BG Discussion: Reply to RC2

We would like to thank the second anonymous reviewer for the in-depth and constructive feedback. A detailed response to questions raised are provided below.

General Comments:

This manuscript considers the trait trade-offs of the haploid and diploid life phases of Coccolithophores under different environmental conditions (e.g.changes in light, nitrogen and temperature) and provides important contributions to the literature.

HOL type strains appear more sensitive to nitrogen stress (in terms of Fv/Fm and ETR), although under replete conditions HOL strains have higher max growth rates/Fv/Fm/ETR. While HOL strains have higher max growth rates, their nitrogen content per cell is lower under N replete conditions. Nitrogen deplete conditions reduce N content in both life cycle types to similar levels, but the allocation of N quota to DNA content is increased in HET strains.

Experiments using a chemostat model are used to determine if trait-tradeoffs confer competitive advantages to HOL vs HET under varying nutrient supply. The authors conclude that higher growth rates and smaller nitrogen storage seen in the haploid phase are advantageous under consistent nitrogen supply, while larger Qmax with lower growth rates is advantageous under conditions of sporadic nitrogen input. This work has applicability to informing ecosystem models with life cycle distinctions in coccolithophore populations.

Overall, this manuscript presents data comparing coccolithophore stains and life cycle types under difference environmental conditions, and shows that there are clear differences in the physiological response and growth advantages of HOL vs HET lifestyles. Furthermore, modeling these trait differences provides a unique tool for broader applicability across time and space.

However, the photosynthetic response data needs more explanation and discussion, as low Fv/Fm can imply nutrient stress but is sensitive to nutrient history and length of acclimation to new nutrient regimes. Further detail of culture methods during these experiments would be helpful. The chemostat modeling experiments also require

clarification regarding the sensitivity experimental methods (not the model construction), and better explanation of the results when presenting Fig. 8.

We will improve the explanation and discussion of the photosynthetic response and chemostat model as described in the response to the specific comments below.

Figures 1 and 9 are beautiful, but may contain more than the scope of the data presented in this paper. And in a similar sense, the discussion section should remain more closely linked to data presented in the results section. Some comments on other related topics are appropriate at the end of the section, but should be minor additions to the core discussion of the data presented.

We will move Fig 1 to the supplement and clarify the caption of Fig 9 to clarify which traits are discussion points and which traits are supported by our results.

Specific Comments:

Line 10/11: What is the difference between nitrogen requirement and nitrogen quota?

Nitrogen requirement refers here to qmin and nitrogen quota to qmax. However, as technically both Qmin and Qmax are nitrogen quotas we will update this.

Line 14: what model? Hasn't been introduced yet...

We will clarify this.

Line 41/43/47: C. braarudii, already stated full name in line 37

We will update.

Line 53: Match body text and figure text more closely? ie genome N content, transport proteins (nitrogen uptake?)...

We will standardize phrasing better.

Line 60: ...for changes in coccolithophore traits in response to light, nutrients....

We will update.

Line 67: Section 2.1 and 2.2 are both experimental?: perhaps 2.1 is specified as strain comparison experiments and 2.2 is nutrient limitation experiments? Or combine all the culture conditions under one section here? This feels like a helpful clarification for when you present the results that switch back and forth between comparing differences in strains, life-cycle phases and nutrient status...

We will rephrase the two sections:

1. Strains and culture conditions
2. Nutrient limitation experiments

Line 74-77: Does the use of HOL vs HET strains isolated from mostly coastal locations influence the ability to fully understand "low nutrient/high light/stratification adapted" vs "turbulence adapted" life cycles? i.e. how do these strains compare to more open ocean strains? and is there comparison of light cycles collected from a single location?

Yes, this is a good point. This is a clear limitation as we are limited by the strains available to us. We will add this consideration to the discussion

Line 78: It may be helpful to use "nitrogen" in place to "nutrient" to clearly specify that these experiments are nitrogen limitation experiments. Perhaps the model equations below could remain generalized to any "nutrient", but once your data is applied, I think using "nitrogen" may be more appropriate/specific.

This also came up in review 1 and we will replace nutrient with nitrogen.

Line 80: 20uM NO3 is usually a quite adequate amount of N in the surface ocean, where max concentrations may only reach 35uM below the nitracline, and could be considered replete in other studies. I'm guessing early exponential nitrogen growth physiology would be similar in both your deplete and replete conditions and differences would be seen in a batch culture only once nitrogen begins to be much lower.

The reviewer is correct that during exponential growth phase both experiments are identical. We do not take measurements for the deplete culture conditions until growth rate has ceases (i.e., at which point nitrogen is depleted to close to 0 uM NO3). We will clarify this in the text.

