Reply to the issues raised by Reviewer 2:

Dear editor,

We thank Reviewer 2 for their constructive view on our modeling work and providing thorough feedback and explicit suggestions for improvement. We reply below to the issue they raised.

Sincerely, also on behalf of my co-authors,

Yannick Bats

Black = Reviewer

Blue = Response

The manuscript describes a new model for phytoplankton carbon isotope fractionation, which includes parameterizations of carbon uptake and intercompartment transport based on light intensity and photoperiod. This is a complex topic for which a mechanistic understanding is still very much in discussion, because the processes are set within the cell where there are few direct constraints. The model is interesting and explores some interesting new parameterizations for cellular carbon fluxes and I think the model is worth being published and put on the table as part of ongoing dialogue to better understand the processes.

REPLY: We thank the reviewer for their appreciation of our work.

There are a few issues that should be addressed. The referencing of previous multi-compartment models needs to be expanded and more balanced in the introduction. In some parts of the discussion it would be worth contrasting again the parameter solutions in this model with the approaches of some previous models.

A key scientific limitation is that the model for coccolithophores relies heavily on light modulation of calcification rates, but there is not presented any validation data of the implied PIC/POC ratios or calcification rates compared to experimental data. Therefore it is currently hard for the reader to assess if this mechanism is a viable novel mechanism or not – and it is the key process invoked to produce greater POC fractionation than possible from Rubisco as the only enzyme. The authors should address this using calcification data for experiments where they are available (such as experiments of Rost) and for experiments without measured calcification the authors should show that the model simulated rates and PIC/POC are within realistic ranges for

those culture conditions, given the great wealth of calcification data produced over the last two decades by the ocean acidification communities.

REPLY: please see our reply to the reviewers' in-depth comment below on this specific point.

Detailed suggestions are provided below.

The introduction provides a simple explanation of the original diffusive model, however it needs to provide a comparable overview of the subsequent step in the development of multi-compartment models accounting for active carbon uptake and CCM. The current paragraph is not effectively organized to build up to the new issue taken up in this paper. And, line 42, the first sentence of the paragraph is not a fair synthesis of the array of mechanistical models developed (and no one really knows what it means to " accurately simulate" the mechanistic processes because the actual mechanistic parameters remain very underconstrained). The authors should approach this more fairly – previous models made advances in simulating mechanistic processes, and the author's new model offers a new parameterization of some of the mechanistic processes in these multi-component models.

So I suggest starting line 42, revising the paragraph in a more constructive approach (as taken with the previous paragraph) to highlight:

Several models have evaluated the organic matter fractionation resulting from the uptake of both CO2 and HCO3 and the intracellular transport and conversion of these carbon sources. Carbon flows into cytosol and intracellular compartments such as the cholorplast and pyrenoid have been simulated in models of diatoms (Hopkinson et al 2014). An array of multi-component models including chloroplast and calcification compartments for coccolithophores have used diverse parameterizations to investigate the impact of active bicarbonate uptake and intracellular transport on fractionation, focusing on the effects of CO2 and growth rate and cellular carbon allocation (Bolton and Stoll, 2013; Holtz et al 2017; McClelland et al 2017). A few models have additionally sought to evaluate potential mechanisms by which light availability affects fractionation, since culture experiments with coccolithophores (Rost et al) and with diatoms (eg refs in Cassar) show unique impacts of light on fractionation. Cassar et al (2006) assessed mechanisms by which light limitation impacted energetics of active HCO3 transport for diatoms. Wilkes and Pearon, 2019) proposed that light dependent enzymes supporting active HCO3 transport constituted a key mechanism for the light-dependence of ep.

In this study, we extend previous work to further explore potential processes which may be responsible for the observation of ep dependence on light intensity and photoperiod. We develop a four component model…

REPLY: We thank the reviewer for pointing this out. In particular, we appreciate the proposed revision of the paragraph. We agree that this is a more structured version with a better presentation of previous mechanistic modeling efforts. We will implement this in the revised version.

Line 109 – clarify what is the evidence that the electron transport rate is forced by light. Also keep in mind the surprising finding of Torres et al 2025 PNAS suggesting that deuterium fractionation did not provide support a greater chloroplast CO2 concentration in higher light.

