Dear Referees,

We thank you for your supportive comments and the constructive reviews on our manuscript. Our detailed responses in blue text to your comments are attached. **The changed contents in the revised manuscript are underlined.**

Responses to comments of referee 1 are shown as following:

General comments:
The present manuscript presents a comprehensive dataset investigating the interactive effects of carbonate chemistry, light intensities and nutrient availabilities on the coccolithophore E. huxleyi. The dataset consists of an impressively high number of treatments, replicates and measured parameters therein. Given the fact that interaction of multiple drivers are often impossible to predict from simpler experiments, datasets as this one are indispensable to understand the expected natural complexity in climate change effects.
Response: We thank this referee for his (her) kind words.

This vast amount of data is, however, somewhat overwhelming and it seems to me that the authors also got lost in it a bit. In its current form, the manuscript is poorly written (also with respect to language) and lacks a story line. While I do acknowledge the difficulty to find overarching patters in such a complicated dataset, the current manuscript does not make it easy for the reader to take home any conclusions. In the discussion, individual paragraphs are often not connected to each other (and sometimes even not between sentences therein). I suggest the authors to focus on the main aspects they want to interpret and discuss these in more than a few sentences, and to omit some of the other (side-)aspects. Likewise, parameters that do not get discussed in detail also do not need to be described in great detail in the (currently quite long) results section. In my opinion, quite some of this information could be sufficiently described in tables and the supplement.
Response: We agree with the suggestions of this referee. The manuscript has been refocused on growth rate, POC and PIC production rates, and fitted alpha (a) and maximum values for growth, POC and PIC production rates. We omitted a description of the rETR. The take home conclusions are that: (1) low dissolved inorganic nitrogen (LN) concentration and high CO_2 level synergistically reduced growth and POC production rate; (2) At high light intensity, low dissolved inorganic phosphate (LP) concentration did not limit growth rate at LC but led to increased high-light inhibition of growth rate at HC; (3) low nutrient concentrations (DIN or DIP) increased the maximum value and the light-use efficiencies of calcification rate. These changes are in **Lines 35–39** and **Lines 43–44** on page 2.

With respect to the general interpretation of the data, I disagree with the way the nutrient treatments are regarded. Despite the fact that cells divided 1-2 times per day (μ>1 in almost all cases) and were clearly exhibiting non-limited exponential growth, the data is discussed as if the cells were nutrient limited and compared to previous studies that investigated strong

nutrient limitation. Regarding nitrogen limitation, for example, residual DIN was 1.0 0.4 μmol L-1 in LN treatments, which is known to not limit growth, and the molar drawdown in HN and LN treatments is actually similarly high. The same is true for the molar drawdown of DIP. Thus, the discussion needs to be refocussed by considering different but not strongly limiting nutrient concentrations rather that limiting vs. non-limiting conditions. This is particularly the case as growth rates are integrated over the whole duration of the experiment (i.e. mixing phases of non-limited growth with potential limitation towards the end of the experiment), while photophysiological measurements are only taken at the final (potentially more limited) stage.

Response: Thanks for the important and supportive comments of this referee. We agree with this referee that growth, POC and PIC production rates of cells were not limited by low DIN or DIP concentration. We refocused on differences in growth, POC and PIC production rates caused by high and low nutrient concentrations rather than on liming and non-limiting nutrient conditions.

For different growth rates between HNHP and LN conditions, we described that:
LN concentration was shown to down-regulate transcripts of genes related to nitrate reductase (NRase) activity, synthesis of amino acids, RNA polymerases and nitrogen metabolism in *E. huxleyi* (Bruhn et al., 2010; Rouco et al., 2013; Rokitta et al., 2014), which led to lower overall biosynthetic activity and decreased the growth rates (Fig. 1). These changes are in **Lines 620–625** on page 29.

For subsection in the discussion section: 'Effect of low dissolved inorganic phosphate concentration on growth rate was modulated by light intensity and CO_2 level', we described that:
1. In this study, low light intensity not only limited cell growth but also was suggested to limit phosphate uptake rates (Nalewajko and Lee, 1983). In this case, compared to the HNHP condition, growth rates of *E. huxleyi* at LP condition were more likely to be limited by low-light intensity (Fig. 1a,c). High light intensity provided energy for cells to take up P, and cells at LP condition need to consume more energy to up-regulate P uptake (Nalewajko and Lee, 1983) which may lead to decreased high-light inhibition of growth rate at LP than at HNHP condition under LC. Furthermore, growth rate of *E. huxleyi* was nearly saturated at 0.25 µmol L^{-1} DIP and was saturated at 0.5 µmol L^{-1} DIP and above. This demonstrated that *E. huxleyi* possesses a high affinity for DIP (Fig. 5) which allowed *E. huxleyi* to take up PO_4^{3-} efficiently. Rokitta et al. (2016) showed that even though PO_4^{3-} concentration in the culture media declined to zero (undetectable), cell number sustained to increase for 4 days, which indicates that *E. huxleyi* cells could store phosphorus for later use. Consequently, high energy consumption mechanisms, efficient uptake and storage capacity for phosphorus in *E. huxleyi* could account for there being no significant differences in growth rates between LP and HNHP at LC and high light intensities. These changes are in **Lines 639–662** on pages 29 and 30.

2. Rising CO_2 was found to lead to higher phosphorous requirements for growth, carbon fixation and nitrogen uptake in *E. huxleyi* (Matthiessen et al., 2012; Rouce et al., 2013). At HC, higher

phosphorous requirements may lead to lower growth rates at LP in comparison to HNHP (Fig. 1a,c). In addition, at LP, cell volume was 17% larger at HC than at LC under the highest light intensity (Table S1). Large cell volume can directly lead to lower growth rates and reduce nutrient uptake by cells, thereby limiting growth Another possible reason for low tolerance to high-light intensity in growth rate at LP and HC might be a combined effect of LP and HC on the carbon concentrating mechanism (CCM) of *E. huxleyi*. LP or HC is hypothesized to down-regulate the activity of CCM in the green algae *Chlorella emersonii* and in *E. huxleyi*, respectively (Rost and Riebesell, 2004; Beardall et al. 2005). When grown at HC, LP may minimize the activity of CCM of *E. hulxeyi*, which could lead to less energy cost for maintaining high efficient CCM. The saved energy in the HC- and LP-grown cells might have exacerbated photo-inhibition. In summary, high phosphorous requirement, large cell volume and less energy consumption at LP and HC conditions may lead to increased high-light inhibition of growth rates of *E. huxleyi* (Fig. 1). These changes are in **Lines 670–692** on pages 31 and 32.

Nalewajko, C., Lee, K. : Light stimulation of phosphate uptake in marine phytoplankton, Mar. Bio., 74, 9–15, https://doi.org./10.1007/BF00394269, 1983.
Beardall, J., Roberts, S., Raven, J. A. : Regulation of inorganic carbon acquisition by phosphorus limitation in the green alga *Chlorella emersonii*, Can. J. Bot., 83, 859–864, https://doi.org/10.1139/b05-070, 2005.

Specific comments:

L33-34: The interaction between CO2 and N is actually the least significant term, why do you focus on this interaction and not the others?

Response: Synergistic effects of low dissolved inorganic nitrogen (DIN) concentration and high CO_2 level on growth and POC production rates are one of main results in this study. We also refocused on interactive effects of DIP concentration, CO_2 level and light intensity on growth and POC production rates, and effect of low nutrient concentrations on PIC production rate.

This sentence '*HC and LN synergistically decreased growth rates of E. huxleyi at all light intensities.*' were replaced by 'LN and HC synergistically reduced growth and POC production rates.' These changes are in **Line 34–36** on page 2.

L36-37: The authors do not provide any data that would allow to conclude on the competitive abilities of this species. If they want to, they would need to either conduct competition experiments, or compare nutrient uptake kinetics with those of competing species.

Response: We thank to this referee for their suggestions.

This sentence '*These results indicate that the ability of E. huxleyi to compete for nitrate and phosphate may be reduced in future oceans with high CO_2 and high light intensities.*' was replaced by 'These results showed that effects of nutrient concentrations on physiological rates of *E. huxleyi* were modulated by CO_2 level and light intensity.' These changes are in **Lines 39–42** on page 2.

L56: Why only from media, not generally from seawater?

Response: 'Coccolithophores take up CO_2 and/or HCO_3^- from seawater for carboxylation'. These

changes are in **Line 66** on page 3.

L60-65: I do not understand why the authors mention two opposing interpretations of multiple stressor effects (i.e. linearly increasing/decreasing/non-affected vs. optimum curve response) without clarifying why they use the linear trends even though they are aware of the fact the responses follow more complex optimum curves.

Response: The text '*Growth rate, particulate organic (POC) and inorganic carbon (PIC) production rates of Emiliania huxleyi, the most abundant calcifying coccolithophore species, usually display optimum responses to a broad range of CO_2 concentration, with growth, POC and PIC production rates increased, decreased or unaffected by rising CO_2 treatments (Langer et al., 2009; Richier et al., 2011; Bach et al., 2015; Jin et al., 2017).*' was replaced by 'Growth rate, particulate organic (POC) and inorganic carbon (PIC) production rates of *Emiliania huxleyi*, the most abundant calcifying coccolithophore species, usually display optimum responses to a broad range of CO_2 concentration (Bach et al., 2011). Growth, POC and PIC production rates could increase, decrease and be unaffected by rising CO_2 treatments across a narrow CO_2 range, which is dependent on the optimal CO_2 levels of these physiological rates and the selected CO_2 range (Langer et al., 2009; Richier et al., 2011; Bach et al., 2015; Jin et al., 2017).' These changes are in **Lines 70–78** on page 4.

L65-67: Really? These is also plenty of evidence for the opposite effect, also published by some of the authors.

Response: We deleted this sentence '*Increased light levels could counteract the negative effects of rising CO_2 on calcification in E. huxleyi when grown under natural fluctuating sunlight (Jin et al., 2017).*' These changes are lin **Lines 78–80** on page 4.

L67-70: Intraspecific differences are another well-established reason for differing responses (e.g. Langer et al. 2009).

Response: Differences in sampling locations, experimental setups, deviations in the measuring methods and intraspecific differences can generally be responsible for the differential responses of growth, POC and PIC productions to rising CO_2 in *E. huxleyi* (Langer et al., 2009; Meyer and Riebesell, 2015). These changes are in **Lines 80–84** on page 4.

Langer, G., Nehrke, G., Probert, I., Ly, J., and Ziveri, P.: Strain-specific responses of *Emiliania huxleyi* to changing seawater carbonate chemistry, Biogeosciences, 6, 2637–2646, https://doi.org/10.5194/bg-6-2637-2009, 2009.

L75: Photo-acclimation to HL or LL? Both are photo-acclimative processes

Response: Reduction in pigment content and effective photochemical quantum yield (F_v'/F_m') are characteristics of photo-acclimation to high light intensity (Geider et al., 1997; Gao et al., 2012). These changes are in **Lines 89** on page 4.

L86-92: The same information is presented in the discussion. Is it really necessary to present it twice with the same level of detail?

Response: This text '*Nevertheless, low nutrient concentrations often enhance the PIC quotas of E. huxleyi. This is due to the fact that low nutrient concentrations hold the cells in the G1 cell cycle phase where calcification occurs (Müller et al., 2008). A recent proteome study on E. huxleyi also shows that nutrient limitation arrests cell cycling (McKew et al., 2015). At molecular levels, nitrate or phosphate limitations down-regulate expression of genes involved in cell cycling, RNA and protein synthesis in E. huxleyi (Rokitta et al., 2014, 2016).*' were replaced by 'Nevertheless, low nutrient concentrations often enhance the PIC quotas of *E. huxleyi* because low nutrient concentrations arrest cell cycling and lengthen the G1 phase where calcification occurs (Müller et al., 2008; McKew et al., 2015).' These changes are in **Lines 100–108** on page 5.

L180: How did you measure the pressure inside the syringe filter?

Response: We cannot measure the pressure inside the syringe filter. But we used an instrument to pump seawater, which was filtered by the syringe filter. The pressure of the pump was 200 mbar.

In the final days of incubation, 25 mL samples for TA measurements were filtered (0.22 μm pore size, Syringe Filter) by gentle pressure with 200 mbar in the pump (GM-0.5A, JINTENG) and stored at 4 ^{o}C for a maximum of 7 days. These changes are in **Lines 214** on page 10.

L193: How similar were the PAM light values to those during the incubation? Please provide a quantitative comparison.

Response: PAM light values are shown in the table R1. But we deleted the description of ETR in lines **232 –243** on page 11.

Table R1. Comparison between PAM light values and incubation light intensity

Light values (μmol photons m^{-2} s^{-1})	1	2	3	4	5	6	7	8	9
PAM light	42	92	133	210	300	450	850	1126	1600
Incubation light		80	120	200	320	480			

L198-203: How was the "cellular absorption value" determined? This parameter most likely changes strongly with light-acclimation, so I do not think that one constant value can be used to convert relative ETR to absolute ones for all treatments. If the authors did not determine this values for each treatment, they should rather report the ETR in their relative unit.

Response: We agree with this referee that cellular absorption value changes strongly with light-acclimation. But we deleted the description of ETR in lines **232 –243** on page 11.

L214: Is it really true that the authors did not even measure the initial cell count but just assumed inoculation to be perfectly equal among all bottles? I do not trust the growth rate estimates at all if this is the case, especially as small differences in the low abundance range will have huge effects on the final counts.

Response: The bottles were filled with Aquil with no headspace to minimize gas exchange. The volume of the inoculum was calculated (see below) and the same volume of Aquil was taken out from 500 mL bottles before inoculation. These changes are in **Lines 179–182** on page 9.

There was 625 ml seawater in the 500 ml polycarbonate (PC) bottles. Before cells were inoculated to new seawater, finial cell concentrations (C_0) were measured. Then we calculated the inoculated volumes (V) according to V = (200 cell/ml x 625 ml)/C_0. And we don't think this method cause errors.

L234-235: Why were the two nutrient treatments analysed separately?

