Responses to Referee Comment 2

Dear Editors and Reviewers,

We thank the reviewers for their supportive and constructive comments on our manuscript. Our point–by–point responses in blue text to your comments are attached. The changed contents in the revised manuscript are underlined.

Yours sincerely,
On behalf of all coauthors
Yong Zhang

RC2

I would thank editor Dr. Koji for inviting me as a co-reviewer for this manuscript submitted in BG. Indeed, I am not an expert in coccolithophore cultivation, also not familiar with the experimental processes carried out. But I am still interested in how the algae will response in a dark and cold world, so I accept the review.

At a first glance of the title, coccolithophores were cultured in low light and temperature…, what should they response? Since, numerous papers have report how coccolithophore physiology will change with high temperature and high light. Would they present an opposite behavior in lower L and T? It is not surprised that the authors found that the major physiological properties, e.g., growth rate and most kinds of POM contents reduce in low L and low T. But importantly, they found changes in cellular stoichiometric ratio, e.g., C:N:P and PIC:POC, which could in turn regulate ocean nutrient and carbon biogeochemical cycles.

My major comments are:

(1) Such low L and low T conditions are well known in the modern oceans. For example, in the oligotrophic tropical and sub-tropical open oceans. Algae dwell in deep layers, for example 150 to more than 250 m depth forming a deep chlorophyll maximum, because of an absence of macro-nutrients in the surface. It results from a balance of nutrient and light availabilities. In the MS, the authors have selected three shelf or coastal strains of which their physiological properties may exhibit differently from those living ones. It might be better to compare the cultivation results with those living coccolithophores in modern surface and subsurface oceans.

Response: We agree and thank you for your comments. Sheward et al. (2023) synthesized data from 115 articles, reporting average cellular contents for *E. huxleyi* of 11.0 ± 6.2 pg POC, 1.7 ± 1.2 pg PON, 0.23 ± 0.12 pg POP, and 8.6 ± 6.3 pg PIC per cell. Despite the inherent influence of environmental factors like temperature and light, our findings remain consistent with these benchmarks. In lines 423–426, we added "While our experimental POC, PON, POP and PIC values align closely with the global

averages synthesized by Sheward et al. (2023), the physiological characteristics of phytoplankton in situ are further reshaped by complex environmental drivers."

Sheward, R. M., Liefer, J. D., Irwin, A. J., and Finkel, Z. V.: Elemental stoichiometry of the key calcifying marine phytoplankton *Emiliania huxleyi* under ocean climate change: A meta–analysis, Glob. Change Biol., 29, 4259–4278, doi:10.1111/gcb.16807, 2023.

(2) The authors found low lipid and carbohydrate contents per cell in low L and low T conditions. However, readers cannot distinguish what cellular physiological changes have driven the lipid and carbohydrate per cell. It may result from (1) lower total organic matter contents per cell, or (2) lower lipid density per picogram organic carbon. I would suggest dividing the lipid and carbohydrate per cell by POC per cell to distinguish such origins. This is important!

Response: We agree and thank you for your comments. "Consequently, the cell reallocates surplus energy into carbohydrates and lipids as a photoprotective mechanism. This is supported by the significantly lower carbohydrate : POC and lipid : POC ratios under low light (Figure S2), whereas the higher ratios observed at low temperature under high light reflect a strategy favoring energy storage (Zhang et al., 2021)." These contents were added in lines 349–353.

Zhang, Y., Li, Z., Schulz, K. G., Hu, Y., Irwin, A. J., and Finkel, Z. V.: Growth-dependent changes in elemental stoichiometry and macromolecular allocation in the coccolithophore *Emiliania huxleyi* under different environmental conditions, Limnol. Oceanogr., 66, 2999–3009, doi: 10.1002/lno.11854, 2021.