REPLY: The cited studies in this paragraph (Grubb et al 2024, and Zhang and Gao 2021) show that electron transport rates through photosystem II in the thylakoid membrane during photosynthesis increase with light intensity, for G. huxleyi. This is direct evidence for the principle that light forces ATP production for this species, as the magnitude of the electron transport rate is directly coupled to the proton motive force resulting in ATP production (i.e., photophosphorylation). We will clarify this in the revised version.

Line 115 – are you suggesting more diffuse with increasing light intensity (add increasing or decreasing for clarity)?

REPLY: We meant to suggest that the pyrenoid's permeability for CO_2 increases with light, as the structure becomes less condensed. We will clarify this in the revised version.

Add another sentence to explain why you interpret that the pyrenoid volume is related to its permeability,

REPLY: We will implement this in the revised version.

and below the equation give the range of fpd and its specific relation to day length.

REPLY: This is given in Table A1, we will refer to this table more clearly in the revised version.

Lines 128 and Figure 1 imply that CA is present at every step. Include some citations on the presence and location of CA in the modeled organisms and/or indicate that it is an assumption based on the the location of CA in other organisms , or if it is a pure assumption.

REPLY: Bach et al (2013; DOI: 10.1111/nph.12225) have shown there are multiple genes encoding CA. By analogy with various diatom and green algae species, they predict the locations of CAs, which generally span all the compartments as implemented in our model. Therefore, as a straightforward approach, we implemented CA catalysis at every step. We will include this in the revised version.

What I am missing between Fig 1 (schematic) and Fig2 (plots of model ep) is a figure where you illustrate the variation of they key parameters with light and photoperiod –

your equation mentions only " proportional to" but a figure is needed to illustrate the range of variation in your parameter for a given range in PFD and day length.

REPLY: This is a useful suggestion. We will leave Fig. 1 unchanged, as to avoid making it too dense with information. We will include two subplots to figure 6 showing how light/photoperiod affect calcification and bicarbonate transport in our model, and how this ultimately affects Ep.

Line 176 – indicate how significant is the temperature dependent fractionation between HCO3 and CO2. Eg over a typical range in culture from 10C to 25C, is it 1 permil or 5 permil? From Fig 2a this looks minimally significant, so the explanation can be brief and indicate this is done for completeness but is not a main factor.

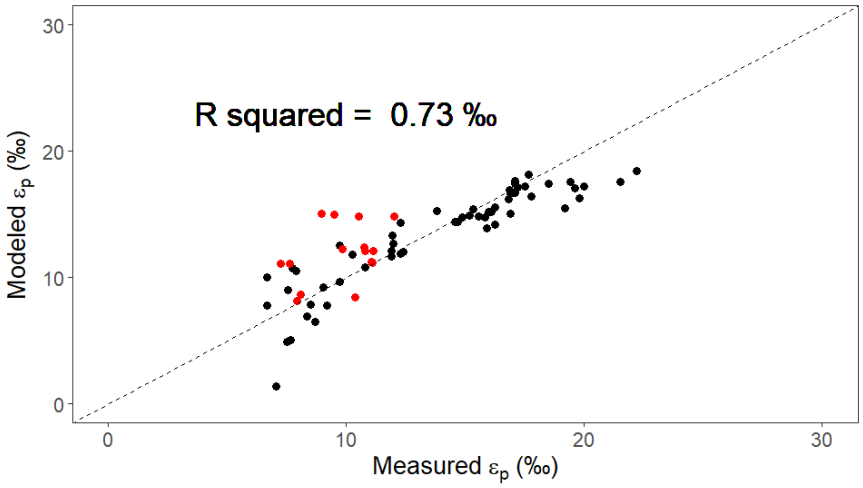
REPLY: We will implement this in the revised version.

Line 195 – Explain the choice of the culture datasets to simulate vs datasets excluded (Tchernov 2014, McClelland et al 2017).

REPLY: Tchernov 2014 did not constrain POC content, which prevented us from accurately constraining cell size and carbon fixation rates, which are important parameters determining carbon isotope fractionation. Therefore, we decided to not use these data here.