Response: We re-analyzed the data with a 3-way ANOVA, which shows individual and interactive effects of nutrient concentration, CO_2 level and light intensity, and compares differences among HNHP, LN and LP conditions.

A three-way ANOVA was used to determine the main effect of dissolved inorganic nutrient concentration, pCO_2, light intensity and their interactions for these variables. A two-way ANOVA was performed to test the main effect of dissolved inorganic nutrient concentration, pCO_2 and their interactions on fitted α and V_{max} of growth, POC and PIC production rates. When necessary, a Tukey Post hoc (Tukey HSD) test was used to identify the differences between two CO_2 levels, nutrient concentrations or light intensities. These changes are in **Lines 290–298** on page 14.

Table 2. Results of three-way ANOVAs of the impacts of dissolved inorganic nutrient concentration, pCO_2, light intensity and their interaction on growth rate, F_v/F_m, F_v'/F_m', POC and PIC production rates, and PIC:POC ratio.

	Factor	*F* value	*p* value
Growth rate (d^{-1})	Nut	264.7	<0.01
	C	875.6	<0.01
	L	2035.8	<0.01
	Nut×C	53.6	<0.01
	Nut×L	84.2	<0.01
	C×L	9.3	<0.01
	Nut×C×L	26.8	<0.01
F_v/F_m	Nut	68.6	<0.01
	C	184.7	<0.01
	L	225.8	<0.01
	Nut×C	10.3	<0.01
	Nut×L	8.1	<0.01
	C×L	15	<0.01
	Nut×C×L	5.2	<0.01
F_v'/F_m'	Nut	63.9	<0.01
	C	181.8	<0.01
	L	1161.8	<0.01
	Nut×C	51.9	<0.01
	Nut×L	15.3	<0.01
	C×L	9.9	<0.01
	Nut×C×L	8.1	<0.01
POC production rate	Nut	11.8	<0.01

(pg C cell^{-1} d^{-1})	C	128.9	<0.01
	L	293.7	<0.01
	Nut×C	4.9	=0.01
	Nut×L	19.0	<0.01
	C×L	8.47	<0.01
	Nut×C×L	1.94	=0.06
PIC production rate	Nut	624.4	<0.01
(pg C cell^{-1} d^{-1})	C	142.0	<0.01
	L	147.2	<0.01
	Nut×C	1.9	=0.16
	Nut×L	17.3	<0.01
	C×L	8.1	<0.01
	Nut×C×L	4.6	<0.01
PIC:POC ratio	Nut	326.7	<0.01
	C	57.7	<0.01
	L	41.8	<0.01
	Nut×C	8.3	<0.01
	Nut×L	12.5	<0.01
	C×L	4.0	<0.01
	Nut×C×L	3.3	<0.01

Nut, dissolved inorganic nutrient concentrations (µmol L^{-1}); C, pCO_2 (µatm); L, light intensity (µmol photons m^{-2} s^{-1}); POC and POC production rates, particulate organic and inorganic carbon production rates; F_v/F_m, maximum photochemical quantum yield; F_v'/F_m', effective photochemical quantum yield. These changes are in **Lines 1198–1209** on pages 56–58.

Table 4. Results of two-way ANOVAs of the effects of dissolved inorganic nutrient concentration and pCO_2 on fitted a and maximum value (V_{max}) of growth, POC and PIC production rates. More detailed information is given as in Table 2. These changes are in **Lines 1246–1249** on page 62.

		Factor	*F* value	*p* value
a	Growth rate	Nut	18.08	<0.001
		CO_2	0.186	0.6711
		Nut×CO_2	0.398	0.6776
	POC production rate	Nut	7.21	0.005
		CO_2	7.78	0.0121
		Nut×CO_2	2.50	0.11
	PIC production rate	Nut	21.73	<0.001
		CO_2	2.32	0.145
		Nut×CO_2	2.56	0.105
V_{max}	Growth rate	Nut	24.9	<0.001
		CO_2	572.7	<0.001
		Nut×CO_2	14.8	<0.001
	POC production rate	Nut	7.301	0.0048

	CO_2	15.95	0.0009
	Nut×CO_2	1.91	0.177
PIC production rate	Nut	56.06	<0.001
	CO_2	86.84	<0.001
	Nut×CO_2	0.168	0.85

L266 ff.: It is no clear to me to which of the two tests (i.e. ANOVA vs. post hoc tests) the statements regarding the p values refer to. These are two different things. Please clearly state if you base a statement of "significance" on the ANOVA itself or a posthoc test in the whole results section. If you describe an optimum-curve behaviour, for example, the ANOVA cannot capture both increasing and decreasing phases of it, but would indicate that one of the two is more dominant.

Response: p value in Table 2 (*see* above) in the manuscript refers to ANOVA, and p value in the results section refers to Tukey post hoc test. All Tukey Post hoc test in the results section were stated by 'Tukey HSD'. Alpha (a) and maximum value (V_{max}) of growth, POC and PIC production rates (optimum-curve behaviour) were calculated from fitted parameters a, b and c based on model of Eilers and Peeters (1988). And a two-way ANOVA was used to test effects of nutrient concentration and CO_2 level on a and V_{max} (Table 4).

The apparent light use efficiency, the slope (α), for each light response curve was estimated as $\alpha = 1/c$. The maximum values (V_{max}) of growth, POC and PIC production rates were calculated according to $V_{max} = \frac{1}{b+2\sqrt{ac}}$. These changes are in **Lines 286–289** on pages 13 and 14.

Eilers, P., and Peeters, J.: A model for the relationship between light intensity and the rate of photosynthesis in phytoplankton, Ecol. Model., 42, 199–215, https://doi.org/10.1016/0304-3800(88)90057-9, 1988.

L412 ff: Quite often, single sentences are not clearly connected. The discussion thus seems like a long list of ideas, but without any structure or line of thought.

Response: In the discussion section, we refocused on: (1) low dissolved inorganic nitrogen concentration and high CO_2 level synergistically reduced growth rate; (2) Effect of low dissolved inorganic phosphate concentration on growth rate was modulated by light intensity and CO_2 level; (3) low dissolved inorganic nutrient concentration (DIN or DIP) and high CO_2 level synergistically reduced POC production rate; (4) low nutrient concentrations (DIN or DIP) facilitated PIC production rate. These topics have been shown as subsections of the discussion section in the revised BG manuscript.

L414-416: Why "synergistic negative effects"? This a priory expectation is not stated (nor argued for) in the intro.

Response: As shown in Fig. 4 in the revised manuscript, maximum growth rates were significantly

lower at LN than at HNHP under both LC and HC; and they were lower at HC than at LC. So LN and HC synergistically reduced growth rates. Previous studies generally reported effects of low nutrient concentration and rising CO_2 on POC quota, so this expectation is not stated in the introduction (Sciandra et al., 2003; Rouco et al., 2013). We deleted these contents in **Lines 581–583** on page 27.

Sciandra, A., Harlay, J., Lefèvre, D., Lemée, R., Rimmelin, P., Denis, M., and Gattuso, J. P.: Response of coccolithophorid *Emiliania huxleyi* to elevated partial pressure of CO_2 under nitrogen limitation, Mar. Ecol. Prog. Ser., 261, 111–122, https://doi.org/10.3354/meps261111, 2003.
Rouco, M., Branson, O., Lebrato, M., and Iglesias-Rodríguez, M. D.: The effect of nitrate and phosphate availability on *Emiliania huxleyi* (NZEH) physiology under different CO_2 scenarios, Front. Microbiol., 4, 155, https://doi.org/10.3389/fmicb.2013.00155, 2013.

Line 1322:

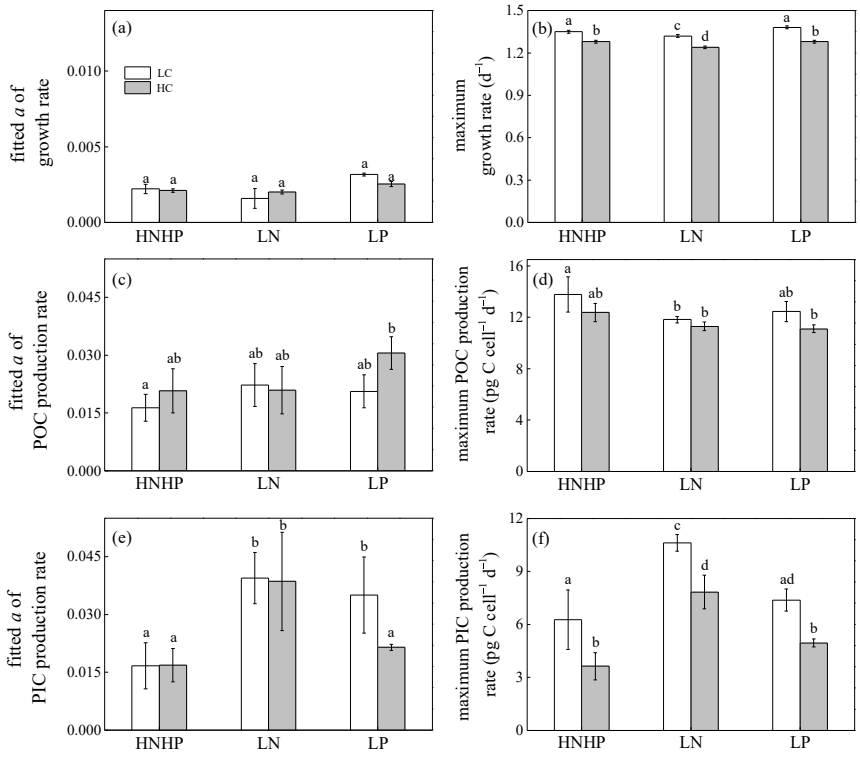


Figure 4. At both LC and HC, fitted *a* (**a**) and maximum (**b**) of growth rate at HNHP, LN and LP conditions. At both LC and HC, fitted *a* (**c**) and maximum (**d**) of POC prodution rate at HNHP, LN and LP conditions. At both LC and HC, fitted *a* (**e**) and maximum (**f**) of PIC production rate at HNHP, LN and LP conditions. *α* was the slope of fitted lines for growth, POC and PIC production rates. Different letters showed statistical differences based on the Tukey post hoc test. The values represent the mean ± standard deviation for four replicates. These changes are in **Lines 1135–1141** on page 52.

L423-430: What does the content of this paragraph mean for the interpretation of the results with respect to nutrient limitation?

Response: In order to show that growth of *E. huxleyi* is in the exponential phase at the fourth to

sixth days during culturing, we cited Langer et al. (2013).

These contents in **Lines 590–597** in page 27: 'Langer et al. (2013) detected that growth of cell on the fourth to sixth days during cultures was in the exponential phase even at 3 μmol L^{-1} NO_3^- or at 0.29 μmol L^{-1} PO_4^{3-} with the same *E. huxleyi* strain. In this study, all parameters were measured on the fourth to the sixth days, and it is most likely that cells at all treatments were sampled in the exponential growth phase' were transferred to the materials and methods section in **Lines 197–202** on pages 9 and 10.

L435-439: This could be explained by an excess of PSII reaction centres (Behrenfeld et al. 1998).
Response: We thank to this referee for his (her) nice suggestion. At high light intensity, increases in electron turnover rate through PSII can protect photosynthesis from photoinhibition. Once electron turnover rate started to decrease after it maximized, light-saturated photosynthetic rates decreased.
'because high light intensity can constantly damage the reaction centers of photosystem II (PSII) of *E. huxleyi* (Fig. 2) and maximize electron turnover rate through PSII centers (Behrenfeld et al. 1998; Ragni et al., 2008).' These changes are in **Line 600–603** on page 28.

Behrenfeld, M. J., Prasil, O., Kolber, Z. S., Babin, M., Falkowski, P. G. : Compensatory changes in photosystem II electron turnover rates protect photosynthesis from photoinhibition, Photosynth. Res., 58, 259–268, http://doi,org/10.1023/A:1006138630573, 1998.

L445-447: See my comment regarding competition above.
Response: We agree with this referee and deleted this sentence in **lines 616–618** on page 28: '*E. huxleyi* appeared to be a poor competitor for inorganic nitrate under low levels of nitrate availability (Fig. 1).'

L466-467: looking at the fit on figure 5, I am not convinced by this, as the fit does not run close to the data in the relevant part of the curve (i.e. the slope).
Response: Agreed. In figure 5 (Line 1361 in page 71), we deleted the fitted line.
We changed these contents '*Under light saturation condition, relationship of growth rates of E. huxleyi with phosphate concentrations indicated a very high affinity for dissolved inorganic phosphate (DIP) with 0.04 μmol L^{-1} half-saturation constant for DIP (Fig. 5).*' to 'Furthermore, growth rate of *E. huxleyi* is nearly saturated at 0.25 μmol L^{-1} DIP and is saturated at 0.5 μmol L^{-1} DIP and above. This demonstrated that *E. huxleyi* possesses a high affinity for DIP (Fig. 5) which allowed *E. huxleyi* to take up PO_4^{3-} efficiently.' These changes are in **Lines 646–655** on page 30.

Line 1361:

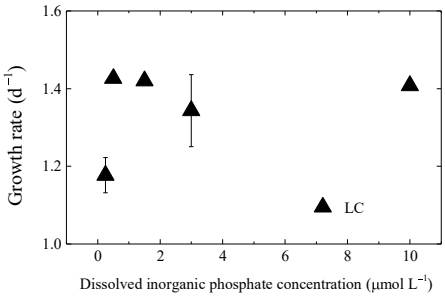


Figure 5. Growth rate of *E. huxleyi* as a function of dissolved inorganic phosphate (DIP) concentration. DIN concentration was 100 μmol L^{-1} in all culture media, and DIP concentrations were set up to 0.25 μmol L^{-1}, 0.5 μmol L^{-1}, 1.5 μmol L^{-1}, 3 μmol L^{-1} and 10 μmol L^{-1} in the culture media. All samples were incubated at 200 μmol photons m^{-2} s^{-1} and at 410 μatm pCO_2 for 4 days, and the values represent the mean ± standard deviation for three replicates. These changes are in **Lines 1150–1156** on page 53.

L480-482: In the natural environment, 10 μM NO3 is definitely not "low nutrients".

