Author's response to comments to "Growth of the coccolithophore *Emiliania huxleyi* in light- and nutrient-limited batch reactors: relevance for the BIOSOPE deep ecological niche of coccolithophores", submitted by L. Perrin to Biogeosciences

We have considered the comments made by reviewers 1 and 2 to our manuscript and the latter was modified as recommended. In addition to these corrections we modified text and repetitions and improved the quality of the scientific message overall the manuscript. Figures and tables as well were improved and changed when the results were not clear enough.

We found the reviewers' comments pertinent and think they improved the manuscript. We have included his suggestions in the revised manuscript. In the following we detail our responses to specific questions and are prepared to implement these corrections/changes should the article be accepted for publication in Biogeosciences.

General comments from referees and author's response

Reviewer 1

- The reviewer 1 suggests to merge certain figures and to place certain figures or tables in a supplementary material.

Figures 1, 8, 10, 11, 12 and table 3 were placed in a supplementary material. Figures 5 and 6 were merged such as figures 13 and 14. Table 5 was removed and values were described in the text of the manuscript.

- The reviewer suggests applying the model to other literature data.

We chose not to include other datasets in the present manuscript because this would have significantly increased the length of the manuscript without adding much in terms of the new modeling method proposed. We think that the modeling we present is strong enough to support our conclusions on the environmental controls on *E. huxleyi* distribution in the deep ecological niche of South Pacific Gyre. We hope our approach will also be used with other datasets in the future by other authors.

- The reviewer points out the language of the manuscript and the long sentences.

We improved considerably the language and make the text more concise avoiding repetition and long sentences. The subheadings of sub-sections 4.1.1 to 4.1.3 were deleted but the text was not deleted. The sub-sections were merged and the text was considerably reduced in order to be a short summary instead of only repetition.

Reviewer 2

- The reviewer 2 states that information on *Emiliania huxleyi* is not equal information on coccolithophores as a whole and suggests being more specific when discussing coccolithophores as a group or *E. huxleyi* as a single species.

We were really careful about this point throughout the manuscript and specified the species *E. huxleyi* when the "coccolithophores" term was not appropriate.

- The reviewer points out the focus on the deep niche and that wider implications of the study are potentially important.

The deep niche focus was chosen for two reasons: (1) little is known about *E. huxleyi* growth in these low-nutrient, low-light conditions despite the fact that they could represent a non-negligible portion of the global *E. huxleyi* population, and; (2) the BIOSOPE transect is unique in the breadth of physical and chemical parameters measured, which makes our joint experimental/modeling exercise easier. However, wider implications of the study for general oligotrophic regions than the deep niche of the South Pacific Gyre was taken into account in conclusions of the work:

"There is potential for our approach to shed light on the functioning of other oligotrophic, low-light phytoplankton ecosystems like cold, dark and nutrient-poor Arctic and Antarctic waters. "

- The reviewer states that the main message from this work is not clear enough and that figures and tables in the manuscript need to be merged, deleted or placed in Supplementary material.

The main message of our work is that batch experiments coupled to simple physiological modeling can help interpret environmental controls on distributions of coccolithophore populations in the ocean. This message was delivered more clearly than in the original manuscript. The deep niche study was chosen to apply our approach is the best possible field situation based on the available published datasets of chemical and physical properties. Figures and tables were reorganized as: figures 1, 8, 10, 11, 12 and table 3 were placed in a supplementary material; figures 5 and 6 were merged such as figures 13 and 14; table 5 was removed and values were described in the text of the manuscript.

- The reviewer suggests using the light dose as a comparison between different experimental studies rather than the amount of light.

This point and the specification of the L:D cycle for each studies taken from the literature was added to the text and discussed in addition to the intensity of irradiance.

- The reviewer points out that organic source of nitrogen could be use by *E. huxleyi* especially in oligotrophic environment.

We added the following text: "A potential influence of organic nitrogen sources, that *E. huxleyi* is capable of using (Benner and Passow, 2010), cannot be excluded, but these would be expected to have been distributed vertically in a similar way to NO_3."

Specific comments and author's changes in manuscript

Reviewer 1

1) The period between units were removed in the manuscript through the text and in figures and tables.

2) Consistent color and marker were used in all figures to be clearer.

3) Line 18: The expression 'coccolithophore ecosystem' was not appropriate here and was changed to "potentially important ecological niche for coccolithophores".

4) L. 22-24: The word "physiology" was changed in "growth".

