# **BG Discussion: Reply to RC1**

We would like to thank the first anonymous reviewer for the kind and constructive feedback. A detailed response to their comments is found below.

# **Overview:**

de Vries et al. present research on the distinct life cycle of *Coccolithus braarudii*, a species of coccolithophores, focusing on the trade-offs between its haploid and diploid phases. They examined how these phases respond to environmental factors such as light, temperature and nutrients. With the laboratory experiments they showed similarities in cell size, nitrogen requirements, uptake rates, and optimal temperature and light conditions between the phases. However, differences were noted in coccosphere size, maximum growth rates, and nitrogen quotas. Authors offered the explanation that trade-off is observed between maximum growth rate and nitrogen quota, with the haploid phase favouring higher growth rates and lower nitrogen storage, while the diploid phase shows the opposite pattern. They also run model simulations that indicated that trade-off allows *C. braarudii* to more effectively utilise varying nitrogen conditions. The ability of the diploid phase to store more nitrogen proves beneficial when nitrogen is intermittently available, while the higher growth rate of the haploid phase is advantageous when nitrogen is constantly available. This study suggests that the trade-off between nitrogen storage and maximum growth rate is a critical factor determining the distribution and functionality of the C. braarudii life cycle.

The results of this study contribute to understanding of how haplo-diplontic life cycle works and I believe it is an important addition to the scientific literature. The paper introduces the concept of trade-off between maximum growth rate and nitrogen quota in the haploid and diploid phases of *C. braarudii*, which has not been identified previously and the models used to test the tradeoffs under different environmental conditions also provide new tools for the field.

However, I do have some major and minor comments and concerns that are provided below. In terms of the writing, it is recommended to revise both the results section and subsequent discussion. The results section currently contains significant conclusions and comparisons, going beyond the objective presentation of the findings. Therefore, it would be beneficial to adjust the content to focus on a more objective reporting of the results. Similarly, the discussion section should be modified to reflect a balanced and unbiased analysis rather than incorporating conclusions that extend beyond the presented data.

# Main remarks:

Abstract

Considering the strong photoinhibition observed in figure 6b under depleted conditions, the authors' claim that HOLs prefer high light and low nutrients becomes questionable. The pronounced differences between high/low nutrient conditions and between HOL/HET phases are noteworthy enough to merit inclusion in the abstract.

The high light/low nutrient line "These life cycle phases vary significantly in inorganic carbon content and morphology, and inhabit distinct niches, with haploids generally preferring low-nutrient and high-temperature and light environments." refers to their environmental niche, we will update the phrasing to reflect this more clearly.

## Introduction

Ls 276, 277, 278: The use of the broad term "nutrient" instead of the specific term "nitrogen" throughout the paper may lead to potential confusion. This generalisation might hold true for nitrogen but not necessarily for other nutrients such as phosphorus. I recommend considering the use of "nitrogen-depleted" specifically in the results section, where other nutrients remain present, and then extrapolating to the term "nutrients" in the discussion to encompass a broader context.

#### We will replace nutrient with dissolved inorganic nitrogen as suggested by the reviewer

L 135-136: The assumption made regarding DNA content is a bit concerning. It is reasonable only if the G2 phase is significantly longer than the G1 phase during the cell cycle and if the stationary phase primarily consists of G1 cells. It is essential to have supporting information to justify this assumption. Without such information, any calculation could potentially be true. For example, if the stationary phase primarily consists of cells in G2, the average DNA content could be even higher than that of a dividing population. Furthermore, if the G1 phase is longer than G2, the situation becomes even more complex. It is important to thoroughly discuss this issue and, if uncertainty exists, take it into account in both the assumption and subsequent models.

We agree that the assumption that DNA content is different in stationary phase cells is not supported by experimental data. We will therefore remove the assumption that DNA content changes from our calculations, recalculate the values and update the conclusion. We will also include some discussion to highlight that we haven't included assumptions about shifts between about G1 and G2 phase that could influence the result. However, we should also point out that this wouldn't change our conclusions despite the magnitude of the difference.

L 280: Please exclude the latter part of the sentence: "Furthermore, the HOL phases show highly reduced ETR during photosynthesis, especially when exposed to high light." This statement holds true only under depleted conditions.

#### We will update this as suggested.

#### Figure 1

In Figure 1e, the chromosomes of the HET phase represent metaphase chromosomes, consisting of two chromatids, indicating a genome still at the haploid state (n). The 2n condition, on the other hand, would typically represent pairs of chromosomes, which can be illustrated side by side but should not be linked by their centromeres.

# The representation of chromosomes in the figure is a minor detail, but something we can fix.

Considering the abundance of figures in the paper, it might be appropriate to consider moving the entire Figure 1 to supplemental material, as Figure 9 provides more comprehensive information. Additionally, certain differences observed in Figure 1, such as cell size, contradict the observations, which could potentially lead to confusion or misinterpretation.

#### We will move Fig 1 to the supplement.

#### **Material and methods**

L 9: Regarding cell size, was the difference between the presence and absence of a coccolith within the cell considered? Were the cell sizes estimated throughout experiments? During the exponential phase, significant coccolith calcification occurs, which could potentially impact the conclusions made. It would be valuable to investigate and account for any potential influence of coccolith presence on cell size when drawing conclusions. Additionally, please provide information on how many cells were counted per image/strain/condition?

We didn't consider whether internal coccoliths contribute to cell volume. However, it is clear from previous experimental manipulations that even when calcification is inhibited cells usually contain an internal coccolith (coccolith formation is paused, but the immature coccolith is retained). So whilst we haven't compared internal coccoliths in exponential and stationary phase cells directly, we wouldn't expect calcification rate to have a direct impact on cell size. For the purpose of the model it is important that we include a measurement of cell size. Whilst calcification could influence this parameter, it does not impact our conclusions.