Response: We agree with this referee that 10 μM NO_3^- is definitely not "low nutrients".

We changed this text '*In natural waters, E. huxleyi usually starts to bloom following diatom blooms (Tyrrell and Merico, 2004). Therefore, our results also indicate that high growth rate of E. huxleyi at low nutrients concentrations may drive the succession of diatom to E. huxleyi.*' to 'In natural seawaters, *E. huxleyi* usually starts to bloom following diatom blooms (Tyrrell and Merico, 2004), which may be related to high growth rate of *E. huxleyi* at low nutrient concentrations.' These changes are in **Lines 665–669** on page 31.

L483-485: What is "alkaline phosphate activity"? There seems to be a word missing. Also, please explain why this is relevant.

Response: We changed 'alkaline phosphate (APase) activity' to 'alkaline phosphatase (APase) activity'. Alkaline phosphatase enzyme cleaves inorganic P from dissolved external organic sources (Dyhrman and Palenik, 2003). In our study, we did not add organic P into seawater. We have deleted '*, and to decrease alkaline phosphatase (APase) activity*' in **Lines 671–672** on page 31.

Dyhrman, S. T., and Palenik, B.: Characterization of ectoenzyme activity and phosphate-regulated proteins in the coccolithophorid *Emiliania huxleyi*, J Plank. Res., 25, 1215–1225, https://doi.org/10.1093/plankt/fbg086, 2003.

L487-492: I do not understand how this is related to LP conditions. Wouldn't one expect that P limitation would increase energy demand due to upregulated P uptake machinery?

Response: In addition, at LP, cell volume was 17% larger at HC than at LC under the highest light intensity (Table S1 or R3, *see* below). Large cell volume can directly lead to lower growth rates and reduce nutrient uptake by cells which also limit growth of cells. Another possible reason for low tolerance to high-light intensity in growth rate at LP and HC might be a combined effect of LP and HC on the carbon concentrating mechanism (CCM) of *E. huxleyi*. LP or HC is hypothesized to down-regulate the activity of CCM in the green algae *Chlorella emersonii* and in *E. huxleyi*, respectively (Rost and Riebesell, 2004; Beardall et al. 2005). When grown at HC, LP may minimize the activity of CCM of *E. hulxeyi*, which could lead to less energy cost for maintaining high efficient CCM. The saved energy in the HC- and LP-grown cells might have exacerbated photo-inhibition. In summary, high phosphorous requirement, large cell volume and less energy consumption at LP and HC conditions may lead to increased high-light inhibition of growth rates of *E. huxleyi* (Fig. 1). These contents are changed in **Lines 679–692** on pages 31 and 32.

In this study, low light intensity not only limited cell growth but also was suggested to limit phosphate uptake rates (Nalewajko and Lee, 1983). In this case, compared to HNHP condition, growth rates of *E. huxleyi* at LP condition were more likely to be limited by low-light intensity (Fig. 1a,c). High light intensity provided energy for cells to take up P, and cells at LP condition need to consume more energy to up-regulate P uptake (Nalewajko and Lee, 1983) which may lead to decreased high-light inhibition of growth rate at LP than at HNHP condition under LC. These changes are in **Lines 639–646** on pages 29 and 30.

Beardall, J., Roberts, S., Raven, J. A. : Regulation of inorganic carbon acquisition by phosphorus limitation in the green alga *Chlorella emersonii*, Can. J. Bot., 83, 859–864, https://doi.org/10.1139/b05-070, 2005.
Nalewajko, C., Lee, K. : Light stimulation of phosphate uptake in marine phytoplankton, Mar. Bio., 74, 9–15, https://doi.org./10.1007/BF00394269, 1983.
Rost, B., and Riebesell, U.: Coccolithophores and the biological pump: responses to environmental changes, in: Coccolithophores – From Molecular Biology to Global Impact, edited by: Thierstein, H. R. and Young, J. R., Springer, Berlin, 99–125, https://doi.org/10.1007/978-3-662-06278-4_52004, 2004.

L498-499: This can be solely explained by increasing levels of energy saturation of C acquisition and fixation with increasing light.
Response: Kottmeier et al., (2016) provided a nice explanation for increased carbon acquisition and fixation with increasing light.

At LC, *E. huxleyi* mainly uses external HCO_3^- as an inorganic carbon source to synthesize POC and PIC and increasing light intensity increases the HCO_3^- uptake rate (Kottmeier et al., 2016) which results in large POC and PIC production rates at high light intensity (Fig. 3). However, at HC, expression of gene related to the HCO_3^- transporter was down-regulated and the HCO_3^- uptake rate was reduced (Rokitta et al., 2012; Kottmeier et al. 2016), which lead to lower PIC production rates at HC than at LC. Meanwhile, cells at HC can increase CO_2 uptake to compensate

for low HCO_3^--uptake for photosynthetic C fixation (Kottmeier et al., 2016), which explains the similar POC quotas between HC and LC (Fig. S3). These changes are in **Lines 702–711** on pages 32 and 33.

L506-509: Seems completely unrelated to the presented and discussed data.
Response: We deleted these contents in **Lines 712–714** on page 33: '*LN down regulates expression of the rbcL gene coding for the large subunit of the ribulose-1,5-biphosphate carboxylase/oxygenase (RUBISCO) (Bruhn et al., 2010; Rokitta et al., 2014).*'

L509-511: Seems completely unrelated to previous discussion.
Response: We changed these contents in **Lines 714–724** on page 33: '*To conserve nitrogen, cells at LN prefer to shut down the synthesis of RUBISCO and then reduce carbon fixation (Falkowski et al., 1989) (Fig. 2b)*' to 'LN was found to reduce the enzymatic function and cellular metabolic rates, such as reduced synthesis and activity of ribulose-1,5-biphosphate carboxylase/oxygenase (RUBISCO), which decreases POC quota at both LC and HC (Falkowski et al., 1989; Rokitta et al., 2014) (Fig. S3 and S6). Furthermore, in comparison to LC, lower cell division rates at HC further reduce POC production rates at LN. On the other hand, large cell volume at LP and HC condition was responsible for low cell division rate and low POC production rate (Figs 1, 3 and S3).'

L515-527: Here, results from really nutrient-limited cultures are compared to the data from this study without discussing the lack of considerable nutrient-limitation of growth. Please rewrite this section by taking this into consideration. Also, take into account that under intermediate light levels, growth rates under P limitation and LC are as high as in the full media.
Response: We agree with this referee and rewrite this paragraph.
This text '*Müller et al. (2008) found that calcification (PIC production) occurred only in the G1 cell cycle phase, and that LN or LP held cells in the G1 phase longer, which led to larger PIC quotas and calcification rates at LN or at LP than at HNHP (Figs. 2 and S5). LC and LP treatment decreased cell division rates, elongated cell cycle, and increased coccolith production of E. huxleyi in the darkness (Paasche and Brubak, 1994). In the present work, however, we found slightly faster cell division (growth) and identical calcification rates at LP and high light intensities (Figs. 1c, 2f and S5). LP has been shown to up-regulate the genes involved in calcium binding proteins such as the glutamic acid related to synthesize of coccolith, calcium homeostasis and transcription factor (cmyb) (Wahlund et al., 2004; Dyhrman et al., 2006), and facilitates the formation of cytoplasmic membrane bodies (Shemi et al., 2016). These are related to the pathways associated with production of coccoliths (Young and Henriksen, 2003) and may also be responsible for larger PIC quotas at LP.*' were replaced by 'Nimer and Merrett (1993) reported that decreased DIN concentration facilitates calcification rate of *E. huxleyi*. This is consistent with our result. Due to lower photosynthetic carbon fixation rate and larger calcification rate at LN in comparison to HNHP (Fig. 3), we could expect that at LN, a high proportion of intracellular HCO_3^- or CO_2 was reallocated to synthesize particulate inorganic carbon. On the other hand, at

LP, slightly larger PIC production rate is likely due to larger cell volume in comparison to HNHP (Fig. 3).' These changes are in **Lines 728–747** on pages 33 and 34.

In addition, we provided three reasons for similar growth rates between LP and HNHP at LC and intermediate light levels. These contents were shown in **lines 639–662** on pages 29 and 30: 'In this study, low light intensity not only limited cell growth but also was suggested to limit phosphate uptake rates (Nalewajko and Lee, 1983). In this case, compared to HNHP condition, growth rates of *E. huxleyi* at LP condition were more likely to be limited by low-light intensity (Fig. 1a,c). High light intensity provided energy for cells to take up P, and cells at LP condition need to consume more energy to up-regulate P uptake (Nalewajko and Lee, 1983) which may lead to decreased high-light inhibition of growth rate at LP than at HNHP condition under LC. Furthermore, growth rate of *E. huxleyi* was nearly saturated at 0.25 µmol L^{-1} DIP and was saturated at 0.5 µmol L^{-1} DIP and above. This demonstrated that *E. huxleyi* possesses a high affinity for DIP (Fig. 5) which allowed *E. huxleyi* to take up PO_4^{3-} efficiently. Rokitta et al. (2016) showed that even though PO_4^{3-} concentration in the culture media declined to zero (undetectable), cell number sustained an increase for 4 days, which indicates that *E. huxleyi* cells could store phosphorus for later use. Consequently, high energy consumption mechanism, efficient uptake and storage capacity for PO_4^{3-} in *E. huxleyi* could account for no significant differences in growth rates between LP and HNHP at LC and high light intensities.'

Nalewajko, C., Lee, K. : Light stimulation of phosphate uptake in marine phytoplankton, Mar. Bio., 74, 9–15, https://doi.org./10.1007/BF00394269, 1983.
Rokitta, S. D., von Dassow, P., Rost, B., and John, U.: P- and N-depletion trigger similar cellular responses to promote senescence in eukaryotic phytoplankton, Front. Mar. Sci., 3, 109, https://doi.org/10.3389/fmars.2016.00109, 2016.

L535-536: ETRmax were measured at high light, so it cannot be limited by low energy input. Instead, previous acclimation to low light may have hampered usage of the provided energy.
Response: We have deleted these contents in **lines 755–760** on page 35: '*At low light intensities, the ETR_{max} values were severely limited by low energy input. Supraoptimal light intensities have been found to significantly reduce the abundance of several proteins involved in repair and assembly of PSII, such as repair of photodamaged Psb D1 proteins in the reaction center of PSII of E. huxleyi (McKew et al., 2013). These suggest that high light intensity is likely to do great damage to the PSII structure and then reduce the ETR_{max}.*'

L541: Please clarify that you have no data on CCM down-regulation but that this is speculation based on previous publications
Response: We have deleted this text in **lines 760–762** on page 35: '*Especially at HC, supraoptimal light intensity and saved energy from down-regulation of CCM activity synergistically decreased ETR_{max} (Fig. 3).*'

L547-550: Of course these processes are correlated. Can you provide something new that further elucidates this fact?

Response: We have deleted these contents in **lines 763–770** on page 35: '*A previous study found that calcification can be an additional sink for electrons in E. huxleyi (Xu and Gao 2012). Compared with HNHP, larger ETR_{max} at LN or at LP and at saturating light intensities likely resulted from larger calcification rates (Figs. 2 and 3). On the other hand, growth, photosynthetic carbon fixation and nitrogen uptake need energy originating from electron transport (Zhang et al., 2015). At LP and at limiting levels of light intensity, lower growth, photosynthetic carbon and nitrate assimilation rates coincided with lower ETR_{max} (Figs. 1–3), implying correlations of these physiological processes.*'

L555-558: I do not understand this line of thought. Please explain in more detail.

Response: Calcite process within vesicle is shown in equation 1. To calcify, *E. hulxeyi* cells need to take up HCO_3^- and Ca^{2+} from the seawater, which consumes energy. Besides that, they also need to extrude H^+ generated during calcification into the cytosol to favour the conversion of HCO_3^- to CO_3^-, which also needs some energy. Thus, calcification is a high-energy consumption process, and *E. huxleyi* needs to possess higher light-use efficiencies for their calcification.

$$HCO_3^- + Ca^{2+} \rightarrow CaCO_3 + H^+ \quad \text{equation 1.}$$

The text '*Calcification is an energy-dependent process (Riebesell and Tortell, 2011), and increased calcification rates at low nutrient concentrations could be aided by higher light-use efficiencies (Fig. 4). In addition, besides taking up inorganic carbon sources and Ca^{2+} from the seawater to calcify, cells need extra energy to expel H^+ generated during calcification from the cells (Jin et al., 2017), these may also account for higher light-use efficiencies for PIC production rates.*' was replaced by 'To calcify, *E. huxleyi* cells need to take up HCO_3^- and Ca^{2+} from the seawater, which consumes energy. Besides that, they also need to extrude H^+ generated during calcification into the cytosol to favour the conversion of HCO_3^- to CO_3^-, which also needs some energy (Paasche 2002). Thus, calcification is an energy consuming process. To maintain large calcification rate at low nutrient concentration, cells possess high light-use efficiencies and can then obtain more energy to take up HCO_3^- and Ca^{2+}, and extrude H^+ into the cytosol.' These changes are in **Lines 773–785** on pages 35 and 36.

Paasche, E. : A review of the coccolithophorid Emiliania huxleyi (Prymnesiophyceae) with particular reference to growth, coccolith formation, and calcification-photosynthesis interactions, Phycologia, 40, 503–529, 2002.

L563-566: The authors correctly state that highly labour-intensive experiments like the

current one are necessary because interactions between multiple stressors cannot be inferred from isolated effects. I therefore do not understand why they speculate on an interaction they did not investigate.

Response: Thanks for the comments of this referee. We have deleted these contents in **Lines 804–806** on page 37: '*In comparison to the current ocean environment, under HC and HL conditions as expected in future oceans, effects of LN and LP on carbon fixation of E. huxleyi may partly negate each other (Fig.2, Table 3).*'

Figure 5 legend: The method description should move into the method section and be more detailed, e.g. were growth rates integrated over 4 days? Were the cultures preacclimated to the conditions? If not, which conditions were they acclimate to before?

Response: We agree with this referee and moved method description in figure 5 legend to the materials and methods section in **Lines 270–280** on page 13.