5) L. 40-41 vs L. 44-45: The sentence “Together, these effects modulate the impact of coccolithophores on ocean-atmosphere CO_2 fluxes“ in Ln. 44-45 was removed because of the repetition with the Ln. 40-41.

6) L. 113: We specified that we only added nitrate and phosphate to the medium and that we did not add the NH_4Cl indicated in the reference medium in order to avoid the problem of multiple nitrogen sources.

7) L. 115: This sentence was changed to “Cells were acclimated to light, temperature and nutrient conditions for at least three growth cycles prior to experiments.”

8) L. 120: Light intensity was expressed in µmol photons m^{-2} s^{-1}.

9) L. 140: Samples were always taken in the afternoon between noon and 4pm, and always in the same sampling order. Each culture bottle sampling operation took approximately 45 min, preventing samples from different bottles from being taken at the same time. However, each analytic value was averaged over the three replicates. This was specified in the manuscript.

10) L. 149: This part of the sentence about coccolith width measurements was deleted.

11) L. 151: We mentioned that the error for pH measurements is 0.02 pH units.

12) L. 167: We added “PIC was obtained considering a 1:1 stoichiometry between Ca^{2+} and PIC, i.e. all of the calcium on the filters was considered to have come from calcium carbonate (Fagerbakke et al., 1994).”

13) L. 193: We made a mistake on this point. The C/N ratio for the nitrate uptake calculation is not necessary for this calculation because the PON data are available for the control experiments. To correct this point we changed the Monod plot (Fig. 5) and the part in the text that describes this point. This entailed only minor difference in the model results because the C/N ratios for the control experiments were near the Redfield ratio: for example the C/N ratio for the control NO_3 experiment of Langer et al. (2013) was 5.72.

14) L. 199: To clarify notation, we used K_R for nutrients in general, K_N for the nitrate half-saturation constant and K_P for the phosphate half-saturation constant.

15) L. 210: The “Q_N^{min}” was changed in “Q_N^{max}”.

16) L. 277: This point was discussed in the discussion part of the manuscript: “The stationary phase was not attained in the P-limited low light culture, but it can be inferred that cells were P-limited from: (a) the POP quota, which was lower than that of the control, (b) the POC:POP ratio, which was higher than that of the control, and (c) a deviation of the growth curve from exponential growth starting (at the latest) on day 16 of 19. While a decline in POP quota is an early sign of limitation, the decline in growth rate occurs later, indicating more severe limitation. The cessation of cell division

(stationary phase) would be the last stage in the process of becoming fully P-limited over the course of a batch culture".

17) L. 294: We made a mistake in calculating the magnitude of the error bar which is in fact smaller that we thought; thus we removed this sentence.

18) L. 379: The part of the sentence was changed to "numerically dominant coccolithophore *E. huxleyi".*

19) L. 381-382: We mentioned the Leonardos and Geider (2005) experiment because it is the only experiment to our knowledge where nutrient-and light co-limitation was carried out. We mentioned that this experiment was carried out with a non-calcifying strain.

20) L. 387: The sentence was changed in "…to ensure that changes in the carbonate system were within a minimal range (< 10% except for the P-limited experiment where the DIC change were 12 and 13%; Table 1)".

21) L. 396-399: This sentence was rephrased according to comment 16.

22) L. 452-458: This sentence was splited in several sentences.

23) L. 469: We removed "as well" in the sentence.

24) L. 527-529: This part was deleted to make shorter this part of the discussion.

25) L. 529-531: This part was deleted to make shorter this part of the discussion.

26) L. 550-554: A part of this sentence was deleted. The other part of the sentence was modified as "Claustre et al. (2008) reported a nitrate concentration <3 nM (i.e. below the detection limit) in the 0-100 m water column, whereas phosphate concentration was always above 0.1 µM in surface layers (Raimbault and Garcia, 2008). Moutin et al. (2008) concluded that phosphate was apparently not the limiting nutrient for phytoplankton along the BIOSOPE transect".

27) L. 557-559: The sentence was changed in "Nitrification and the vertical diffusivity of nitrate through the nitracline (Holligan et al., 1984) needs to be taken into account and could potentially be a source of dissolved nitrate in the deep niche of coccolithophores."

28) L. 572-574: A sentence was added to mention the grazing and vertical export: "The maximum estimated growth rate at the GYR station (0.024 d^{-1} at 175 m depth) corresponds to an *E. huxleyi* generation time of 29.3 days, suggesting that division rate at the DCM was extremely slow, all the more so since this estimate does not consider grazing and vertical export of cells.".

