Reply to the Associate Editor
We have carefully addressed all the points raised by the reviewers and the editor, revising our manuscript accordingly. We sincerely appreciate the reviewers' insightful comments, which have helped us improve the clarity and quality of our work.

Both Referee 1 and the Associate Editor highlighted some minor aspects related to the clarity of certain sentences and Figure 2. We have made the necessary adjustments to the text and the figure to enhance readability and ensure clarity.

Rebuttal letter referee nr. 1

Overall

The aim of the study is worthwhile, as it focuses on a relatively understudied coccolithophore (compared to model species such as *Emiliania/Gephyrocapsa huxleyi* and *Gephyrocapsa oceanica*). The authors investigated whether elevated pCO_2 impacts *Helicosphaera carteri*, assessing coccolith morphology and particulate inorganic and organic carbon (PIC and PIC, respectively). The authors claim that the results of this study suggest that *H. carteri* may have a constant contribution to the rain ratio under ocean acidification.

The revised manuscript is significantly improved over the previous version. The authors' did a good job incorporating feedback from myself and the other reviewer, which has strengthened the manuscript. Notably, they have added text to the methods and discussion clarifying their experimental setup and the lower DIC under OA conditions. I only have one major concern remaining (Fig. 2), as well as some minor revisions and recommendations for the text.

REPLY: We appreciate the reviewer's positive assessment of our revised manuscript. We have added the error to Fig. 2 and prepared a second revision incorporating the reviewer's suggestions.

Figure 2.

I appreciate the authors' desire to improve the readability of the manuscript by present the data in multiple formats, but my concern is that, currently, the figure is misleading and detracts from the conclusion—that there is no difference in the percentage of malformed coccoliths under increase CO_2. Many (if not most) readers tend to look at figures first when reading results. It is necessary to incorporate error in some way so that readers cannot be misled by the figure. There are methods to incorporate error bars on stacked bar plots, but if that is not feasible, perhaps the type of figure should be changed.

REPLY: We adjusted the fig. 2 following the suggestions.
NEW FIGURE:

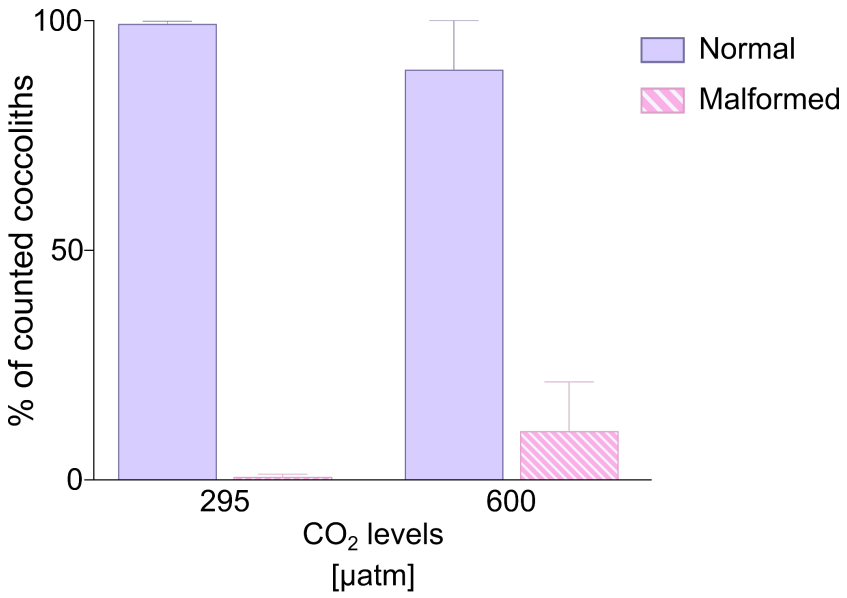

Figure 2. Percentages (%) of normal and malformed coccoliths of *H. carteri*. Values reported represent the averages of the three replicates. Error bars show standard deviation.

Minor Comments and Recommendations

Introduction

- Line 58: "...*H. carteri* produces between ~80 120 pg cell^{-1} day^{-1}". Missing an 'and' or dash
  REPLY: We adjusted the sentence.
  NEW TEXT: "..*H. carteri* produces between ~80 and ~120 pg cell^{-1} day^{-1}".

- Line 70: "Indeed, despite the coccosphere's function is still unclear...". The grammar is a bit unclear. Rephrase for clarity.
  REPLY: We replaced this sentence.
  NEW TEXT:
  Lines 71-72: "Both calcite production and coccospheres are beneficial for coccolithophores in terms of eco-physiology and evolution (e.g., Henriksen et al., 2003; Langer et al., 2021; Monteiro et al., 2016; Walker et al., 2018)."

Materials and Methods

- Table 1 caption: Define SD in caption.
  REPLY: We added the SD definition in the caption.

- Line 176: "CL^3 is the coccolith length." Is there a typo here? Should it be C_L, C_{L3}?
  REPLY: The coccolith length is represented by C_L. We adjusted the typo.

Results

- Lines 224-226: I don't think it is necessary to state that the high standard deviation (SD) is due to high variability among the replicates (and vice versa for low SD and low variability). It's a bit redundant.
  REPLY: We have modified the sentences in order to avoid redundancy.
  NEW TEXT:
  Lines 224-225: "The percentage of malformed coccoliths at 600 µatm is characterized by a high standard deviation (SD). On the contrary, at 295 µatm, SD is quite low in all the considered categories (Table 2)."

- Lines 241-242: I recommend condensing into one sentence and rephrasing.
  REPLY: We have modified the text.
  NEW TEXT:
  Lines 240-241: "Cellular POC returns an average of 108.14±5.42 pg $cell^{-1}$ at 295 µatm and 118.51±6.41 pg $cell^{-1}$ at 600 µatm of CO_2, with an unpaired t-test showing no significant change between CO_2 levels (t-test p value>0.05; Table 3)."