We added these contents:

'2.6 Response of growth rate of *E. huxleyi* to different dissolved inorganic phosphate (DIP) concentrations

5 L Aquil media were enriched with 100 μmol L^{-1} DIN, aerated for 24 h at 20 ^{o}C with air containing 400 μatm pCO_2, sterilized by filtration (0.22 μm pore size, Polycap 75 AS, Whatman) and then pumped into autoclaved 250 mL PC bottles. 10 μmol L^{-1}, 3 μmol L^{-1}, 1.5 μmol L^{-1}, 0.5 μmol L^{-1}, 0.25 μmol L^{-1} DIP (finial concentration) were respectively added into Aquil media with three replicates at each DIP concentration. 200 cells mL^{-1} was inoculated to Aquil media and all samples were cultured at 200 μmol photons m^{-2} s^{-1} for 4 days before starting the experiment. Finial cell concentration was measured by using a Z2 Coulter Particle Count and Size Analyzer (Beckman Coulter).'

Technical corrections:

Generally, there are a lot of instances where grammar and wording need to be improved. I strongly suggest the native speakers in the author list to thoroughly correct the final revised version of this manuscript. Below a few examples:

Response: The native speakers in the author list have corrected the grammar and wording in the final revised manuscript.

L34-35: Please correct/rephrase this sentence.

Response: This sentence '*High light intensities compensated for inhibition of LP on growth rates at LC, but exacerbated inhibition of LP at HC.*' was replaced by 'At high light intensity, LP did not limit growth rate at LC, but led to increased high-light inhibition of growth rate at HC.' These changes are in **Lines 36–39** on page 2.

L48-49: Please correct/rephrase this sentence.

Response: Agreed. This sentence: '*Anthropogenic emission of CO_2 is taken up by the oceans, decreasing pH of seawater and resulting in ocean acidification (OA)*' was replaced by 'Rising atmospheric CO_2 level leads to increasing seawater CO_2 concentration and decreasing pH, which is known as ocean acidification (OA).' These changes are in **Lines 54–57** on page 3.

L55: Replace “in the UML” by “therein”

Response: In **Line 62** on page 3: ‘in the UML’ was replaced by ‘therein’.

L57-60: Please correct/rephrase this sentence. Why “counteract”?

Response: We have deleted ‘*which counteracts with photosynthetic CO_2 fixation,*’ in **Lines 68–69** on page 4.

L84: Consider replacing “decreased” by “suboptimal”

Response: ‘decreased’ was replaced by ‘suboptimal’ in **Line 98** on page 5.

L86-92: Combine first two sentences into one.

Response: This text ‘*Nevertheless, low nutrient concentrations often enhance the PIC quotas of E. huxleyi. This is due to the fact that low nutrient concentrations hold the cells in the G1 cell cycle phase where calcification occurs (Müller et al., 2008). A recent proteome study on E. huxleyi also shows that nutrient limitation arrests cell cycling (McKew et al., 2015). At molecular levels, nitrate or phosphate limitations down-regulate expression of genes involved in cell cycling, RNA and protein synthesis in E. huxleyi (Rokitta et al., 2014, 2016).*’ were replaced by ‘Nevertheless, low nutrient concentrations often enhance the PIC quotas of *E. huxleyi,* because low nutrient concentrations arrest cell cycling and lengthen the G1 phase where calcification occurs (Müller et al., 2008; McKew et al., 2015).’ These changes are in **Lines 100–108** on page 5.

Müller, M. N., Antia, A. N., and LaRoche, J.: Influence of cell cycle phase on calcification in the coccolithophore *Emiliania huxleyi*, Limnol. Oceanogr., 53, 506–512, https://doi.org/10.4319/lo.2008.53.2.0506, 2008.

McKew, B. A., Metodieva, G., Raines, C. A., Metodier, M. V., and Geider, R. J.: Acclimation of *Emiliania huxleyi* (1516) to nutrient limitation involves precise modification of the proteome to scavenge alternative sources of N and P, Environ. Microbiol., 17, 4050–4062, https://doi.org/10.1111/1462-2920.12957, 2015.

L93-101: Indicate at which pCO2 levels these studies were conducted.

Response: Zhang et al. (2015) reported that at 50–800 µmol photons m^{-2} s^{-1}, 1050 µatm CO_2 decreased the maximum growth rate, POC and PIC production rate of *Gephyrocapsa oceanica* compared to 510 µatm. These changes are in **Lines 111–113** on pages 5 and 6.

Under natural solar radiation, Jin et al. (2017) reported that compared to 395 µatm, 1000 µatm CO_2 increased the growth and POC production rates of *E. huxleyi* at high sunlight levels. These changes are in **Lines 116–118** on page 6.

L119: Why “Even”?

Response: ‘*Even*’ was replaced by ‘And’in **Line 138** on page 7.

L398-403: This is not discussion later on. Is it needed then?

Response: Contents in **Lines 771–785** on pages 35 and 36 explained why light-use efficiency of POC and PIC production rates was larger than that of growth rates, which is relevant with **Lines 533–536** on page 25.

L142-145: Please correct/rephrase this sentence.

Response: This sentence in **Lines 162–169** on page 8: '*The synthetic seawater medium Aquil was prepared according to Sunda et al. (2005), added by 2200 µmol L^{-1} bicarbonate (as opposed to 2380 µmol L^{-1} in the original recipe), in order to reflect the alkalinity in the South and East China Seas of about 2200 µmol L^{-1} (Chou et al., 2005; Qu et al., 2017).*' was replaced by 'The Aquil medium was prepared according to Sunda et al. (2005) with the addition of 2200 µmol L^{-1} bicarbonate, resulting in initial concentrations of 2200 µmol L^{-1} total alkalinity (TA). This reflects 2200 µmol L^{-1} alkalinity in the South and East China Seas (Chou et al., 2005; Qu et al., 2017).'

L158: For clarity, please add "For each nutrient treatment, [: : :]"

Response: We added 'For each nutrient treatment, ' in **Line 184** on page 9.

L158-159: Add standard errors for light levels.

Response: For each nutrient treatment, 20 bottles at each pCO_2 level were incubated at light intensities of 80±5, 120±8, 200±17, 320±16, and 480±30 µmol photons m^{-2} s^{-1} of photosynthetically active radiation (PAR) (4 replicates each) measured using a PAR Detector (PMA 2132, Solar Light Company, Glenside). These changes are in **Lines 184–187** on page 9.

L165-167: Please correct/rephrase this sentence.

Response: This sentence: '*Bottles were rotated two times per day at 10:00 a.m. and 6:00 p.m. to make the cells can obtain light homogeniously.*' was replaced by 'Culture bottles were rotated twice at 10:00 a.m. and 6:00 p.m..' in **Lines 192–195** on page 9.

L178: "CO2 System" should read "CO2SYS"

Response: '*CO2 System*' was replaced by 'CO2SYS' in **Line 211** on page 10.

L182: "Dickson et al. 2003" should read "Dickson et al (2003)".

Response: '*Dickson et al. 2003*' was replaced by 'Dickson et al. (2003)' in **Line 216** on page 10.

L185: "equimolal" should read "equimolar".

Response: '*equimolal*' was replaced by 'equimolar' in **Line 219** on page 10.

L186-187: I assume you did not calculate K1 and K2, but used these constants from Roy et al. for your calculations: : : If so, please correct accordingly.

Response: 'Carbonic acid constants K_1 and K_2 were taken from Roy et al. (1993).' This change is in **Lines 221** on page 10.

L194-196: Please correct/rephrase this sentence.

Response: 3 mL samples were kept in the dark for 15 min at 20 °C, and F_v / F_m values were determined at a measuring light intensity of 0.3 µmol photons m^{-2} s^{-1} and at a saturation pulse of 5000 µmol photons m^{-2} s^{-1} with 0.8 s.' These changes are in **Lines 228–231** on page 11.

L226: Replace "their" by "cellular".

Response: '*their*' was replaced by 'cellular' in **Line 266** on page 13.

L256-264: Estimates of uncertainty are missing.

Response: Table 1 in the original manuscript was replaced by Table S2 in the original supplement in the main text. The text '*The carbonate system parameters (mean values for the beginning and end of incubations) are shown in Table 1. For low CO_2 (LC) condition, the pCO_2 levels of the media were about 435 µatm at HNHP, 410 µatm at LN and 370 µatm at LP conditions, and the pH_T values (reported on the total scale) were about 8.10 at HNHP, 8.11 at LN and 8.16 at LP. For high CO_2 (HC) condition, the pCO_2 levels of the media were about 970 µatm at HNHP, 935 µatm at LN and 850 µatm at LP, and the pH_T values were about 7.80 at HNHP, 7.80 at LN, and 7.85 at LP conditions.*' was replaced by 'The carbonate system parameters of the seawater at the beginning and end of the incubation are shown in Table 1. Within the low CO_2 (LC) treatment, pCO_2 levels of the seawater declined by 16% at HNHP, 19% at LN and 8% at LP, and pH values increased by 0.07 at HNHP, 0.06 at LN and 0.02 at LP (Tukey HSD, all $p < 0.05$). Within the high CO_2 (HC) treatment, pCO_2 levels of the seawater declined by 23% at HNHP, 21% at LN and 32% at LP, and pH values increased by 0.1 at HNHP, 0.09 at LN and 0.15 at LP (Tukey HSD, all $p < 0.05$).' These changes are in **Lines 315–328** on page 15.

Table 1 (S2 in the original supplement)**.** Carbonate chemistry parameters of the seawater at the beginning and end of incubations at different nutrient conditions and pCO_2 levels.

			pCO_2 (µatm)	pH (total scale)	TA (µmol L^{-1})	DIC (µmol L^{-1})	HCO_3^- (µmol L^{-1})	CO_3^{2-} (µmol L^{-1})	CO_2 (µmol L^{-1})	Ω calcite
HNHP	LC	Before	510±17^a	8.04±0.01^a	2228±17^a	2004±20^a	1829±21^a	159±2^a	16±1^a	3.8±0.1^a
		End	428±57^b	8.11±0.05^b	2225±24^a	1967±22^b	1773±34^b	180±18^a	14±2^b	4.3±0.5^a
	HC	Before	1210±53^a	7.71±0.02^a	2219±19^a	2131±22^a	2010±22^a	81±2^a	39±2^a	1.9±0.1^a
		End	935±139^b	7.81±0.06^b	2225±24^a	2098±12^b	1966±17^b	102±14^b	30±4^b	2.4±0.3^b
LN	LC	Before	483±23^a	8.06±0.02^a	2204±10^a	1973±10^a	1796±13^a	162±6^a	16±1^a	3.9±0.1^a
		End	391±39^b	8.12±0.03^b	2123±38^b	1866±45^b	1679±48^b	175±9^b	13±1^b	4.2±0.2^b
	HC	Before	1126±66^a	7.73±0.02^a	2201±3^a	2105±7^a	1983±9^a	85±4^a	36±2^a	2.02±0.1^a
		End	888±114^b	7.82±0.05^b	2142±38^b	2016±47^b	1890±49^b	98±8^b	29±4^b	2.4±0.2^b
LP	LC	Before	397±16^a	8.14±0.02^a	2248±30^a	1982±22^a	1777±17^a	192±8^a	13±1^a	4.6±0.2^a
		End	365±24^b	8.16±0.02^a	2219±20^b	1942±22^b	1731±25^b	199±8^a	12±1^b	4.8±0.2^a
	HC	Before	1140±110^a	7.73±0.04^a	2215±41^a	2128±46^a	2005±46^a	86±7^a	37±4^a	2.1±0.2^a
		End	780±43^b	7.88±0.02^b	2228±14^a	2084±11^b	1941±12^b	117±6^b	25±1^b	2.8±0.1^b

HNHP, 101 µmol L^{-1} dissolved inorganic nitrogen (DIN) and 10.5 µmol L^{-1} dissolved inorganic phosphate (DIP); LN, 8.8 µmol L^{-1} DIN; LP, 0.4 µmol L^{-1} DIP. Different letters represent

statistically differences between the beginning and end of the experiments (Tukey Post hoc, $p < 0.05$). The values are expressed as mean values with standard deviation for four replicates. These changes are in Lines **1171–1181 on pages 54 and 55.**

L274-279: Units of the treatments are missing.

Response: At LC, growth rate at LN was similar with that at HNHP under limited light intensity with 80 µmol photons m^{-2} s^{-1} (Tukey HSD, df = 1, $p = 0.82$), and was significantly lower than at HNHP under optimal and supra-optimal light intensities (Tukey HSD, both df = 1, $p < 0.01$ for 200 µmol photons m^{-2} s^{-1}; $p = 0.005$ for 480 µmol photons m^{-2} s^{-1}). At HC, growth rates at LN were significantly lower than those at HNHP under limited, optimal and supra-optimal light intensities (Tukey HSD, all df = 1, $p < 0.01$ for 80, 200, 480 µmol photons m^{-2} s^{-1}). These changes are in **Lines 339–345** on page 16.

L346-349: Replace "At each nutrient condition, at both LC and at HC" by "At all nutrient and CO2 levels".

Response: '*At each nutrient condition, at both LC and at HC*' was replaced by 'At all nutrient and CO_2 levels,' in **Lines 414** on page 19.

L356: Why "both"?

Response: 'both' indicates 'at both LC and HC'. At both LC and HC, at 80–480 µmol photons m^{-2} s^{-1} F_v/F_m did not show significant differences between LN and HNHP (Tukey HSD, all df = 1, all $p > 0.05$), and at 480 µmol photons m^{-2} s^{-1}, they were lower at LP than at HNHP at both LC and HC (Tukey HSD, both df = 1, both $p < 0.05$) (Fig. 2a,c). These changes are in **Lines 422–425** on page 20.

L439-441: This sentence sounds as if the authors would have observed the first statement, and the reference refers to the latter, while the opposite is true. Please rephrase.

Response: This sentence: '*At HC, the negative effect of high [H^+] on growth rate was larger than positive effects of increased CO_2 and HCO_3^- concentrations, which could be attributed to lower growth rates at HC than at LC (Fig. 1) (Bach et al., 2011).*' was replaced by 'Lower growth rates at HC than at LC are due to the fact that at HC the negative effect of high [H^+] on growth rate was larger than positive effects of increased CO_2 and HCO_3^- concentrations (Bach et al., 2011).' These changes are in **Lines 607–612** on page 28.