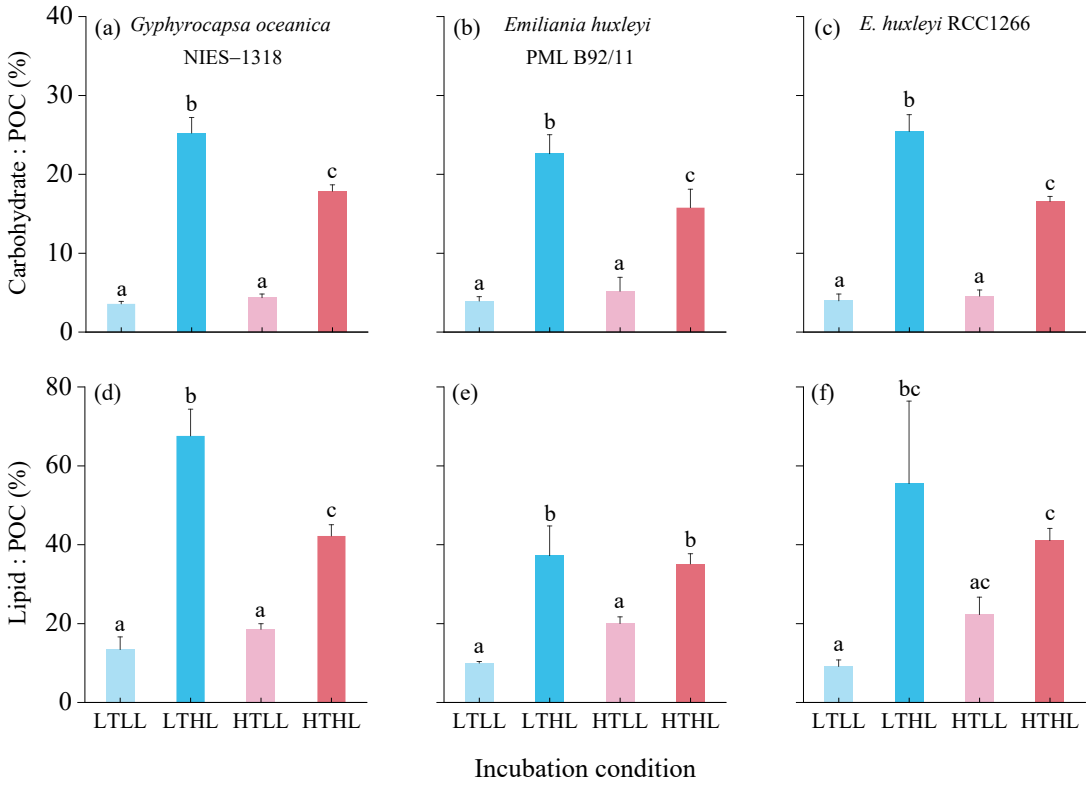

Figure S2 Under the treatments of low temperature (LT, 9 ºC) and low light (LL, 15 µmol photons m^{-2} s^{-1}), LTHL (9 ºC, 150 µmol photons m^{-2} s^{-1}), HTLL (21 ºC, 15 µmol photons m^{-2} s^{-1}) and HTHL (21 ºC, 150 µmol photons m^{-2} s^{-1}), the ratios of carbohydrate : POC and lipid : POC of *Gephyrocapsa oceanica* NIES–1318, *Emiliania huxleyi* PML B92/11 and *E. huxleyi* RCC1266. Data are presented as mean ± standard deviation for three replicates (n = 3). Different letters (a, b, c) represent significant differences between treatments (Tukey, $p < 0.05$).

(3) In the cultures, I found extremely high nutrient conditions, 64 µmol/L for nitrate and 4 µmol/L for phosphate. But we know that coccolithophores inhabit in deep subsurface waters because of nutrient limitation. I am not sure the cultivation could simulate the "reality" world.

Response: Thank you for your comments. In lines 447–452, We added "In addition, the nutrient concentrations in this study exceed typical levels found in the open ocean's surface or subsurface waters. These nutrient–replete conditions were intentionally maintained to decouple the physiological effects of temperature and light from nutrient

stress. Consequently, when extrapolating our findings to natural environments, one must account for the potential of nutrient limitation to modulate or attenuate the observed responses."

Minor suggestions:

Line 55-56: which enhances…

Response: We agree and have changed it.

Line 57: are important contributors to the biological and carbonate counter pumps.

Response: We agree and have changed it.

Line 59-60: the North Atlantic and Southern Ocean are not oligotrophic, though phytoplankton growth can be limited by iron availability.

Response: We agree and changed "sectors of the North Atlantic and Southern Ocean" to "Eastern Mediterranean" in line 60.

Line 63-64: but some authors state that coccolithophores in deep layers may be heterotrophic or mixotrophic, rather than just autotrophic.

Response: We agree and added "and osmotrophy (heterotrophic)" in line 63.

Using subsections in Discussion may improve the readability of the article.

Response: We agree and added subsections in Discussion section.