At least 100 cells were counted per strain. We will update the methods to reflect this.

L 118: Although I am not an expert in quotas, I find it surprising that the estimation of Qmax and Qmin is based on completely distinct methods. Please provide the specific equations used for calculating Qmax and Qmin in the given context, especially as these are a major part of modelling efforts.

We agree that using CN for Qmin would have been ideal, but a previous comparison by Perin et al., 2008 demonstrated that these two methods give very similar results.

We will specify the equations for clarity.

L 125-129: The values of Fv/Fm and ETR are "computed" rather than directly measured, particularly ETR, which relies on parameters that are subject to certain hypotheses, such as the efficiency of light capture (cell concentration, antenna size, etc.). While it is appropriate to present the differences in ETR in the results section, the underlying cause and significance of these differences should be thoroughly discussed.

#### We will add this discussion.

L 126: In section 2.2 on nutrient limitation, it is mentioned that the "deplete" concentration is 20  $\mu$ M. Therefore, the condition indicated as 220.5  $\mu$ M should be referred to as "replete." The same clarification should be made in the legend of Figure 7.

## This was a typo, thanks for spotting it. We will update this.

#### **Results**.

Several portions of the text extensively discuss the findings and would be better placed in the discussion section. While not explicitly mentioning all of them, it is crucial for the authors to consider that the results section should solely describe the outcomes of the data analysis. Conclusions, comparisons, and in-depth discussions should be reserved for the dedicated discussion section.

L 251-254: This paragraph belongs in the discussion section.

We will do this

L 253: Cell surface (no "'s ")

We will fix this

L257: Duplicate: cycle cycle

## We will fix this

L 259: The phrase "unlike temperature" is awkwardly worded. Both for temperature and light, the differences are not life cycle phase-specific.

#### We will remove "unlike temperature"

L 263-269 This section belongs in the discussion section.

L 270-271 This sentence belongs in the discussion section.

#### We will move 263-271 to the discussion

**Figure 3**: Could you please provide the information on the number of cells counted for each condition in this section here? Additionally, remove the conclusions "Both the HOL and HET ..." from the figure legend and especially if mentioning significantly provide the appropriate data supporting this wording. It would be more appropriate to include a statistical test and describe that.

We will add the number of cells counted and the p-value for the significance statement. We feel the text helps with the interpretation of the figure, so we have retained the text "Both the HOL and HET ..." but clarified the nature of the statistical tests that support these conclusions.

L 276: Please insert "maximal", as in: "similar maximal photosynthetic efficiency"

#### We can update this

#### L 286: What is "Fig. A2" ?

The caption of Fig. A2 should read "absolute DNA content (pg N per cell)" we will update this.

L 292-294: This sentence belongs in the discussion section. In addition: considering the DNA content and its potential "cost," I find it very speculative, as evolutionary pressure in such cases would likely lead to the development of more compact genomes. However, it appears that this is not the case here, and therefore, the connection between DNA content and its cost might be somewhat far-fetched. - Discuss.

We will move this to the discussion and contextualize it with previous studies. However, we disagree that this is too far-fetched. Nitrogen requirement is a strong selector for phytoplankton fitness in oligotrophic regions, and there are trade-offs associated with having a very compact genome. Given that the genome contributes substantially to the total cellular nitrogen budget, it's reasonable to assume that switching between life cycle phases may help to lower the minimal N quota.

We agree that genomic reduction would be an effective alternative strategy to lower N quotas, although this is primarily seen in organisms adapted to ultra-oligotrophic regions. In an organism exhibiting a haplo-diplontic life cycle that inhabits a much wider range of nutrient regimes, it's important to consider how genome reduction would influence both life cycle phases.

Interestingly, there is evidence of genome reduction (or at least extensive gene loss) in the HET phase of some coccolithophores. Multiple environmental isolates of Emiliania huxleyi

have lost multiple genes associated with the HOL life cycle phase and are therefore stuck in the HET phase (von Dassow 2015 ISME). Moreover, this gene loss was observed primarily in oligotrophic isolates. So genomic reduction could be an alternative strategy for lowering N quotas in coccolithophores in oligotrophic regions, but with the major consequence of the loss of sexual reproduction.

#### Figure 5

The summarizing Figure 5 is interesting. It would be helpful to include the conditions associated with each maxima.

#### This is a good suggestion and something we could add.

Figure 6:

a) Consider renaming Fv/Fm as "Maximal yield" or a similar term, as it represents an estimation of the maximal yield, but theoretical, since it is measured in the "dark"(F0).

#### We are happy to rename Fv/Fm with maximal yield as suggested.

b) Remove "light inhibition" from the title.

#### We will do this.

Clarify whether the average values presented are based on all HET/HOL strains or just one of each. This information is important.

#### It is the average. We will update this.

Provide details on the duration of cell exposure to each light level. Are different samples used at each level?

## We will add this information to the methods section.

In Figure 6b, ensure consistency with the positioning of deplete and replete conditions. Currently, they are presented on the left and right sides in (a) but are opposite in (b), which could be misleading.

This is a good point and we will update the figure.

#### L 301-302, what is "Figure A1"?

We are unsure about this comment, but figure A1 is part of the appendix

Additionally, the results of Table 1 and Figure 8 are briefly described and may benefit from a more comprehensive explanation.

We can expand the captions, especially for Table 1.

#### Discussion

Please revise the write-up, with the intention of systematically integrating the parts that were previously discussed in the results section.

We will integrate the sections the reviewer suggested should be moved into the discussion.