L465: "saturation condition, relationship" should read "saturated conditions, the relationship".

Response: We have deleted this sentence: '*Under light saturation condition, relationship of growth rates of E. huxleyi with phosphate concentrations indicated a very high affinity for dissolved inorganic phosphate (DIP) with 0.04 µmol L^{-1} half-saturation constant for DIP.*' in **Lines 646–649** on page 30.

L467-471: Please correct/rephrase this sentence.

Response: these contents '*Under light saturation condition, relationship of growth rates of E. huxleyi with phosphate concentrations indicated a very high affinity for dissolved inorganic phosphate (DIP) with 0.04 µmol L^{-1} half-saturation constant for DIP (Fig. 5). Since LP was reported to enhance expression of gene with a role in phosphorus assimilation or metabolism and synthesis of inorganic* PO_4^{3-} *transporters (Dyhrman et al., 2006; McKew et al., 2015; Rokitta et al., 2016), which allowed E. huxleyi to take up* PO_4^{3-} *efficiently enough, so that LP did not result in reduced growth rate at LC in this study (Fig. 1).*' was replaced by 'Furthermore, growth rate of *E. huxleyi* was nearly saturated at 0.25 µmol L^{-1} DIP and was saturated at 0.5 DIP and above. This demonstrated that *E. huxleyi* possesses a high affinity for DIP (Fig. 4), which allowed *E. huxleyi* to take up PO_4^{3-} efficiently.' These changes are in **Lines 646–655** on page 30.

L480-482: Please correct/rephrase this sentence.

Response: In natural seawaters, *E. huxleyi* usually starts to bloom following diatom blooms (Tyrrell and Merico, 2004) which may be related to a high growth rate of *E. huxleyi* at low nutrient concentrations.' These changes are in **Lines 665–669** on page 31.

L496-502: Please correct/rephrase this sentence.

Response: This text '*At LC, E. huxleyi mainly uses external* HCO_3^- *as an inorganic carbon source for photosynthesis and calcification, and increasing light intensities are able to increase* HCO_3^- *uptake rates (Kottmeier et al., 2016). This may explain why POC and PIC quotas and production rates increased with increasing light intensity (Figs. 2 and S5). HC down-regulates gene expression related to the* HCO_3^- *transporter (Rokitta et al., 2012) and decreases the* HCO_3^- *uptake rate in E. huxleyi (Kottmeier et al. 2016), leading to lower PIC quotas at HC than at LC (Fig. 2).*' were replaced by 'At LC, *E. huxleyi* mainly uses external HCO_3^- as an inorganic carbon source to synthesize POC and PIC and increasing light intensity increases the HCO_3^- uptake rate (Kottmeier et al., 2016), which results in large POC and PIC production rates at high light intensity (Fig. 3). However, at HC, expression of gene related to the HCO_3^- transporter was down-regulated, and the HCO_3^- uptake rate was reduced (Rokitta et al., 2012; Kottmeier et al. 2016), which lead to lower PIC production rates at HC than at LC.' These changes are in **Lines 696–708** on pages 32 and 33.

L503: omit first "-"

Response: '*low-* HCO_3^- *-uptake*' was replaced by 'low HCO_3^- -uptake' in **Line 709** on page 33.

L516: Insert “could have” between “which” and “led”.

Response: We have deleted this content ‘*which led to*’ in **Lines 729** on page 33.

L553-555: I do not understand this sentence. Please rephrase.

Response: This sentence ‘*Calcification is an energy-dependent process (Riebesell and Tortell, 2011), and increased calcification rates at low nutrient concentrations could be aided by higher light-use efficiencies (Fig. 4). In addition, besides taking up inorganic carbon sources and Ca^{2+} from the seawater to calcify, cells need extra energy to expel H^+ generated during calcification from the cells (Jin et al., 2017), these may also account for higher light-use efficiencies for PIC production rates.*’ was replaced by ‘To calcify, *E. hulxeyi* cells need to take up HCO_3^- and Ca^{2+} from the seawater, which consumes energy. Besides that, they also need to extrude H^+ generated during calcification into the cytosol to favour the conversion of HCO_3^- to CO_3^-, which also consumes energy (Paasche 2002). Thus, calcification is an energy comsuming process. To maintain large calcification rates at low nutrient concentration, cells possess high light-use efficiencies and can then obtain more energy to take up HCO_3^- and Ca^{2+} and extrude H^+ into the cytosol.’ These changes are in **Lines 773–785** on pages 35 and 36.

Figures: Consider using a dashed line for one of the fits to distinguish between the two CO2 levels.

Response: Thanks for this nice suggestion of this referee. Dashed lines represent the fits at HC in all figures.

L869: Based on which test?

Response: ‘Different letters showed statistical differences based on the Tukey post hoc test.’ These changes are in **Lines 1139–1140** on page 52.

L902: Explain letters to abbreviate pCO2 and light intensity.

Response: LC represented 410 µatm pCO_2, and light intensity was 200 µmol photons m^{-2} s^{-1} in **Line 1154–1156** on page 53.

This sentence ‘*All samples were incubated at 200 µmol photons m^{-2} s^{-1} and at LC for 4 days.*’ was replaced by ‘All samples were incubated at 200 µmol photons m^{-2} s^{-1} and at 410 µatm pCO_2 for 4 days, and the values represent the mean ± standard deviation for three replicates.’ These changes are in **Line 1154–1156** on page 53.

L920-921: Please rephrase to make it a sentence.

Response: These contents ‘*All samples were incubated at 200 µmol photons m^{-2} s^{-1} and at 410 µatm pCO_2 for 4 days. The values represent the mean ± standard deviation for three replicates.*’ were replaced by ‘All samples were incubated at 200 µmol photons m^{-2} s^{-1} and at 410 µatm pCO_2 for 4 days, and the values represent the mean ± standard deviation for three replicates.’ These changes are in **Line 1154–1156** on page 53.

Figure 2: Indicate if the PIC:POC is molar- or weight-based.
Response: PIC:POC ratio is based on weight in figure 3 in **Line 1284** on page 65.

Responses to comments of referee 2 are shown as following:

The study by Zhang et al. is an important effort to address multifactorial control over the response to acidification of an important phytoplankton species, using an ambitious matrix of treatments. However, there are some major problems that must be resolved, as currently I am unsure of a major portion of the data or results interpretations as presented.
Response: We thank this referee for the positive comments. We refocused on growth rate, POC and PIC production rates. The conclusion of this study are that: (1) low dissolved inorganic nitrogen concentration and high CO_2 level synergistically reduced growth rate; (2) Effect of low dissolved inorganic phosphate concentration on growth rate was modulated by light intensity and CO_2 level; (3) low dissolved inorganic nutrient concentrations (DIN or DIP) and high CO_2 level synergistically reduced POC production rate; (4) low dissolved inorganic nutrient concentrations (DIN or DIP) facilitated PIC production rate. These conclusions have been shown as subsections in the discussion section in the revised BG manuscript.

1. In the Introduction the authors plant the study as if it aims to mimic the natural environment presently or in the future in the laboratory, that is, that the nutrient, light, and CO2 conditions they chose are truly representative. I think this is not unnecessary and risks setting up an incorrect context for interpreting the study results. For example, the authors justify the choice of light regimes in the first paragraph by claiming that phytoplankton in the future ocean will be exposed to higher light levels in the mixed layer, citing two studies. I note also that neither of the studies cited (Gao et al. 2012 and Hutchins and Hu 2017) is relevant to cite, as one is a lab study and the other is a review of lab studies, and neither is a model study predicting average light fields at which phytoplankton will be exposed in the future ocean. In any case, it is difficult to imagine that changes in the stratification of the central ocean basins can only lead to an increase in light exposure. Light exposure is highly dynamic and depends on mixing regime, so yes, the light regime should change, but to model that in the lab with constant light levels is not reasonable. My comment here does not at all negate the study design: Even though we can never mimic the ocean in the lab, it still serves to understand how factors may interact. In the case of trying to predict the response to acidification, it at least serves us to understand how robust the lab results might be for predicting the direction of possible responses, and often as well helps provide insight into mechanisms underlying the responses. I do suggest that they consider revising the Intro.
Response: We agree with this referee that it is difficult to imagine that changes in the stratification of the central ocean basins can only lead to an increase in light exposure. However, it is true that light availability is tied to the mixed layer depth and sea ice fraction, and reduced primary

production correlates with increased stratification in the tropical, southern Pacific and North Atlantic in the CSM1.4 model (Steinacher et al. 2010). Thus, two references: Gao et al. (2012), and Hutchins and Hu (2017) were replaced by Steinacher et al. (2010) in **line 62** on page 3.

Some contents in the introduction were changed (underlined are altered text).
Rising atmospheric CO_2 level leads to increasing seawater CO_2 concentration and decreasing pH, which is known as ocean acidification (OA) (Caldeira and Wickett, 2003). On the other hand, rising atmospheric CO_2 also leads to global and ocean warming, which enhances water column stratification and shoals the upper mixed layer (UML) (Wang et al., 2015). This affects light exposure of phytoplankton dwelling therein (Steinacher et al. 2010). In addition, enhanced stratification reduces the transport of nutrients from deep oceans to the UML (Behrenfeld et al., 2006), which reduces the nutrient concentrations in the UML. These changes are in **Lines 54–65** on page 3.

Coccolithophores take up CO_2 and/or HCO_3^- from seawater for carboxylation, and use HCO_3^- for calcification which produces coccoliths. Calcification processes generate CO_2 due to production of protons, and therefore influencing CO_2 influx into the oceans (Rost and Riebesell, 2004). Growth rate, particulate organic (POC) and inorganic carbon (PIC) production rates of *Emiliania huxleyi*, the most abundant calcifying coccolithophore species, usually display optimum responses to a broad range of CO_2 concentration (Bach et al., 2011). Growth, POC and PIC production rates could increase, decrease and be unaffected by rising CO_2 treatments across a narrow CO_2 range, which is dependent on the optimal CO_2 levels of these physiological rates and the selected CO_2 range (Langer et al., 2009; Richier et al., 2011; Bach et al., 2015; Jin et al., 2017). Differences in sampling locations, experimental setups, deviations in the measuring methods and intraspecific differences can generally be responsible for the differential responses of growth, POC and PIC productions to rising CO_2 in *E. huxleyi* (Langer et al., 2009; Meyer and Riebesell, 2015). These changes are in **Lines 66–84** on pages 3 and 4.

These contents '*This is due to the fact that low nutrient concentrations hold the cells in the G1 cell cycle phase where calcification occurs (Müller et al., 2008). A recent proteome study on E. huxleyi also shows that nutrient limitation arrests cell cycling (McKew et al., 2015). At molecular levels, nitrate or phosphate limitations down-regulate expression of genes involved in cell cycling, RNA and protein synthesis in E. huxleyi (Rokitta et al., 2014, 2016).*' were replaced by 'because low nutrient concentrations arrest cell cycling and lengthen the G1 phase where calcification occurs (Müller et al., 2008; McKew et al., 2015).' in **Lines 101–108** on page 5.

Recently, several studies investigated interactive effects of rising CO_2 and light intensity on physiological rates of coccolithophores (Feng et al., 2008; Jin et al., 2017). Zhang et al. (2015) reported that at 50–800 μmol photons m^{-2} s^{-1}, 1050 μatm CO_2 decreased the maximum growth rate, POC and PIC production rates of *Gephyrocapsa oceanica* compared to 510 μatm. At low light levels, coccolithophores increase CO_2 uptake to compensate for inhibition of HCO_3^- uptake on photosynthesis, while at high light intensity they don't increase CO_2 uptake (Kottmeier et al.,

2016). Under natural solar radiation, Jin et al. (2017) reported that compared to 395 μatm, 1000 μatm CO_2 increased the growth and POC production rates of *E. huxleyi* at high sunlight levels. These indicate that during growth under different experimental conditions, rising CO_2 showed contrasting effects on growth and POC production rates of *E. huxleyi* and *G. oceanica*. These changes are in **Lines 109–122** on pages 5 and 6.

Steinacher, M., Joos, F., Frö licher, T. L., Bopp, L., Cadule, P., Cocco, V., Doney, S. C., Gehlen, M., Lindsay, K., Moore, J. K., Schneider, B., Segschneider, J. : Projected 21st century decrease in marine productivity: a multi-model analysis, Biogeosciences, 7, 979–1005, 2010.

2. There is at least one major problem with the growth rates reported, possibly many more:
a. It makes no sense to report a single growth rate as the response to nutrient-limitation in batch culture experiments. At inoculation of cultures, cells should be nutrient replete even in the LP and LN conditions. If they have been "acclimated" to growing previously in the same media, the inoculums likely are from cultures that have already exhausted the phosphate (in LP) or nitrate (in LN), so the cells will have to re-configure nutrient uptake and metabolism, begin to grow, then exhaust the nutrients, re-configuring nutrient and connected metabolism again. The growth rate most certainty will NOT be constant. A recent study where these effects can be seen would be that of Rokitta et al. (2014). The authors report only a single growth rate, not changes in cell density over time, no indication of when nutrient limitation may start nor how long cells have been in nutrient limited conditions. In this sense, a good study to look at would be the recent one by Müller et al. (2017) using a continuous culture approach to understand the interaction/independence of nutrient limitation and acidification effects (curiously, the authors cite the study in the Intro but do not discuss at all, despite its central relevance!). The results presented in the current manuscript are therefore completely uninterpretable.
Response: Thanks for the comments and suggestions of this referee. To prevent seawater-air CO_2 exchange, incubation bottles were filled with seawater with no headspace and tightly closed during incubations. This is one of the reasons for measuring cell concentration at the end of the incubation and reporting a single growth rate. More importantly, studies of Rokitta et al. (2014) reported that cell number of *E. huxleyi* increased exponentially on the third to sixth days during incubation, and showed that growth rates were similar at the fourth, fifth and sixth days. We agree with this referee that growth rates are not constant, however, variation in growth rates at different days were much lower than variation in growth rates between different treatments.