- Line 246: Define protoplast and coccosphere sizes as 'dimensionless' in methods. Eliminate 'µm/µm'.
  REPLY: We eliminated 'µm/µm' and added a specific in the methods section.
  NEW TEXT:
  Lines 166-168: "Since AR and RD are based on the ratio between major and minor axes of the coccosphere and/or the protoplast, they are considered dimensionless. Hence, the unit for these parameters is not reported."

- Lines 246-249: There only seems to be one set of values reported (600 µatm?). Is it meant to include the values at 295 µatm as well?
  REPLY: Yes, the values included both the values from 295 and 600 ppm. We adjusted the text to make it more clear.
  NEW TEXT:
  Lines 241-250: "A non-significant change is also observed in cellular PIC and in the PIC:POC ratio, showing an average value of 151.86±4.23 pg $cell^{-1}$ at 295 µatm and 149.47±9.49 $cell^{-1}$ at 600 µatm of CO_2 (t-test p value>0.05; Table 3) and of 1.37±0.072 at 295 µatm and 1.27±0.013 at 600 µatm of CO_2, respectively (t-test p value >0.05; Table 3).

Helicosphaera carteri protoplast (0.90±0.02 and 0.90±0.01 at 295 µatm and 600 µatm, respectively) and coccosphere (0.89±0.02 at 295 µatm and 0.88±0.003 at 600 µatm) roundness does not show any significant variation with increasing CO_2 (t-test p value>0.05), indicating the maintenance of a constant shape at different CO_2 levels (Fig. 3a, b; Appendix A Table A1). No changes have been detected for protoplast (11.45±0.19 µm at 295 µatm and 11.81±0.27 µm at 600 µatm; t-test p value>0.05; Fig. 3c; Appendix A Table A1) and coccosphere size (18.18 ±0.25 µm and 17.92±0.66 at 295 µatm and 600 µatm, respectively; t-test p value>0.05; Fig. 3d; Appendix A Table A1)."

- Line 250-251: "The range of..." This seems to repeat the previous sentence, except it also states trends that are not statistically significant (i.e., "slightly higher range"). I recommend eliminating.
  REPLY: We appreciate the suggestion. We eliminated the repetition.

Discussion

- Line 259: Section 4.1 header may be a bit misleading as written. Consider rephrasing to clarify that elevated CO_2 did not lead to increased malformations.
  REPLY: We replaced "malformations" by "morphology" (**Line 258**).

- Lines 330-331: It's awkward as written. Consider rephrasing and avoid 'good health'.
  REPLY: We changed the sentence.
  NEW TEXT:
  Lines 329-330: "Daily observation of the living culture under a light microscope showed in both CO_2 treatments that *H. carteri* remained in a good condition, with good motility of the cells."

- Lines 338-342: "A non-significant variation" sounds awkward. It seems like you are drawing attention to the variability, not the lack of a significant difference. Clarify that Le Guevel et al (2024) did not observe a difference in PIC:POC with changing CO_2, but coccosphere size was impacted.
  REPLY: We adjusted the text.
  NEW TEXT:
  Lines 336-338:"The maintenance of a stable PIC:POC ratio in the same *H. carteri* strain and at similar CO_2 levels (300 µatm and 600 µatm) has recently been observed also by Le Guevel et al. (2024) (Fig. 5), who also recorded a slight increase in coccosphere size within this CO_2 range (+0.69 µm from 200 to 600 µatm). These authors grew the species under even higher CO_2 levels, recording a decrease in coccosphere size (-1.05 µm) moving from 600 µatm to 1400 µatm of CO_2. However, this decrease in coccosphere size with

increasing CO_2/decreasing pH was not associated with a significant trend in the PIC:POC ratio (Le Guevel et al., 2024)."

- Line 346: "global decreasing trend in CO_2". Consider providing the range of CO_2 to make it more relevant (i.e., is ~600µatm represented in this study?)
  REPLY: In the cited works, the CO_2 range considered spans from the warm, high-CO_2 world of the Middle Miocene to the cooler, low-CO_2 conditions of the Pleistocene. Specifically, CO_2 levels ranged from 350–500 ppm during the Middle Miocene to as low as 200 ppm in the Pleistocene.

  We adjusted the text by adding the range of CO_2.
  NEW TEXT:
  Lines 345-348: "These authors documented a stable PIC:POC ratio of this genus along with a reduction of coccolith (and coccosphere) size in response to the global decreasing trend in CO_2, which ranged from ~350–500 ppm during the Middle Miocene to ~200 ppm in the Pleistocene (Herbert et al., 2016; Sosdian et al., 2018; Super et al., 2018; Zachos et al., 2001; Zhang et al., 2013)."

- Lines 335-337: "could represent an advantage in future oceans where the species could play a stable role in the C cycle despite changes in CO_2 concentrations." What kind of advantage? Is it an advantage to *H. carteri*? Isn't the point that stable PIC:POC means the contribution to the rain ratio should remain stable over elevated CO_2 concentrations?
  REPLY: We clarified this by rephrasing as follows:
  NEW TEXT:
  Lines 357-358: "…could stabilize the future C-cycle despite changes in CO_2 concentrations."

- Lines 385-387: "The most likely explanation of these observations is that other aspects than the PIC:POC ratio influence the species' response to increased CO_2 levels." Isn't this reversed? Aspects other than CO_2 influence the PIC:POC ratio.
  REPLY: No, it is not reversed. However, we changed the phrasing to make it clearer.
  NEW TEXT:
  Lines 386-388: "The most likely explanation for these observations is that the PIC:POC ratio is not a sufficient predictor for the strain's sensitivity to increased CO_2."

References

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