29) L. 585-590: The sentence in question was splited into several sentences.

30) L. 610-614: This sentence was modified. While it is not possible to obtain reliable half-saturation constants for nutrient uptake in a batch experiment (a chemostat experiment is necessary), other parameters such as the maximum growth rates and maximum uptake rates can indeed be estimated in a batch experiment. As far as we know the only literature found to estimate the half-saturation constant for nutrient uptake for *E. huxleyi* using a batch culture is from Eppley et al. (1969). However, we think that the transient character of batch cultures makes the determination of half-saturation constants very difficult. We propose to circumvent this difficulty by modeling the batch experiments with a simple Droop model that enables us to extract information on nutrient affinity (the half saturation constant) from the transient results of the batch experiment

31) L. 679: Reference to the final revised version of Beaufort et al. (2008) was made.

32) Table 2: POC: PON and POC:POP was reported rather than PON:POC and POP:POC and the decimal point was used instead of the comma in the final manuscript (Table 2).

Reviewer 2

Ln 18: The expression ’coccolithophore ecosystem’ was not appropriate here and was changed to “potentially important ecological niche for coccolithophores”. The sentence was modified as “Alongside the well-known, shallow-water coccolithophore blooms visible from satellites, the lower photic zone is a poorly known but potentially important ecological niche for coccolithophores in terms of primary production and carbon export to deep ocean”.

Ln 18-19: We changed the sentence as follow : “In this study, the physiological responses of an *Emiliania huxleyi* strain to conditions simulating the deep niche in the oligotrophic gyres along the BIOSOPE transect in the South Pacific oceanic gyre were investigated”.

Ln 30: This sentence was modified to “This study contributes more widely to the understanding of *E. huxleyi* physiology and behavior in a low-light and oligotrophic environment of the ocean.”

Lns 38-39: The word “contribute” was used rather than “participate”.

Ln 40-41: The reviewer did a correct comment here and we modified the sentence as: “The relative importance of calcification and photosynthesis is one of the factors that dictates the effect of coccolithophores on ocean-atmosphere CO_2 fluxes (Shutler et al., 2013). Environmental conditions such as temperature, irradiance, nutrient concentrations and pCO_2 exert a primary control on the calcification/photosynthesis ratio in coccolithophores and also affect cellular growth rates, which, together with grazing, mortality, sinking of cells and oceanic transport, define the biogeography of coccolithophores.”.

Ln 42-43: We added “in coccolithophores” to avoid confusion with the whole phytoplankton community.

Ln 44: As detailed in the comments “Ln 40-41”, the sentence was changed.

Ln 47: We started the list with “e.g.” as well in the Ln 50.

Ln 60: The term “discovered” was changed to “observed”. Deep photic zone (low light) communities of coccolithophores have been observed in the North and Central Pacific at least since the work of Okada and Honjo (1973).

Ln 62: The sentence was modified as “This deep coccolithophore niche occurred at about 200 m depth, at a very low irradiance level (< 20 µmol photons m^{-2} s^{-1}) and at a depth corresponding to the nitrate and phosphate nutricline with dissolved nitrate (NO_3) and phosphate (PO_4) concentrations of about 1 µM and 0.2 µM, respectively.”

Ln 114: We chose to work with a surface strain from the BIOSOPE transect because no *E. huxleyi* strains were isolated inside the gyre at 200 m depth. This is a limitation of our study that we will mention.

Lns 120-123: A model of the PAR daily cycle at the date and the coordinates of the GYR station was used to calculate the L:D cycle. This was between 14:10 and 12:12 along the whole transect. Thus, the 12:12 cycle used in our experiments is representative of the in situ situation. This point was specified in the manuscript: “taken from a calculation of L:D cycle at the GYR station at the date of the sampling”.

Ln 159: Samples for nutrients were analyzed on a Seal Analytical auto-analyzer model AA3. This was modified in the text of the manuscript. http://www.seal-analytical.com/Products/AA3HRAutoAnalyzer/tabid/59/language/en-US/Default.aspx.

Ln 168-170: The details were added to the manuscript: “POP was determined as the difference between the total particulate phosphorus and particulate inorganic phosphorus, analyzed using a auto-analyser Seal Analytical AA3, after the filters were placed in a solution of hydrochloric acid, according to the method of Labry et al. (2013).”.