Low DIN and DIP concentration did not limit growth in this study, the reasons are that:
In this study, growth rates of *E. huxleyi* were larger than 1 in almost all treatments, and cells divided 1–2 times per day (Fig .1), which indicates non-limiting nutrient conditions during the incubation. Based on measured PON quota and cell concentration in this study (Figs. 1 and S6 in the manuscript), PON concentrations at the end of incubations were estimated to be 7.8–9.3 μmol L^{-1} at different nutrient conditions (Table S2). These data were closely correlated with molar drawdown of dissolved inorganic nitrogen (DIN) during the incubations. Furthermore, residual 1 μmol L^{-1} DIN in the final day of the incubation showed non-limitation of growth and POC production rates by nitrogen. On the other hand, Rokitta et al. (2016) reported that F_v/F_m of *E.*

huxleyi was 50% lower at P-depleted than at P-replete conditions. In this study, F_v/F_m and POC quota were very similar between LP and HNHP treatments (Figs. 2 and S3), which suggests that LP did not limit growth and carbon fixation. This text was added in the first paragraph in the discussion section in **Lines 568–579** on pages 26 and 27.

Comparison between the study of Müller et al. (2017) and ours are shown as following:
Using a chemostat culture, Müller et al. (2017) reported that DIN or DIP limitation decreased the POC and PIC production rates (in pg C $cell^{-1}$ d^{-1}) by 50% and rising pCO_2 levels did not affect POC production rates. However, when normalized to cell volume, nutrient limitation did not affect POC and PIC production rates (in pg C $cellV^{-1}$ d^{-1}), and rising pCO_2 levels reduced POC and PIC production rates. In our study, decreased DIN or DIP concentration reduced the normalized POC production rates (in pg C $cellV^{-1}$ d^{-1}), and increased the normalized PIC production rates at both LC and HC (Fig. S5). Differing results between the study of Müller et al. (2017) and ours may result from different experimental setup. Growth was really limited by N or P, cells were cultured in a continuous photon flux, and cell growth was in the stable phase when POC and PIC samples were taken in the study of Müller et al. (2017). While we took POC and PIC samples in the exponential growth phase, and LN or LP did not really limit growth of *E. huxleyi* in our study. These contents were added in the discussion section in **Lines 786–798** on pages 36 and 37.

Rokitta, S. D., von Dassow, P., Rost, B., and John, U.: *Emiliania huxleyi* endures N-limitation with an efficient metabolic budgeting and effective ATP synthesis, BMC Genomics, 15, 1051–1064, https://doi.org/10.1186/1471-2164-15-1051, 2014.
Müller, M. N., Trull, T. W., and Hallegraeff, G. M.: Independence of nutrient limitation and carbon dioxide impacts on the Southern Ocean coccolithophore *Emiliania huxleyi*, ISME J., 11, 1777–1787, https://doi.org/10.1038/ismej.2017.53, 2017.

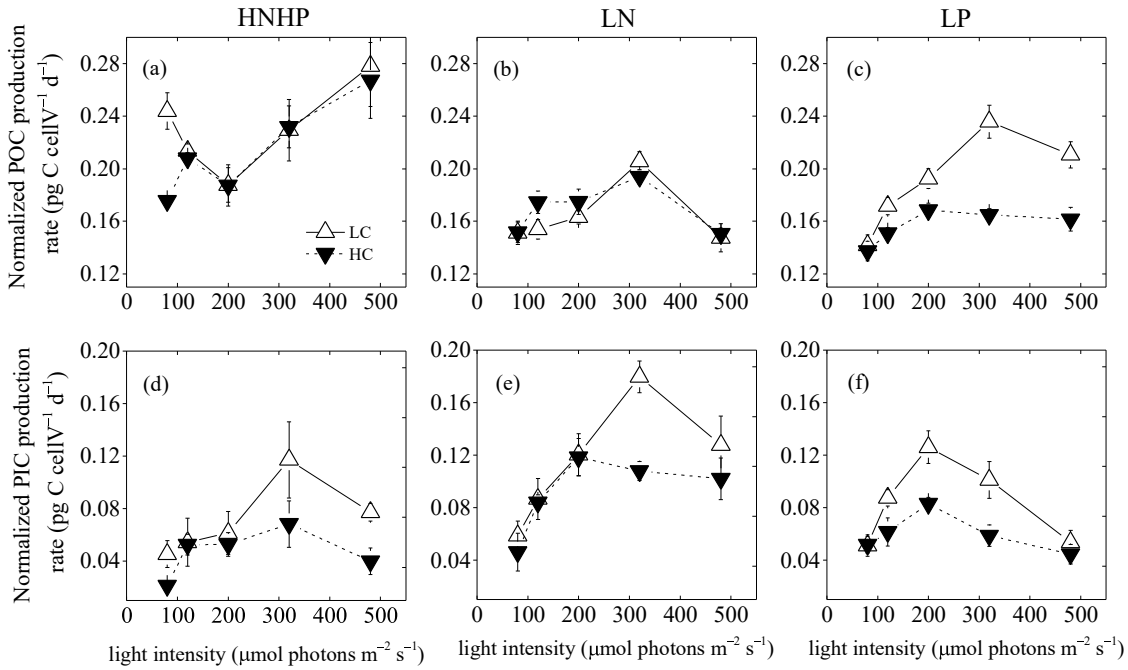


Figure S5. At both LC and HC, normalized POC production rate (pg C cellV^{-1} d^{-1}) of *E. huxleyi* as a function of light intensity at HNHP (**a**), LN (**b**) and LP (**c**) conditions. At both LC and HC, light response of normalized PIC production rate (pg C cellV^{-1} d^{-1}) of *E. huxleyi* at HNHP (**d**), LN (**e**) and LP (**f**) conditions. The values represent the mean ± standard deviation for four replicates. These contents were added in the supplement.

b. The growth rate presented appears to be calculated only from an initial cell concentration and a final one, which is generally not adequate even in batch culture experiments when nutrient limitation is avoided, because it is necessary to understand if growth rate changes or not during the experiment

Response: When cell growth is in the exponential phase, cell concentration increased exponentially with incubation days, and growth rates should be very similar.

Langer et al. (2013) found that growth of cells on the fourth to sixth days of batch cultures was in the exponential phase even at 3 μmol L^{-1} NO_3^- or at 0.29 μmol L^{-1} PO_4^{3-} with the same *E. huxleyi* strain. In this study, all parameters were measured on the fourth to the sixth days, so it is most likely that cells in all treatments were sampled in the exponential growth phase. These contents are shown in **Lines 197–202** on pages 9 and 10.

Langer, G., Oetjen, K., and Brenneis, T.: Coccolithophores do not increase particulate carbon production under nutrient limitation: A case study using *Emiliania huxleyi* (PML92/11), J. Exp. Mar. Biol. Ecol., 443, 155–161, 2013

c. The initial cell concentration appears not to have been measured, but to have been calculated, which causes many errors.

Response: The bottles were filled with Aquil with no headspace to minimize gas exchange. The volume of the inoculum was calculated (see below) and the same volume of Aquil was taken out from 500 mL bottles before inoculation. These changes are in **Lines 179–182** on page 9.

There was 625 ml seawater in the 500 ml polycarbonate (PC) bottles. Before cells were inoculated to new seawater, finial cell concentrations (C_0) were measured. Then we calculated the inoculated volumes (V) according to V = (200 cell/ml x 625 ml)/C_0. And we don't think this method cause errors.

d. The growth rates provided seem high in comparison to most previous studies of this species. Most authors report that the maximum growth rate of Emiliania huxleyi in batch cultures under "optimum" nutrient and light conditions and a day:night lighting is in the range of 0.7-0.9, a little more than one doubling per day (for just a sampling of studies, see van Bleisjwiik et al. 1994; Zondervan et al. 2002; Rokitta et al. 2014; Müller et al. 2015). Higher growth rates are occasionally reported, but under longer light cycles, e.g. Langer et al. 2009, or a very nice study by the same first author (Zhang et al. 2014). The rates here seem quite high for a 12:12 light:dark cycle, and for that reason it's important to see the data (at least in supplementary), to have full confidence in the methods, and to have at least a brief mention of this.

Response: Growth rate of *Emiliania huxleyi* was affected by light intensity, light cycle, temperature and dissolved inorganic nitrogen and phosphate concentrations and so on. I summarized the culture conditions of some studies (Table R2 in the response letter), and found that high incubation temperature (20 °C) in our study may lead to higher growth rates compared studies of Bleisjwiik et al. (1994); Zondervan et al. (2002); Rokitta et al. (2014) and Müller et al. (2015). Final cell concentration in this study was shown in Table R3 (or Table S1 in the supplement).

van Bleijswijk, J. D. L., Kemper, R. S., Veldhuis, M. J., Westbroek, P. : Cell and growth characteristics of types A and B of *Emiliania huxleyi* (prymnesiophyceae) as determined by flow cytometry and chemical analyses, J. Phycol., 30, 230–241, 1994.

Zondervan, I., Rost, B., Riebesell, U. : Effect of CO2 concentration on the PIC/POC ratio in the coccolithophore *Emiliania huxleyi* grown under light-limiting conditions and different daylengths, J. Exp. Mar. Biol. Ecol., 272, 55–70, 2002.

Müller, M. N., Trull, T. W., and Hallegraeff, G. M.: Differing responses of three Southern Ocean *Emiliania huxleyi* ecotypes to changing seawater carbonate chemistry, Mar. Ecol. Prog. Ser., 531, 81–90, 2015.

Langer, G., Nehrke, G., Probert, I., Ly, J., and Ziveri, P.: Strain-specific responses of *Emiliania huxleyi* to changing seawater carbonate chemistry, Biogeosciences, 6, 2637–2646, 2009.

Zhang, Y., Klapper, R., Lohbeck, K. T., Bach, L. T., Schulz, K. G., Reusch, T. B. H., and Riebesell, U.: Between- and within-population variations in thermal reaction norms of the coccolithophore *Emiliania huxleyi*, Limnol. Oceanogr., 59, 1570–1580, 2014.

Table R2. Growth rates and experimental culture conditions of some studies.

Reference	Growth rate (d^{-1})	Light intensity (μmol photons m^{-2} s^{-1})	Light cycle (Light/Dark)	Temperature (°C)	DIN concentration (μmol L^{-1})	DIP concentration (μmol L^{-1})
Bleijswijk et al. 1994	0.8	70 or 140	16:8	10 or 15	30 to 39	0.2 to 0.4
Zondervan et al. 2002	1.1	150	16:8	15	100	6.25
Rokitta et al. 2014	0.8	250	16:8	15	100	6.25
Müller et al. 2015	0.3–0.6	100–115	24:0	14	88	3.6
Langer et al. 2009	**1.2–1.6**	400	16:8	**17–20**	100	6.25
Zhang et al. 2014	**1.1–1.6**	160	16:8	**15–22**	64	4
This study	**1.2–1.3**	200	12:12	**20**	100 or 8	10 or 0.4

Table R3 (S1). Final cell concentration and cell volume at the end of the incubation, and incubation period. Data in the brackets are the standard deviations for four replicates. These contents were added in supplement as Table S1.

Initial N/P	pCO_2	L	Final cell concentration (cell mL^{-1})	Incubation time (d)	cell volume (μm^3)
101/10.5	435	80	153,960(14,490)	6	39.82(1.33)
		120	86,910(11,650)	5	51.67(0.96)
		200	40,060(5,180)	4	62.22(0.97)
		320	35,250(4,280)	4	54.88(1.13)
		480	22,010(2,860)	4	52.47(3.08)
	970	80	119,180(9,560)	6	46.99(1.49)
		120	76,330(13,560)	5	50.49(0.52)
		200	38,950(1,620)	4	57.36(0.68)
		320	25,050(1,480)	4	51.92(0.78)
		480	20,390(616)	4	50.58(2.34)
8.8/10.5	410	80	131,030(7,160)	6	52.50(0.55)
		120	86,350(3,350)	5	66.66(0.80)
		200	37,630(1,810)	4	65.00(0.31)
		320	125,460(6,320)	5	62.08(1.74)
		480	53,920(4,930)	5	59.94(4.42)
	936	80	83,060(3,410)	6	51.79(0.27)
		120	50,630(1,520)	5	56.65(0.67)

		200	29,110(1,030)	4	59.27(0.79)
		320	86,510(1,680)	5	60.52(1.40)
		480	42,240(11,370)	5	56.16(3.16)
101/0.4	372	80	81,230(11,000)	6	61.75(2.19)
		120	98,630(4,490)	5	59.65(0.91)
		200	51,750(1,920)	4	58.28(0.58)
		320	38,220(3,120)	4	53.70(1.16)
		480	75,040(16,940)	5	60.93(1.83)
	852	80	67,400(8,450)	6	48.56(3.20)
		120	43,320(2,130)	5	64.40(0.88)
		200	116,630(1,760)	5	61.35(0.81)
		320	90,170(2,960)	5	64.1(0.95)
		480	44,490(2,150)	6	71.66(1.33)

e. I'm especially concerned in the Methods when they say that 4-6 days corresponds to 14 generations. That would correspond to growth rates between 1.62 day-1 (at a "low" light level previous studies have found to nearly saturate growth rate) or 2.42 day-1, a level unachievable even for most diatoms (and not readily believable for a coccolithophore, even E. huxleyi). Perhaps this is a typographical error?

Response: Cells were cultured at each experimental treatment for 4 to 6 days, which corresponds to 7 to 8 generations, and then inoculated to new seawater and cultured for another 4 to 6 days.

'cells were acclimated to the experimental treatments for at least 7 generations before starting the experiment' in **Lines 189–190** on page 9.

f. For cell counts they use a particle counter (presumably based on the Coulter principle, although the information provided is inadequate to identify the type of instrument). This is potentially very problematic particularly in the case of E. huxleyi. How can living cells be distinguished from detached coccoliths, agglomerations of detached coccoliths, and/or empty coccospheres, all of which are very abundant in E. huxleyi cultures? In limited conditions these other particles can actually dominate the suspended particles found in cultures and it can be difficult to distinguish cells. With all these issues, I really am not sure from the information provided that they are actually measuring cells. Cells should be counted under a microscope or with a flow cytometer (a Coulter-type particle counter can be used, if it is being checked, compared, calibrated with microscope or cytometer counts throughout the experiment). Details are needed.