The data of McClelland et al 2017 has very high instantaneous (i.e., dark period corrected) growth rates (around and above 2 cell division per day). This makes the data on itself very interesting. For example, the Ep values of McClelland et al are on the lower range, which is what you expect with high growth rates.

If we do take these data into account, the model still produces a reasonable fit (R^2 = 0.73) (see figure below, red points are McClelland et al data). However, considering the ultimate goal of our modeling work is to use it on open ocean dinocyst and alkenone Ep measurements, we made the decision to not include the McClelland et al data, because of these high growth rates, which are unlikely to be representative of natural G. huxleyi populations. We will clarify this decision in the revised version of the manuscript.



Also eExplain why only G. huxleyi but not other Gephytrocapsa culture data (Rickaby, McClelland, Torres) were targeted for simulation.

REPLY: The ultimate goal of our model is to use it inversely on sedimentary dinocysts and alkenone Ep measurements of open ocean sites, for CO2 and/or light reconstructions. Considering G. huxleyi is the primary alkenone producer in open ocean sites (e.g., Schmidt et al 2013, Sawada et al 1996, Winter et al 2002, Haidar and Thierstein 2001), we made the decision to only use G. huxleyi data. We do acknowledge the role of G. oceanica alkenone production in specific settings. To fully grasp the underlying processes determining sedimentary alkenone Ep observations, this species has to be taken into account in future modeling efforts. We will implement a more elaborate discussion on this in the outlook section of the discussion.

Section 3.1 – This is model behavior and a sensitivity study. I am not sure that bullet points are stylistically the best way to present this. I suggest going to a paragraph format

We agree that this is an unconventional way of presenting the results of testing model behavior. On the other hand, we find it a very clear way of presenting the different model sensitivity parameters. Therefore, we decided to leave this unchanged, but are of course open to editorial advice on this matter.

and it would be helpful to cite the Figure and panel (Fig 2a) after each statement.

REPLY: We agree, and will implement this in the revised version.

Is the removal of 13C enriched HCO3 from the cytosol into coccoliths consistent with the observed range of d13C of coccoliths?

REPLY: Yes, as can be observed in Figure 8, and this will be indicated in the revised version.

Fig 3 – calcification rate needs more clarification of the units, what PIC/POC is implied.

REPLY: We will clarify the units in the Methods section of the revised version, in the paragraph of line 95.

Also I think an additional post-hoc comparison of the modeled vs observed PIC/POC is very important, please add this. Also in 3.2.2 clarify how does the modeled calcification rate increase with PFD and daylength change (if at all) the PIC/POC production rate in the model? Is the modeled PIC/POC consistent with the observations in Rost et al? This needs to be illustrated in Fig. 5 – there are many, many experiments of calcification of Ehux at different

REPLY: We thank the reviewer for pointing this out, also considering the comment of the general feedback given in their introductory paragraph.

Indeed, comparing simulated PIC:POC values with PIC:POC measurements would provide good additional post-hoc validation. However, PIC and POC measurements represent 'standing stock' values of the inorganic and organic carbon content of algae and not actual fluxes. In other words, they represent PIC and POC content in a steady state situation in which carbonate production and coccolith shedding (in the case of PIC), and organic carbon fixation and respiration (in the case of POC) are balanced. However, calcification is formulated extremely simple in our model: cytosolic HCO_3^- precipitates into $CaCO_3$ with rate *calc* (in s^{-1}). This does not take into account any of the known complexities of calcification in the strongly physiologically-controlled coccolith vesicle, let alone coccolith shedding. We are hesitant to implement this at this point because of multiple reasons. First, our focus is Ep. Second, implementation would make the model more complex and increase the degrees of freedom, because shedding rates (in moles per cm^3 $CaCO_3$) are relatively unconstrained. For this reason, an accurate simulation of steady state PIC values is not possible.

We found the suggestion, however, interesting. So, to investigate this remark by the reviewer, we made an attempt to compare modeled gross calcification (dPIC/dt = 0.85*calc*Hc, see lines 95-98), with gross calcification measurements based on ^{14}C-labeled sodium bicarbonate (Grubb et al 2024), alkalinity drawdown (Grubb et al 2024), or gross PIC production rates based on PIC quota and growth rates (Bach et al 2013, Kottmeier et al 2016).