Line 82: Triplicate bottles? Culture volumes? Vessel type? temperature control method? More detail on culture methods would be helpful.

We apologise for not including this fundamental information and will include it in the new text. All experiments were conducted in triplicate with 50ml tissue culture flasks with 20ml of volume. All experiments were done inside a temperature-controlled room.

Line 95: BMG?

BMG is the manufacturer we will rephrase:

"fluorescence microplate (BMG Labtech)"

Line 102/192: It would be nice to see that raw cell density data over time in the supplement/appendix… how many days does it take to get to 10-20k cells?

Good suggestion, we can include this in the supplement.

Line 120: Q^{max} is also divided by cell count? Q^{min} calculation uses initial media nitrogen concentration? Unclear

Qmax is also per cell and Qmin uses initial media nitrogen concentration. We will clarify this.

Line 125: Citation for fluorescence. Parkhill 2001? Falkowski?

We will add Maxwell and Johnson JXBot 2000 as the citation for fluorescence

Line 136: Is there further explanation of this assumption or a citation? Assuming half the DNA content is assuming ALL replete cells are actively in the DNA replication stage…?.

We will remove this assumption as discussed in the reply to the other referee.

Line 163: units for P and N and Q

We will add this

Line 167: is Q^N the same as Q? Specify if N in equations is for nutrients or nitrogen.

Yes, we will fix this

Line 169: Flynn 2002?

The reviewer is correct that this was initially proposed in Flynn et al., 2002, we will update this.

Line 200: What is m^{inf} ?

Mu infinity is the maximum theoretical growth rate (as opposed to the realized maximum growth rate). We will explain this in the new manuscript.

Line 213: Is *E.hux* spelled out earlier in manuscript?

No, we will update it to the full name.

Line 226: mortality term (m, + units)

We will add this.

Line 228: redefine terms that have i subscripts (?), or define i once at the start of the chemostat model section.

We will define i as suggested (it refers to the different strains in the chemostat model)

Line 237: "Using the chemostat model, we…."

We will update this.

Line 237: does this mean main difference between experimental nutrient treatments?

This refers to the main difference for all experiments (I.e. nutrients, and light and temperature optima) but not PAM measurements. We will clarify this.

Line 238-242: Some of the justification and theory of this model experiment may be better in the discussion section? Then, this model experiment needs more explaination. I am having trouble interpreting Fig. 8 when I don't know how long the model was run, what an "input scenario" is or how you determine and implement "metabolic cost" differences.

Each model was run until steady state. Nitrogen input was constant and above saturation, although the exact number did not impact relative contributions.

Metabolic cost was defined as a lower growth rate for HET (0.5) relative to HOL (0.7). This number was based on the observed difference in maximum growth rate. We will clarify this in the Methods section of the final manuscript.

We will keep the description in the method so readers can better understand Figure 8.

Line 248: The cell size and coccosphere size were measured in replete and depleted nitrogen conditions (methods), so is this showing average across all nitrogen treatments? Or is this under culture maintenance conditions (15C + 50 mE $m^{-2}s^{-1}$) or respective temp and light optima? Clarify

This is under maintenance conditions. We will clarify this.

Line 253: Would a larger coccosphere volume have a higher metabolic cost for production? It may not influence uptake rates, but would it possibly influence growth rates in addition to grazing susceptibility?

Yes, this is a possibility as discussed in line 315

Line 259: Are average maximum growth rates observed between HET and HOL strains statistically different? Sort of looks like HOL growth rates have higher maxima within your treatment ranges for both temp and light.... (oops, addressed in next section!)

Line 262: Was there a reason for not testing higher irradiances in the lab? Eg. artificial lighting can only reach 150?

We did try higher irradiances but had trouble removing temperature effects

Fig.4: Not sure box plots are appropriate for n=3??

This is a valid point. We will update the plot to show means with a standard deviation error bar.

Line 275: Are the Fv/Fm tests conducted in the first transfer generation into N depleted media? I think there are difference in observable change in Fv/Fm based on transient

changes in N supply vs balanced growth when cells are fully acclimated to low N supply…. (Parkhill?)

For the “deplete” culture conditions the strains were maintained at exponential phase under nutrient replete conditions and then inoculated in media which contained nitrogen concentrations which were initially non-limiting, but low enough to allow the full draw down of nitrogen once the cultures reached stationary phase.

Fv/Fm was then measured at stationary phase to represent “deplete” culture conditions and measured at exponential phase to represent “replete” culture conditions.

The experiments were conducted in batch culture, so we were unable able to effects of transient changes in nutrient concentration.