Response: We thank this referee for their suggestion.

Cell densities were measured using a Z2 Coulter Particle Count and Size Analyzer (Beckman Coulter). The diameter of detected particles was set to 3 to 7 μm in the instrument, which excludes detached coccoliths because the diameter of coccolith is less than 3 μm (Müller et al., 2012). These changes are in **Lines 247–250** on page 12.

Recently, we measured cell concentration using a Cell Lab Quanta SC flow cytometer (Beckman Coulter) and a Z2 Coulter Particle Count and Size Analyzer. Cell concentration was 14,550 cells mL^{-1} when it was measured by a flow cytometer (Fig. R1 and R2 in the response letter) and was 15, 210 cells mL^{-1} when it was measured by the Z2 Coulter Particle Count and Size Analyzer (Fig. R3 in the response letter). Variation in measured cell concentration between two methods was 4.3%. Thus, we don't think that the cell concentration measured using a Z2 Coulter Particle Count and Size Analyzer cause error.

Cell concentration was also measured by a Cell Lab Quanta SC flow cytometer (Beckman Coulter), and variation in measured cell concentration between two methods was about 4.3%. This sentence was added in **Lines 250–253** on page 12.

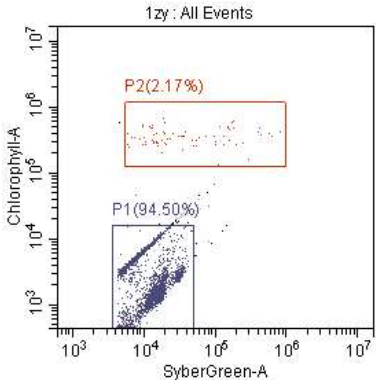


Figure R1 Signal shown in flow cytometer.

Tube Name: 1zy
Sample ID:

Population	ents/μL(V)	% T...	% P...	Mean FSC-A	Mean SSC-A	Median FSC-A	Median SSC-A	CV FSC-A	CV SSC-A
0.2um	130.99	19.5...	19.5...	4839.3	2082.9	4702.0	2112.8	131.43%	14.
10UM	0.00	0.00%	0.00%	####	####	####	####	####	#
3UM	0.00	0.00%	0.00%	####	####	####	####	####	#
3UM-1	0.40	0.06%	0.06%	802676.6	907325.0	802676.6	907325.0	42.89%	8.
10UM-1	0.00	0.00%	0.00%	####	####	####	####	####	#
6UM	0.40	0.06%	0.06%	806525.1	1929917.1	806525.1	1929917.1	75.85%	13.
P4	506.61	75.5...	75.5...	7334.9	4553.8	6904.6	3084.0	111.04%	144.
P2	14.55	2.17%	2.17%	650772.1	149084.1	647158.3	123957.0	21.58%	79.
P1	634.01	94.5...	94.5...	12763.8	19283.6	8495.8	3651.6	234.81%	263.

Figure R2 Calculated cell concentration by a flow cytometer.

Figure R3 Cell concentration shown by a Z2 Coulter Particle Count and Size Analyzer

Müller, M. N., Beaufort, L., Bernard, O., Pedrotti, M. L., Talec, A., Sciandra, A. : Influence of CO_2 and nitrogen limitation on the coccolith volume of *Emiliania huxleyi* (Haptophyta), Biogeosciences, 9, 4155–4167, 2012.

Because of these unresolved methodological issues in measuring cell abundance, at the present time I cannot trust growth rate data or cell elemental quotas reported.

Response: As mentioned above, cell abundance was measured using a suitable method and growth rate was correctly calculated. Cellular carbon content was measured using a Perkin Elmer Series II CHNS/O Analyzer 2400 instrument (Perkin Elmer Waltham, MA).

Variations in measured carbon content between the four replicates were calculated to be 1–13% in this study. This sentence was added in **Lines 267–268** on page 13.

3. There is no way to know when nutrients became depleted. In the case of nitrate, it is not clear if that nutrient became limiting or sampling occurred when cells were just about to use up the last uM. In this sense, it is essentially impossible to interpret differences in any of the measured parameters between HNHP, LN, and LP conditions. The Fv/Fm data in Fig. 3 heightens my suspicion that cells never truly reached P starvation under LP conditions, as Fv/Fm doesn't show any clear drop in LP compared to HNHP condition at any light or CO2 treatment (compare to Rokitta et al. 2016, for example). In the case of phosphate, perhaps they became limiting at the end, but when? The fact that the increase in PIC/cell reported in many previous studies wasn't observed, but occurred under LN instead, is consistent with the suspicions that the presumed nutrient status was not limiting (and that cell abundance was not being measured properly).

Response: Low DIN or DIP concentration in this study did not limit growth and carbon fixation rates. The reasons are as follows (**Lines 568–579** on pages 26 and 27): (1) In this study, growth rates of *E. huxleyi* were larger than 1 in almost all treatments, and cells divided 1–2 times per day (Fig .1 in the manuscript), which indicates non-limiting nutrient conditions during the incubation. (2) Based on measured PON quota and cell concentration in this study (Figs. 1 and S6), PON concentrations at the end of incubations were estimated to be 7.8–9.3 μmol L^{-1} at different nutrient conditions (Table S2). These data were closely correlated with molar drawdown of dissolved inorganic nitrogen (DIN) during the incubations. Furthermore, residual 1 μmol L^{-1} DIN in the final day of the incubation showed non-limitation of growth and POC production rates by nitrogen.

(3) On the other hand, Rokitta et al. (2016) reported that F_v/F_m of *E. huxleyi* was 50% lower at P-depleted than at P-replete conditions. In this study, F_v/F_m and POC quota were very similar between LP and HNHP treatments (Figs. 2 and S3), which suggest that LP did not limit growth and carbon fixation.

4. I think the approach for analyzing and interpreting the data could be more powerful:
a. The 3-way ANOVA ignores differences between LP and LN conditions
Response: Thanks for the comments of this referee. We re-analyzed the data with a 3-way ANOVA, which shows individual and interactive effects of nutrient concentration, CO_2 level and light intensity, and compares differences among HNHP, LN and LP conditions.

A three-way ANOVA was used to determine the main effect of dissolved inorganic nutrient concentration, pCO_2, light intensity and their interactions for these variables. A two-way ANOVA was performed to test the main effect of dissolved inorganic nutrient concentration, pCO_2 and their interactions on fitted α and V_{max} of growth, POC and PIC production rates. When necessary, a Tukey Post hoc (Tukey HSD) test was used to identify the differences between two CO_2 levels, nutrient concentrations or light intensities. These changes are in **Lines 290–298** on page 14.

Table 2. Results of three-way ANOVAs of the impacts of dissolved inorganic nutrient concentration, pCO_2, light intensity and their interaction on growth rate, F_v/F_m, F_v'/F_m', POC and PIC production rates, and PIC:POC ratio.

	Factor	*F* value	*p* value
Growth rate (d^{-1})	Nut	264.7	<0.01
	C	875.6	<0.01
	L	2035.8	<0.01
	Nut×C	53.6	<0.01
	Nut×L	84.2	<0.01
	C×L	9.3	<0.01
	Nut×C×L	26.8	<0.01
F_v/F_m	Nut	68.6	<0.01
	C	184.7	<0.01
	L	225.8	<0.01
	Nut×C	10.3	<0.01
	Nut×L	8.1	<0.01
	C×L	15	<0.01
	Nut×C×L	5.2	<0.01
F_v'/F_m'	Nut	63.9	<0.01
	C	181.8	<0.01
	L	1161.8	<0.01
	Nut×C	51.9	<0.01
	Nut×L	15.3	<0.01
	C×L	9.9	<0.01
	Nut×C×L	8.1	<0.01

POC production rate (pg C cell^{-1} d^{-1})	Nut	11.8	<0.01
	C	128.9	<0.01
	L	293.7	<0.01
	Nut×C	4.9	=0.01
	Nut×L	19.0	<0.01
	C×L	8.47	<0.01
	Nut×C×L	1.94	=0.06
PIC production rate (pg C cell^{-1} d^{-1})	Nut	624.4	<0.01
	C	142.0	<0.01
	L	147.2	<0.01
	Nut×C	1.9	=0.16
	Nut×L	17.3	<0.01
	C×L	8.1	<0.01
	Nut×C×L	4.6	<0.01
PIC:POC ratio	Nut	326.7	<0.01
	C	57.7	<0.01
	L	41.8	<0.01
	Nut×C	8.3	<0.01
	Nut×L	12.5	<0.01
	C×L	4.0	<0.01
	Nut×C×L	3.3	<0.01

Nut, dissolved inorganic nutrient concentrations (μmol L^{-1}); C, pCO_2 (μatm); L, light intensity (μmol photons m^{-2} s^{-1}); POC and POC production rates, particulate organic and inorganic carbon production rates; F_v/F_m, maximum photochemical quantum yield; F_v'/F_m', effective photochemical quantum yield. These changes are in **Lines 1198–1209** on pages 56–58.

b. The 3-way ANOVA approach followed by a posthoc test to identify pairwise differences can be valid, but it doesn't help for identifying patterns. In this case, the Eilers and Peeters model they fit would help, but they only look at the fit of the alpha parameter, when the curves shown in their figures clearly indicate that the other fitted parameters (a, b, c) may be interesting as well.

Response: As suggested by this referee, we used the model of Eilers and Peeters (1988) to fit growth, POC and PIC production rates, and calculated alpha (a) and maximum values (V_{max}) of growth, POC and PIC production rates. Then a 2-way ANOVA was used to test effects of nutrient and CO_2 level on a and V_{max}.

A two-way ANOVA was performed to test the main effect of dissolved inorganic nutrient concentration, pCO_2 and their interactions on fitted a and V_{max} of growth, POC and PIC production rates. This sentence was added in **Lines 294–296** on page 14.

Table 4. Results of two-way ANOVAs of the effects of dissolved inorganic nutrient concentration and pCO_2 on fitted a and maximum value (V_{max}) of growth, POC and PIC production rates. More detailed information is given as in Table 2. These changes are in **Lines 1246–1249** on page 62.

		Factor	F value	p value
a	Growth rate	Nut	18.08	<0.001
		CO_2	0.186	0.6711
		Nut×CO_2	0.398	0.6776
	POC production rate	Nut	7.21	0.005
		CO_2	7.78	0.0121
		Nut×CO_2	2.50	0.11
	PIC production rate	Nut	21.73	<0.001
		CO_2	2.32	0.145
		Nut×CO_2	2.56	0.105
V_{max}	Growth rate	Nut	24.9	<0.001
		CO_2	572.7	<0.001
		Nut×CO_2	14.8	<0.001
	POC production rate	Nut	7.301	0.0048
		CO_2	15.95	0.0009
		Nut×CO_2	1.91	0.177
	PIC production rate	Nut	56.06	<0.001
		CO_2	86.84	<0.001
		Nut×CO_2	0.168	0.85

5. What about cell volume effects? As reported recently by Müller et al. (2017), these could be crucial. If I understood that previous study correctly, nutrient limitation seemed to act independently rather than synergistically with ocean acidification when cell volume was accounted for. Of course, that study used continuous culture rather than batch culture/starvation conditions, but still it seems relevant at least to consider. Currently the Discussion seems to ignore some relevant studies such as Müller et al. 2017 that I previously cited, as well as Olson et al. 2016. Further, it needs to be much clearer. Finally, some revision of the English is suggested.

Response: Cell volume is shown in Table R3 (or Table S1 in the supplement). POC and PIC production rates are normalized by cell volume, and the normalized POC and PIC production rates were shown in Figure S5 in the Supplement.

Comparison between the study of Müller et al. (2017) and ours are shown as following:

Using a chemostat culture, Müller et al. (2017) reported that DIN or DIP limitation decreased the POC and PIC production rates (in pg C $cell^{-1}$ d^{-1}) by 50% and rising pCO_2 levels did not affect POC production rates. However, when normalized to cell volume, nutrient limitation did not affect POC and PIC production rates (in pg C $cellV^{-1}$ d^{-1}), and rising pCO_2 levels reduced POC and PIC production rates. In our study, decreased DIN or DIP concentration reduced the normalized POC production rates (in pg C $cellV^{-1}$ d^{-1}), and increased the normalized PIC production rates at both LC and HC (Fig. S5 in the supplyment). Differing results between the study of Müller et al. (2017)

and ours may result from different experimental setups. Growth was really limited by N or P, cells were cultured in a continuous photon flux, and cell growth was in the stable phase when POC and PIC samples were taken in the study of Müller et al. (2017). While we took POC and PIC samples in the exponential growth phase, and LN or LP did not really limit growth of *E. huxleyi* in our study. These contents were added in the discussion section in **Lines 786–798** on pages 36 and 37.

At 15 °C, 140 µmol photons m^{-2} s^{-1}, 28 µmol L^{-1} DIN and 2.4 µmol L^{-1} DIP conditions, rising CO_2 increased POC quota (pg C $cell^{-1}$) of *E.huxleyi* strain s2668, while decreased normalized POC quota (pg C $cellV^{-1}$) in study of Olson et al. (2016). In our study, rising CO_2 did not significantly affect POC quota (Fig. S3) and normalized POC quota (Fig. S4) at 120 µmol photons m^{-2} s^{-1} and HNHP conditions.

Olson, M. B., Wuori, T. A., Love, B. A., Strom, S. L. : Ocean acidification effects on haploid and diploid *Emiliania huxleyi* strains: Why changes in cell size matter, J. Exp. Mar. Biol. Ecol., 488, 72–82, 2017.