The unit of measured gross calcification rates in the literature is in pg C $cell^{-1}$ d^{-1}. Our modeled gross calcification rate (dPIC) is in mol C cm^{-3} s^{-1}. Therefore, for comparison dPIC has to be multiplied by 12.01 g/mol and $1*10^{12}$ (molar weight of carbon, and g to pg), multiplied by 86400 (to go from s^{-1} to d^{-1}), and multiplied by the volume of the site of calcification (to go from pg C cm^{-3} d^{-1} to pg C $cell^{-1}$ d^{-1}), which is the coccolith vesicle. The latter is difficult to constrain, and likely results in substantial uncertainty. However, we can apply the approach of McClelland et al 2017, who assumed the coccolith vesicle has an oblate spheroidal shape based on TEM images. Accordingly, the

coccolith vesicle volume is scaled to cell size, with a specific scaling factor (fv= 0.6) of the equatorial axis in respect to cell radius, and a specific shape factor (sv = 4), scaling the polar axis (see McClelland et al 2017):

$V_{vesicle} = (4\pi * (fv * \text{cell radius})^2 * (fv * \text{cell radius}) / sv) / 3$

$V_{vesicle} = (4\pi * (0.6 * \text{cell radius})^2 * (0.6 * \text{cell radius}) / 4) / 3$

By applying this, we can simulate gross calcification rate in pg C $cell^{-1}$ d^{-1}, and compare it to values measured by Grubb et al (2024), Bach et al (2013) and Kottmeier et al (2016), which show a PIC production range of ~2.5 – 15 pg C $cell^{-1}$ d^{-1}.

Our model, using parameter settings of *G. huxleyi*, a photoperiod of 24 h, and [CO_2] of 20 µM, and a range of cell sizes, simulates PIC production rates increasing with light availability (see figure below), which is also observed in culture experiments (Grubb et al., 2024; Nimer and Merret, 1993). Our PIC production rates are around an order of magnitude lower than the measurements of Grubb et al 2024, Bach et al (2013) and Kottmeier et al (2016) (0.5 - 2 vs. ~2.5 - 15 pg C/cell/d). This could relate to uncertainties in constraining coccolith vesicle shape and size, as shown above. A larger vesicle volume results in higher PIC production rates. However, exact vesicle sizes are not accurately constrained.

However, as mentioned previously, for the sake of our purpose and simplicity, we deliberately implemented a simple calcification term that ignores the nature of the physiology of the coccolith vesicle and calcification. The model accurately simulates organic carbon isotope fractionation, which is validated by three independent post hoc tests. We therefore believe our rather simplistic implementation of calcification suffices for this purpose and adding the unconstrained calcification part would rather distract from our aims

In sum, despite the valuable suggestion, we decided for now to not include this additional post hoc validation, as our model is not developed to accurately simulate this. However, this reply letter will remain available to all who are interested.

