Reviewer 2

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

The authors conducted a series of experiments to determine the oxygen isotope fractionation associated with photosynthetic aquatic organisms using a mesocosm system. In contrast to previous studies that analysed dissolved gases, the present study determined the isotope composition of O_2 from gas samples collected from the chamber headspace. This approach is entirely appropriate provided that the experimental system is adequately controlled. However, I have several concerns regarding the experimental design, analytical procedures, and interpretation of the results, as outlined below.

1) The manuscript describes repeated gas sampling using 5 mL flasks, but it is not clear how the cumulative removal of gas affected the composition and pressure of the closed atmosphere over experiments lasting several months. Please clarify how the withdrawn gas volume was compensated, estimate the cumulative sampled volume relative to the chamber headspace, and discuss whether repeated sampling could influence the calculated O_2 concentration and isotope ratios.
> Indeed, the withdrawn volume was not compensated, resulting in a lowering of pressure inside the chamber headspace. The volume of the headspace is 3L of air and the flasks are 5 mL, and as a consequence, each sampling modifies the pressure of the chamber by 0.17 %, which is small.

We performed some tests by changing the sampling frequency during respiration and photosynthesis phases with no influence on the calculated isotopic discrimination (similar fractionation factors after 5 samplings and after 87 samplings). We also regularly sampled gas from a chamber with only water (no algae) and did not observe any signal.

However, because we were concerned by this effect, we tried to limit our samplings to around 20 times before opening the chamber to recover the atmospheric pressure when running the experiments in a routine way.

Still, we agree with the reviewer : compensation for withdrawn gas volume is indeed an issue which we will need to address in a future set-up and this will be mentioned in the revised version of the manuscript.

2) The manuscript interprets the observed O_2 and CO_2 dynamics in terms of photosynthesis and respiration, but no quantitative information is provided on the biomass of Chlorella vulgaris during the experiments. Since biomass is expected to change substantially over the several-month incubation, it is difficult to assess whether the observed O_2 fluxes are consistent with the physiological activity of the algal culture. Please clarify whether biomass (e.g., cell density, chlorophyll a, dry weight, or particulate carbon) was monitored during the experiments, or discuss this limitation more explicitly.
> Thank you for this comment. It was not possible to monitor biomass in the current system, mainly because we need to work in a closed system. We could only have indications during the growing phase before starting the experiments.

However the general dynamics of the 3 main parameters (CO_2, O_2 and $d^{18}O$) gives us confidence that we are indeed seeing the signal of photosynthesis and respiration processes. Our concerns in early development that we could have thermal effects caused by the light and water outgassing were resolved with the thermostated water tank and with a water only baseline that showed no such variations.

Still your comment is very sound and we could try to have a sampling of the water in a next improved version of our mesocosm. We propose to add this as a perspective.

3) A striking result is that essentially identical photosynthetic discrimination factors are obtained under two biologically very different assumptions: (i) no respiration during the light period and (ii) respiration equal to the dark rate. Since the first assumption is physiologically unrealistic, the weak sensitivity of the calculated discrimination factor to these contrasting assumptions raises concerns about the identifiability of the model. It would be helpful if the authors could explain why the estimated ε^{18}photo is almost insensitive to the assumed respiratory flux, and discuss whether this reflects limited information content in the measurements or an inherent limitation of the experimental design and modelling framework.
> Many thanks for this comment. The reason why the discrimination is not much sensitive to the proportion of respiration occurring during the light phase is the relative values of the photosynthesis and dark respiration fluxes. The photosynthesis flux is much larger than the dark respiration flux which can directly be seen from the difference in duration of the light vs dark phases. The O_2 uptake flux during the dark phase is indeed only about 10% of the photosynthetic flux.
With a strongest rate of O2 uptake during the light phase, we expect a strongest change in calculated isotopic discrimination. To illustrate this effect, we present in Table R2 some more sensitivity tests with unrealistic high O2 uptake during the light phase (3 and 5 times the observed O_2 uptake rate of the dark phase) and we obtain in this case slightly more difference in the calculated isotopic discrimination.

ε_{photo} with -0% of F_{dark} (‰)	ε_{photo} with -100% of F_{dark} (‰)	ε_{photo} with -300% of F_{dark} (‰)	ε_{photo} with -500% of F_{dark} (‰)
-2	-2	-2	-2
-4	-4	-3	-3
-6	-5	-4	-4
-10	-9	-7	-6

Table R2 - Sensitivity tests for the calculation of photosynthetic discrimination factor (ε_{photo}) with several hypothesis of concurrent respiration fluxes : 0%, 100%, 300% and 500% of dark respiration flux (F_{dark})

4) It has been reported that changes in the O_2/N_2 and O_2/Ar ratios can introduce apparent isotope fractionation in measurements of O_2 isotopes (Abe and Yoshida, 2003; Barkan and Luz, 2003). These studies demonstrated that variations in O_2/N_2 and O_2/Ar affect not only $\Delta^{17}O$ but also $\delta^{18}O$ measurements. Since substantial changes in O_2 concentration are expected in the present

incubation experiments, it is important to clarify whether such analytical artefacts were evaluated and corrected. If no correction was applied to the $\delta^{18}O$ data, the authors should explain why this was considered unnecessary and discuss the potential impact on the reported fractionation factors.
> Many thanks for this comment. Indeed, there is an influence on dO2/N2 on the measured d18O that we usually refer to as the "chemical slope". This effect is of second order on the values of the isotopic discriminations (change of 1 ‰). It is estimated by dilution of our standard through addition of pure N2 in different proportions. We will add the details about this correction in the manuscript.

5) I strongly recommend incorporating continuous monitoring of key aquatic parameters, such as dissolved oxygen concentration and chlorophyll a, those are able to monitor by sensors. Such measurements would provide valuable information on changes in biomass and physiological status throughout the experiments, and would greatly strengthen the interpretation of the observed O_2 fluxes and isotope fractionation.
> This is also a good comment and we actually had some sensors for the dissolved O2 concentration (optodes). Unfortunately, we realised that despite calibration of the instruments, they were very sensitive to relatively small variations of temperature induced by the change of light to dark and we could not use the series of measurements. This is the reason why we do not display them in this study but we hope to be able to include these sensors with a smaller sensitivity to temperature in next experiments with the same set-up.