strong discrimination is cyanide-resistant respiration-related, which could be answered by the use of inhibitors. But as a minimum, it seems to me that the temperature effect on the results presented here should be tested before the manuscript is resubmitted.
> Many thanks for this comment. We agree with the different points noted here.
Still, it should be noted that this manuscript presents a fully new experimental system for the study of the different effects noted by the reviewers as written in the perspectives of the first version of the manuscript. A lot of efforts were devoted to the construction and validation of this mesocosm system for the study of aquatic biosphere and the first objective of this paper was to present this system with a first validation and estimate of discrimination factor. As a consequence, we did not dive much here on the different impacts to be studied.

Still, we understand the concern of the reviewer and want to address it. As we could use this validated system after submission of the manuscript, we have some additional results which we could present in a revised version of the manuscript.

1. First, we did experiments with marine diatoms hence validating the system for the study of marine biosphere (only freshwater experiments were performed in the first version of the manuscript).

2. Second, we could perform first tests varying the temperature. Experiments run with diatoms show a discrimination of 18 ‰ at 16 ˚C, i.e. in closer agreement with previous studies of isotopic discrimination during marine respiration. Moreover, the experiments run at different temperatures confirm an increased isotopic discrimination at higher temperature (Figure R1). We propose to include these results and the figure R1 in our revised manuscript.

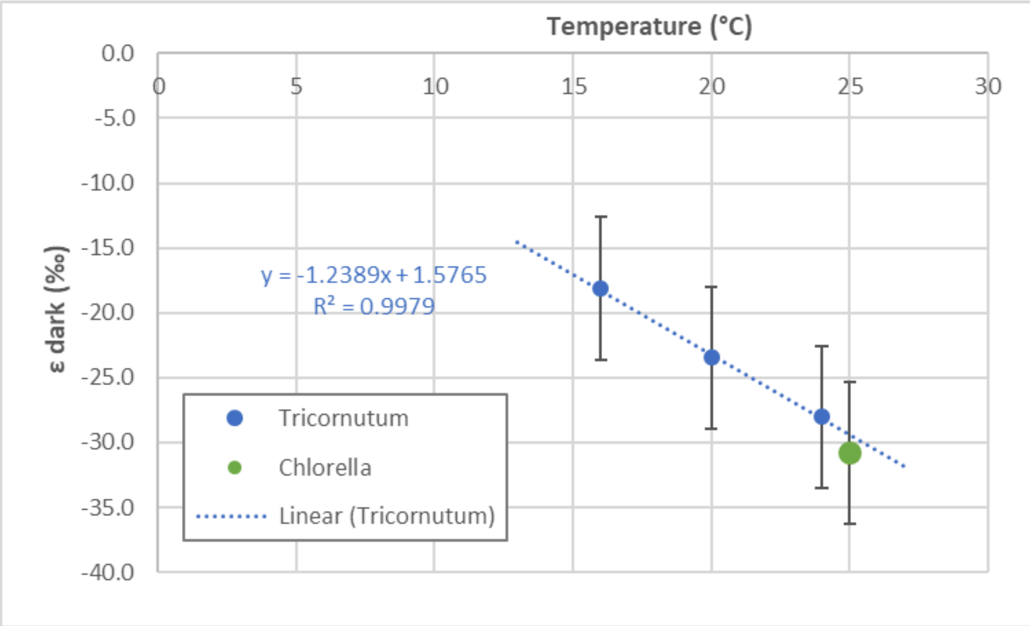

Figure R1 - Discrimination factors for the dark phases (ε_{dark}) for different water temperatures for *Tricornutum* (diatoms) and *Chlorella.*

In addition, some of the reported discrimination values reported here are extreme, about -40 permil. This is well beyond what is known for respiration processes. How is this explained?
> In the first version of the manuscript, we proposed that the temperature or the choice of our species can explain this high value. Our new experiments on diatoms confirm this hypothesis: diatoms at 16°C have discrimination values of -18 ‰, closer to those found in previous studies, whereas the same diatoms at 24°C have values up to -28 ‰ (Figure R1). We propose to include these results in the revised version of the manuscript.

As for discrimination during photosynthesis, the authors acknowledge that their results are too uncertain to shed any new light on this question. So I wonder if including these results and discussing them adds much to the paper.
> We agree that for photosynthesis, the results can not be used yet. Still, as this manuscript aimed to present a new system, its validation and opens perspectives for future studies (some of them to be included in the new version as mentioned above), we thought that it could be interesting to show the photosynthesis results even if they can not be used yet. These results show what improvements can be done to the general setup and/or protocols to perform experiments. We would like to keep this section and propose to shorten the section a bit and mention more explicitly the improvements to be done (length of phases, number of sampling).
Still, we can also remove it if the reviewers and editor think that it does not bring any added value.

In general, the manuscript is well written and easy to follow.
> Thank you

Minor comments:
Title: Instead of “intermediary scale setup”, maybe a “mesocosm” will be a shorter and more often used term?
> Indeed we regularly use the term mesocosm for our setup and we will use it more in the revised version, as well as in the title.

Line 70: In this paragraph, the following papers should be cited and added to the discussion :
Severinghaus, J. P., Beaudette, R., Headly, M. A., Taylor, K., & Brook, E. J. (2009). Oxygen-18 of O2 records the impact of abrupt climate change on the terrestrial biosphere. *Science*, *324*(5933), 1431-1434.

> Thank you, we will add this reference and discuss it.

Tables and figures: Please report epsilon in permil (i.e., -29 instead of -0.029), as in the text.
> Thank you for pointing this, we will modify the table for more readability