Line 282: *fewer* resources? Is Fv/Fm measured during exponential growth phase? I don't think changes in Fv/Fm due to nutrient stress occur until very low level of N….? Are lower growth rates in HOL relevant to the higher Fv/Fm and ETR measured in N depleted cultures?

Fv/Fm for the replete cultures were measured during stationary phase with very low levels of nitrogen.

Fig. 6 put Depleted and Replete panels on consistent side in both a and b

We will fix this.

Line 300: What do you mean by “no apparent difference”? KQ and mmax bars look different in A1….show stats of some kind? I also still want to see the raw cell abundance data that these growth rates were estimated from…

We will rephrase to: Furthermore, there is no apparent difference between the HET and HOL life cycle phases (Fig. A1).

Fig 7. *replete* quota is Q^{max}

Thank you for the correction. We will fix this.

Lines 306-310: I think these comparisons to the literature should be in the discussion section.

We are happy to move this.

Table 1: Order strains in consistent order with other figures. Is this caption correct? I thought Table A1 had the model parameters used. This table is the parameter value estimates from your lab data?

We will update the captions to be consistent with both the table and figure. The model parameters and the measured/estimated parameters have the same values. However, some are represented with different units, we will fix this for clarity.

Line 311: “Trait trade-offs” section feels like discussion.

We include this discussion in the methods section as it is important to understand the methods used for the model.

Line 320: I think you are saying “competitive advantage” to mean the relative abundances of HOL vs HET cells? But it is unclear. This section needs better explanation of the results (possibly clarification in the methods section too). How much nitrogen is in a pulse? Does it go to zero between pulses? What is relative Qmax? How much nitrogen is supplied under continuous supply? Low nitrogen concentrations should also influence abundances of low vs high growth rate strains, I don't see how this is ruled out by your study.

The competitive advantage is the ratio of the HET and HOL cells. We will clarify this.

To answer the other comments: we used a nitrogen pulse with concentration above saturation. Running the experiment with a different concentration will not change our final results and conclusions. This is because the nitrogen concentration affects the absolute abundance of each strain, but not their relative abundances since the values of half saturation constant and maximum uptake rates are the same for both strains.

Between pulses the nitrogen input is zero, which leads the nitrogen concentration to reach equilibrium at zero as well.

The relative Qmax is already defined in the methods. It is the ratio of the Haploid to Diploid Qmax. We clarified this further in the results.

Line 345: Turbulence is not really part of this dataset…

Turbulence and nutrient intermittence are used interchangeably here following several of the cited authors. However, we can add some discussion around this.

Line 352: What’s an “extended maximum uptake rate”?

We can rephrase this to: “Longer periods at which maximum uptake rate is sustained. “

Line 354: When *what* is similar?

Nutrient uptake rates. This will be fixed

Line 356: Explain what Fv/Fm and ETR tell you about photosynthetic ability either here or in the Fv./Fm/ETR methods section.

We can add further details in the methods section

Figure 9. Are motility and calcification data part of this paper?

No but they co-occur and are thus illustrated as dotted lines. We will further clarify this.

"-a" twice in second caption sentence.

Thanks we will fix this

Technical Corrections:

We will fix all the corrections as suggested below by the reviewer.

Line 79: "…modifying the K/2 media from an initial NO_3 concentration of 220.5 mM down to 20 mM."

Line 86: no new paragraph

Line 89: "Cell size was measured for each stain in both nutrient replete and nutrient depleted cultures"

Line 95: "…growth rates were estimated using change in cell abundance over time as estimated using…"

or change "growth rate" to "cell abundance" and leave end of paragraph as is.

Line 112: temperate-sensitivity

Line 125: Walz WATER-PAM (?) Pulse-amplitude modulated

Line 125: nutrient replete and nutrient depleted

Line 126: PAM is not a measurement "…nitrogen replete experimental cultures grown with 220 mM NO_3, Fv/Fm and ETR were measured…."

Line 127: "…For the nitrogen depleted experimental cultures,"……."and Fv/Fm and ETR were measured once cells…"

Line 135: replete

Line 181/185/189/205/210/etc.: subscript K_N in equation and text

Line 241: -it

Line 225: phytoplankton abundance of both HET and HOL strains

Line 275: which temp and light conditions are the nutrient experiments conducted at, assuming respective temp and light optima, but unclear? Fig. 6b is showing the average of both HET and HOL strains…

Line 276: depleted, replete

Fig 6 – why are RCC1200 and RCC3779 not shown in panel a?

Unfortunately, both cultures were lost to a power outage before the PAM measurements were conducted. We will update the manuscript to clarify this.