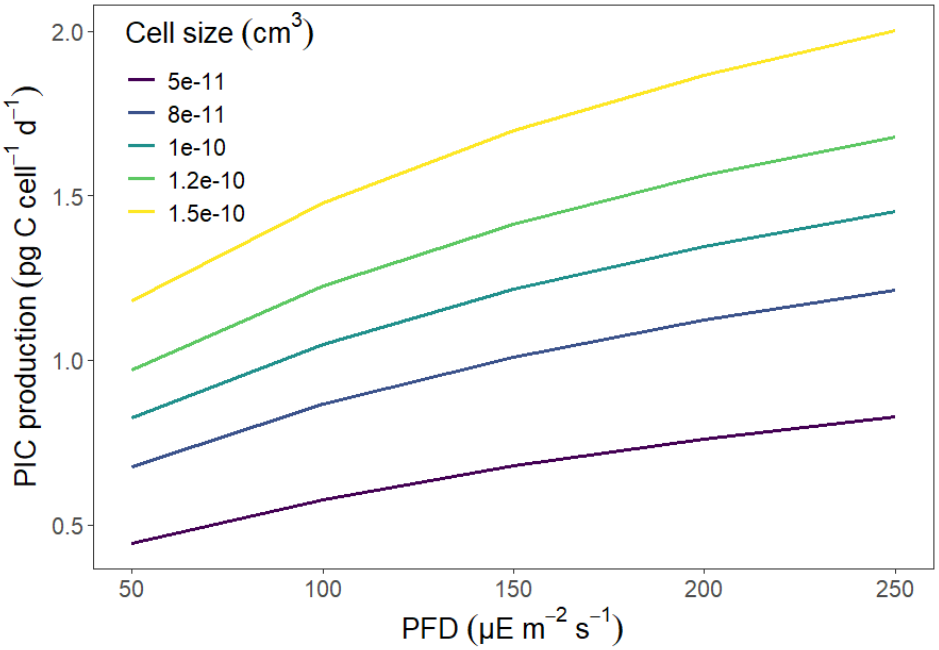


Figure 7 and resulting discussion. For huxleyi, there are significant differences in the inferred HCO3 fluxes and HCO3/CO2 ratios – produced in the same laboratory – based on either the 14C disequilibrium method vs the MIMS method. The two methods make different assumptions about the data processing. It is important to represent these two options in the figures and discussion because this accurately reflects how underconstrained the actual information on uptake and intracellular fluxes is for coccolithophores. This will make the authors discussion a bit easier regarding agreement with estimated fluxes. See the following:

Kottmeier, D.M., et al., *Strong shift from HCO3− to CO2 uptake in Emiliania huxleyi with acidificaon: new approach unravels acclimation versus short-term pH effects*. Photosynthesis research, 2014. **121**(2- 3): p. 265-275.

Kottmeier, D.M., S.D. Rokitta, and B. Rost, *H+-driven increase in CO2 uptake and decrease in HCO3−uptake explain coccolithophores' acclimation responses to ocean acidification*. Limnology and Oceanography, 2016. **61**(6): p. 2045-2057.

REPLY: We thank the reviewer for pointing this out. We will implement this in the revised version of the manuscript. Instead of changing figure 7, we will include an appendix figure showing the contrasting MIMS and 14C results of Kottmeier et al. 2014 and 2016. We will discuss this briefly in the discussion section.

Fig 8 – Refer again to equation 25 to clarify that ePIC is defined relative to CO2 aq since it has often been defined relative to DIC.

REPLY: We will implement this in the revised version.

In section 3.3.2 It would be worth referring to models which used a coccolith vesicle to calculate the ePIC in conjunction with ep (eg Bolton and Stoll, 2013; McClelland et al

2017; Holtz et al 2017) and commenting what additional processes were used in those models or additional tuning which led to better fits and whether such tuning is compatible with your model.

REPLY: We will implement this in a broader and more elaborate discussion on this topic. In particular, the previous modeling studies referred to (Bolton and Stoll, 2013; McClelland et al 2017; Holtz et al 2017) do not necessarily have better fit of ε_{PIC}.

Bolton and Stoll (2013) constrained ε_{PIC} as model input using ε_{PIC} measurements to derive bicarbonate fluxes as the free unconstrained parameter.

McClelland et al (2017) simulated ε_{PIC} relatively well, however, they did not apply different light availability conditions, meaning the relationship between light and ε_{PIC} cannot be validated; which is the main point of criticism on our model output in section 3.3.2.

The Holtz et al. (2017) model actually does not simulate ε_{PIC} well. At least not better than our model.

These examples show that even when implementing a specific coccolith vesicle model compartment, ε_{PIC} is not straightforwardly simulated, showing the complexities of coccolithophore calcification and associated isotope fractionation.

References:

Bach, L. T., Mackinder, L. C. M., Schulz, K. G., Wheeler, G., Schroeder, D. C., Brownlee, C., & Riebesell, U. (2013). Dissecting the impact of CO2 and <scp>pH</scp> on the mechanisms of photosynthesis and calcification in the coccolithophore Emiliania huxleyi. New Phytologist, 199(1), 121–134. https://doi.org/10.1111/nph.12225

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Kottmeier, D. M., Rokitta, S. D., & Rost, B. (2016). H+-driven increase in CO2 uptake and decrease in HCO3− uptake explain coccolithophores' acclimation responses to ocean acidification. Limnology and Oceanography, 61(6), 2045–2057. https://doi.org/10.1002/lno.10352

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