Ln 189: It is N-uptake. This was changed in the text.

Ln 192-194: We made a mistake on this point. The C/N ratio for the nitrate uptake calculation is not necessary for this calculation because the PON data are available for the control experiments. To correct this point we will change the Monod plot (Fig. 5 in the manuscript) and the text that describes this point. This will entail only a minor difference in the model results because the C/N ratios for the control experiments are near the Redfield ratio: for example the C/N ratio for the control NO_3 experiment of Langer et al. (2013) was 5.72.

Ln 202-205: We improved the sentence about these two different methods to determine cell volume and surface area: “The volume and surface of cells (S_{cell}) was obtained either by measurements of cells (both in the control culture and at the end of the nutrient-limited cultures) for the RCC911 strain experiments, or was estimated from Q_C, the cellular organic carbon quota (in $\mathrm{pmol_C\ cell^{-1}}$), and the density of carbon in coccolithophore biomass (approximately equal to 0.015 $\mathrm{pmol_C\ \mu m^{-3}}$; Aloisi, 2015) for the batch experiments of Langer et al. (2013) for which cell measurements were not made".

Ln 214: We changed "NO_3 and PO_4" to "N and P" to avoid confusion.

Ln 216: We changed the nutrients notation in the text because of the existing confusion between nutrient N and nitrogen N. We referred to nutrients in general with the letter R, to the nutrient nitrogen with the letter N and to nutrient phosphate with the letter P.

Ln 243: We changed the notation for the half saturation constants for nutrient uptake: K_N is the constant for nitrate uptake, K_P is the constant for phosphate uptake and K_R is the generalized constant for nutrient uptake. Same thing for the nutrient quotas, e.g. $Q_{N/P}$, that was referred to as Q_R.

Ln 250-251: We mean nutrient cellular quota and we added this point to the text.

Ln 269,279,288,306: We removed these sub-headings.

Ln 293: We expressed ratios as C:P and C:N when revising the manuscript rather than P:C and N:C.

Ln 379-381: We were more careful when we talk about *E. huxleyi* and coccolithophores as a group. Of course this work gives us new insights for the species *E. huxleyi* and maybe for other Isochrysidales or Noelaerhabdaceae but undoubtedly not for all coccolithophore species.

Ln 409: This sentence was removed.

Ln 442: We changed "for decreasing phosphate than for decreasing nitrate" to " for P- limitation than for N-limitation".

Ln 447: We modified this sentence making it clear that Zondervan (2007) is almost entirely based on *E. huxleyi* results.

Ln 452-460: As in general comment, the light dose was added to the text in order to improve the comparison and because of the importance of the light dose and not only the light intensity. Only Feng et al. (2008) used a 12:12 L:D cycle, but the other mentioned studies Rokitta and Rost (2012), Trimborn et al., (2007) and Zondervan et al. (2002) used a 16:8 L:D cycle. We changed this paragraph to be more specific and avoid comparing experiments with very different L:D cycle experiments.

Ln 463-465: As noted by the reviewer the relationship between coccosphere size and coccolith size is very species-specific, thus we decided to remove a part of this sentence and modified it as "The significant correlation between cell and coccosphere volume (Figure 4) and observations of other studies (e.g. Aloisi, 2015; Gibbs et al., 2013) support the conclusion that coccosphere size in the water column and in sediments could be used as a proxy for cell size (and thus POC quota).".

Ln 467,476, 483: These summary sections were combined and written more clearly.

Ln 561: This sentence was rephrased. Other sources of nitrogen might include organic nitrogen, although based on the modeling results (see answer to general comment) we think that inorganic nitrogen dominates over organic nitrogen.
"As *E. huxleyi* is capable to use organic sources of nitrogen as shown by Benner and Passow (2010), this nitrogen source cannot be excluded, but these would be expected to have been distributed vertically in a similar way to NO_3".

Ln 573: A short comparison of this growth rate estimation was made: "Reports of the in situ growth rate of phytoplankton are not common, including for *E. huxleyi*, due to the inherent difficulties in measuring this parameter (Laws, 2013). Goldman et al. (1979) reported phytoplankton doubling times in the North Pacific around 0.36-0.89 per day which corresponds to a growth rate of approximately 0.25 d^{-1}. Selph et al. (2011) estimated growth rates in the equatorial Pacific between 110° and 140°W to be below 0.3 d^{-1} for the phytoplankton community living at 1% of surface irradiance with net growth rates (considering mortality rates) around zero.".

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