Reply to review by Dr. Edward Laws

Dear editor,

We thank Dr. Laws for his constructive view on our modeling work and reply below to the issue he raises.

Sincerely, also on behalf of my co-authors,

Yannick Bats

Black = Reviewer

Blue = Response

Reviewer 1:

I have reviewed the manuscript egusphere-2026-3713 by Bats et al. The authors describe a model to explain carbon fractionation by marine phytoplankton that takes into account most of what is known or presumed to be known about the relevant processes. It is apparent from figure 10 that the model accounts for most of the variance (85%) of measured εp values.

We thank the reviewer for appreciating our work and acknowledging the fact we aimed to take into account most of what is known (or presumed to be known) about processes fundamental to algal organic carbon isotope fractionation.

The authors unfortunately fall into the trap of concluding that if the model does not fit the data, there must be something wrong with the data. For example, the authors say, "Bidigare et al. (1997) found similar εp values between the diploid calcifying and haploid non-calcifying G. huxleyi which suggests no effect of calcification on εp using a chemostat culturing setup with a constant washout rate. However, this method has been criticized for producing εp values that may not reflect natural conditions, as the diluton rate may be too high to allow the DIC pool in the culture medium to reach isotopic equilibrium (Zhang et al., 2022). Therefore, their result may be affected by these equilibrium conditions." This is pure speculation, and in describing their methods, Laws et al. (1995: Dependence of phytoplankton carbon isotopic composition on growth rate and [CO2]aq: Theoretical considerations and experimental results. Geochim. Cosmochim. Acta 59: 1131-1138) state that "Sampling for isotopic analysis of the particulate organic carbon in the growth chamber was not begun until the culture completed at least four doublings at a given growth rate and the δ 13C of dissolved

inorganic carbon (DIC) in the growth chamber stabilized to ±0.1 per mil." That is in fact exactly the recommendation made by Zhang et al. (2022) to avoid the possibility of isotopic disequilibrium. In truth, there is no reason to think that the results of Bidigare et al. (1997) reflect isotopic disequilibrium in the growth medium. An alternative explanation for the results of Bidigare et al. (1997) is that the processes that occur inside the cell are more complex than envisioned by a mathematical model such as that of Bats et al.

REPLY: We agree with the reviewer that model results should be interpreted with caution. Indeed, models, which are an abstraction/simplification of reality and based on specific assumptions, should primarily not be used to refute properly conducted measurements. This was not our intention with this paragraph and we will adapt it accordingly.

Undeniably, the processes underlying coccolithophore calcification, and the physiological differences between the naked haploid and calcifying diploid strains of G. huxleyi, are more complex than envisioned within our model (which only focuses on the diploid strains). We deliberately implemented a rather simple calcification formulation in the model, to avoid making the model too complex for its actual purpose, which is simulating organic carbon isotope fractionation. This can result in inaccuracies regarding calcification simulations. However, considering the primary focus of our model is on organic carbon isotope fractionation, and considering this is simulated, and validated, accurately, we believe this rather simple implementation of calcification suffices for this purpose. We will elaborate on this in the revised version of the manuscript.

Lastly, the reviewer makes a good point about our discussion on the chemostat method and Zhang et al. (2022). We will take this into account in the revised version.

When Laws et al. (Sources of inorganic carbon for marine microalgal photosynthesis: A reassessment of δ13C data from batch culture studies of Thalassiosira pseudonana and Emiliania huxleyi. Limnology and Oceanography 43: 136-142 (1998) re-analyzed the data of Thompson and Calvert (1994, 1995), they concluded that CO2 released through calcification was important at high growth rates in the Thompson and Calvert dataset. Interestingly, the δ 13C values of cellular organic matter decreased at high growth rates – opposite to the expectations of a supply/demand model. The explanation in this dataset was a shift from bicarbonate at low growth rate to CO2 at high growth rates.

REPLY: This is most certainly an interesting point and we will add this to the discussion in the revised version.