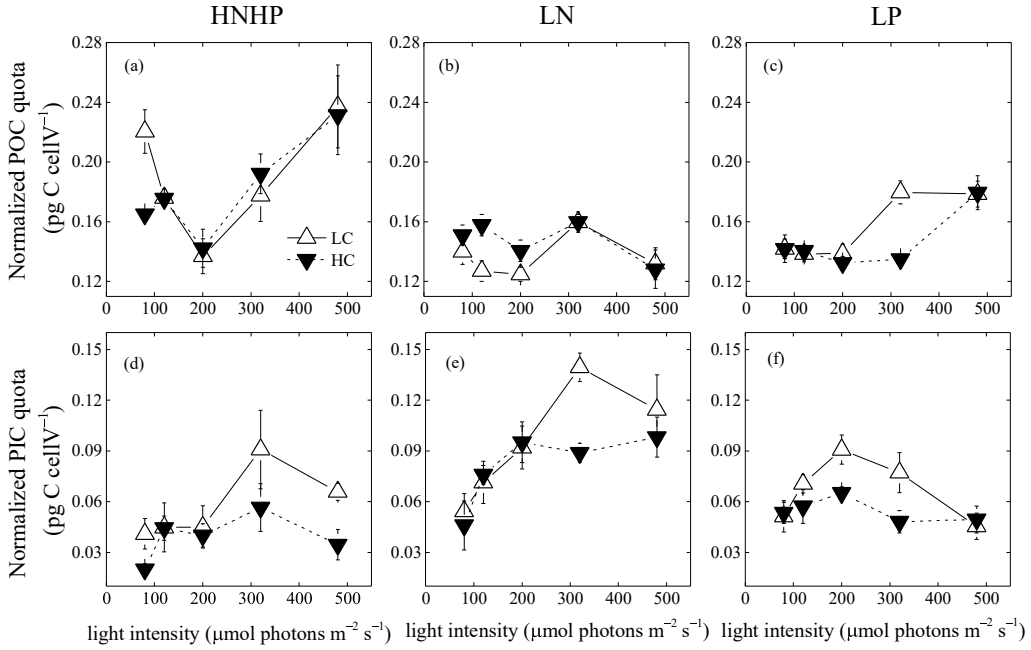


Figure S4. At both LC and HC, normalized POC quota (pg C $cellV^{-1}$) of *E. huxleyi* as a function of light intensity at HNHP (**a**), LN (**b**) and LP (**c**) conditions. At both LC and HC, light response of normalized PIC quota (pg C $cellV^{-1}$) of *E. huxleyi* at HNHP (**d**), LN (**e**) and LP (**f**) conditions. The values represent the mean ± standard deviation for four replicates.

6. The Discussion focuses a lot on ETR and photophysiology (Fv/Fm, Fv'/Fm'), which doesn't make a lot of sense. Effects both of high CO2 and of supposed nutrient limitation on photophysiological parameters seem to be subtle in comparison to what they report on growth rates and cell quotas. The light dependence of photosynthesis in E. huxleyi has actually been

comparatively well studied, and much of the discussion seems overly speculative and not to focus on some of the curious differences with what has been reported previously (e.g., Houdanetal. 2005 reporting that calcified cells are especially resistant to high PAR).

Response: Thanks for this comment of this referee. We have deleted descriptions of *ETR* in **Lines 232–243, Lines 498–523, Lines 755–770.**

The doubts I have about the study are quite serious, and hopefully my major comments (above) and minor comments (below) help the authors determine where to clarify. Nevertheless, I think the study design may not be appropriate for investigating an interaction between nutrient limitation and acidification. The only way such an experimental design could potentially work for the question planted is with daily and trusted cell counts and nutrient measurements showing when nutrient depletion occurred.

Response: The comments and suggestions of this referee are helpful and useful. As mentioned above, we are confident that cell concentration was measured correctly, and that nutrient concentrations did not limit growth and POC production rates. This is clear.

There are many minor points through the manuscript to address as well. I mention a few:

Line 27 "and exposing phytoplankton to increased light intensities" and lines 52-53 later. I think this is too much over-simplification. At the base of the mixed layer and within the pycnocline, nutrients can be obtained by diffusion across the pycnocline, so phytoplankton will grow and increase in biomass until they compete for light. I do not see how the average light exposure of phytoplankton will necessarily increase. The references cited (Gao et al. 2012 and Hutchinson and Fu 2017) do not explain this (as I mentioned earlier).

Response: Thanks for suggestion of this referee.

This content '*exposing phytoplankton to increased light intensities.*' was replaced by 'affecting the light intensity to which phytoplankton are exposed.' in **Line 27–28** on page 2.

These contents '*This exposes phytoplankton dwelling in the UML to higher light intensities (Gao et al., 2012; Hutchins and Fu, 2017).*' were replaced by 'This affects light exposure of phytoplankton dwelling therein (Steinacher et al. 2010).' These changes are in **Lines 60–62** on page 3.

Lines 101-103 "Interaction of rising CO2 with light appears to affect differentially coccolithophores when grown under different experimental setups." The sentence is not clear. What does "differentially affect coccolithophores" mean? Do those factors affect coccolithophores differently than other phytoplankton or do these factors have contrasting effects or ??

Response: Zhang et al. (2015) reported that compared to 510 μatm, 1050 μatm CO_2 decreased growth and POC production rate of *Gephyrocapsa oceanica* at high light intensity. Jin et al. (2017) reported that compared to 395 μatm, 1000 μatm CO_2 increased growth and POC production rates of *E. huxleyi* at high sunlight levels. Thus, the studies of Jin et al. (2017) and Zhang et al. (2015) reported contrasting response of growth and POC production rates of *E. huxleyi* and *G. oceanica* to rising CO_2. And rising CO_2 have contrasting effects on growth and POC production rates of the coccolithophores *E. huxleyi* and *G. oceanica*.

This sentence '*Interaction of rising CO_2 with light appears to affect differentially coccolithophores when grown under different experimental setups.*' was replaced by 'These indicate that during growth under different experimental conditions, rising CO_2 showed contrasting effects on growth and POC production rates of *E. huxleyi* and *G. oceanica*.' These changes are in **Lines 118–122** on page 6.

Lines 142-144: "added by 2200 µmol L^{-1} bicarbonate (as opposed to 2380 µmol L^{-1} in the original recipe), in order to reflect the alkalinity in the South and East China Seas of about 2200 µmol L^{-1}," First, I don't understand why it's important to match the South and East China Seas if they are not specifically using strains isolated from those seas and trying to predict how organisms there will respond. Second, I don't see how bicarbonate concentration is equated with alkalinity, as CO_3^{2-} also contributes to alkalinity, and for alkalinity every unit of CO_3^{2-} counts twice. I think carbonate usually can contribute about a fifth or a fourth or so of total alkalinity (see Zeebe and Wolfgladrow 2001 or other references).

Response: This sentence '*The synthetic seawater medium Aquil was prepared according to Sunda et al. (2005), added by 2200 µmol L^{-1} bicarbonate (as opposed to 2380 µmol L^{-1} in the original recipe), in order to reflect the alkalinity in the South and East China Seas of about 2200 µmol L^{-1} (Chou et al., 2005; Qu et al., 2017).*' was replaced by 'The Aquil medium was prepared according to Sunda et al. (2005) with the addition of 2200 µmol L^{-1} bicarbonate, resulting in initial concentrations of 2200 µmol L^{-1} total alkalinity (TA). This reflects 2200 µmol L^{-1} alkalinity in the South and East China Seas (Chou et al., 2005; Qu et al., 2017).' These changes are in **Lines 162–169** on page 8.

We think this is a logical question, and 2380 µmol L^{-1} bicarbonate can also be added into seawater (Sunda et al. 2005).

In general, HCO_3^- in the natural seawater accounts for more than 90% of the dissolved inorganic carbon (DIC), CO_3^{2-} for about 9%, and CO_2 for less than 1% (Zeebe and Wolf-Gladrow 2001). Alkalinity (TA) is calculated as

$$TA=[HCO_3^-]+2[CO_3^{2-}]+[HPO_4^{2-}]+[OH^-]+2[PO_4^{3-}]-[H^+]\ldots\ldots \quad \text{equation 2}$$

$$HCO_3^- \rightleftharpoons H^+ + CO_3^{2-} \quad \text{equation 3}$$

$$HCO_3^- + H_2O \rightleftharpoons OH^- + H_2CO_3 \quad \text{equation 4}$$

According to equations 2 and 3, when 1 mole HCO_3^- (1 mol TA) dissociates to 1 mol CO_3^{2-} (2 mol TA) and 1 mol H^+ (–1 mol TA), alkalinity did not change. According to equations 2 and 4, when 1 mole HCO_3^- (1 mol TA) reacts with 1 mol H_2O to produce 1 mol OH^- (1 mol TA) and 1 mol H_2CO_3, alkalinity did not change. Thus, bicarbonate concentration is equated with alkalinity.

Sunda, W. G., Price, N. M., and Morel, F. M. M.: Trace metal ion buffers and their use in culture studies, in: Algal culturing techniques, edited by: Andersen R. A., Elsevier Academic Press, London, 53–59, 2005
Zeebe, R. E., Wolf-Gladrow, D. A. : CO_2 in seawater: equilibrium, kinetics, isotopes. Amsterdam, Elsevier, 2001.

Lines 161-165: I mentioned above the problems with these lines
Response: See response to **Lines 162–169 (above).**

Line 172: How often were nutrients measured?
Response: Nutrients concentrations were measured before and at the end of experiments.

Lines 182-184: Was pH measured immediately or after storage? pH should be measured immediately, as I understand (within a couple hours is best).
Response: pH was measured within 10 min after the pH sample was taken.
'The pH_T was immediately measured at 20 ^oC with a pH meter' (**Line 218**, page 10).

Lines 210-215: I already mentioned the major problems I have with the methodology as described here. Perhaps they can fix that.
Response: Recently, we measured cell concentration using a Cell Lab Quanta SC flow cytometer (Beckman Coulter) and a Z2 Coulter Particle Count and Size Analyzer. Cell concentration was 14,550 cells mL^{-1} when it was measured by a flow cytometer (Fig. R1 and R2 in the response letter) and was 15, 210 cells mL^{-1} when it was measured by the Z2 Coulter Particle Count and Size Analyzer (Fig. R1; 2; 3 in the response letter). Variation in measured cell concentration between two methods was 4.3%. Thus, we don't think that the cell concentration measured by using a Z2 Coulter Particle Count and Size Analyzer cause error.
Cell concentration was also measured by a Cell Lab Quanta SC flow cytometer (Beckman Coulter), and variation in measured cell concentration between two methods was about 4.3% (**Lines 250–253**, page 12)

Line 225: Do they mean "difference" instead of "variance"
Response: '*variance*' was replaced by 'difference' (**Line 265**, page 12).

Lines 251-255: It would be invaluable to know when nutrients were depleted. Do they have data on this?
Response: DIN and DIP concentrations were measured before and at the end of incubations, and these data were shown in Table S2. As mentioned above, DIN and DIP concentration did not limit growth and POC production rates in this study.

Lines 256-264: This whole paragraph is basically redundant with Table 1. Also, it seems "(mean values for the beginning and end of incubations)" means that the beginning and ending values have been averaged together, while in Table S2 the beginning and ending values are given

separately. Table S2 is far more useful, especially for assessing the changes in carbonate parameters during the experiment. I would place that table (S2) in the main text, using it to replace the current Table 1. Then in this text the focus should be more on how consistent were carbonate parameters over time and across treatments within the LC and within the HC treatments. Furthermore, when I calculate the averages using the values given in the text immediately before, I get different values (405 for LC and 918 for HC). What is happening? Were some replicates not used?

Response: We agree with this referee that Table 1 was replaced by Table S2 in the main text.

These contents '*The carbonate system parameters (mean values for the beginning and end of incubations) are shown in Table 1. For low CO_2 (LC) condition, the pCO_2 levels of the media were about 435 μatm at HNHP, 410 μatm at LN and 370 μatm at LP conditions, and the pH_T values (reported on the total scale) were about 8.10 at HNHP, 8.11 at LN and 8.16 at LP. For high CO_2 (HC) condition, the pCO_2 levels of the media were about 970 μatm at HNHP, 935 μatm at LN and 850 μatm at LP, and the pH_T values were about 7.80 at HNHP, 7.80 at LN, and 7.85 at LP conditions.*' were replaced by 'The carbonate system parameters of the seawater at the beginning and end of the incubation are shown in Table 1. Within the low CO_2 (LC) treatment, pCO_2 levels of the seawater declined by 16% at HNHP, 19% at LN and 8% at LP, and pH values increased by 0.07 at HNHP, 0.06 at LN and 0.02 at LP (Tukey HSD, all $p < 0.05$). Within the high CO_2 (HC) treatment, pCO_2 levels of the seawater declined by 23% at HNHP, 21% at LN and 32% at LP, and pH values increased by 0.1 at HNHP, 0.09 at LN and 0.15 at LP (Tukey HSD, all $p < 0.05$). Average pCO_2 levels were 410 μatm for all LC conditions, and were 920 μatm for all HC conditions.' These changes are in **Lines 315–328** on page 15.

We checked the carbonate chemistry parameters and found that at LC treatments, the pCO_2 levels of the seawater were 439 μatm at HNHP (435 μatm was written in the manuscript (MS)), 409 μatm at LN (410 μatm in the MS) and 371 μatm at LP conditions (370 μatm in the MS); at HC treatments, the pCO_2 levels of the media were about 973 μatm at HNHP (970 μatm was written in the MS), 936 μatm at LN (935 μatm in the MS) and 852 μatm under LP conditions (850 μatm in the MS). Average pCO_2 levels were 408 μatm (410 μatm in the MS) at all LC conditions, and were 920 μatm (925 μatm in the MS) at all HC conditions. Thus, variation between the written, rounded off data and the original data causes slight differences.

Lines 368-373: This is not a very good description. It seems to exaggerate small differences between HC and LC.

Response: We have deleted the description of rETR in the main text in **Lines 498–523** on pages 23 and 24.

Line 467: I can't find any reference to Fig. 5 in the Results. Why does it appear suddenly in the Discussion? Further, I have problems with this Michaelis-Menten fit: It does not make any sense to fit growth rate in a batch culture (measured from initial and final concentrations) to the initial phosphate concentration. This seems to ignore understanding of phytoplankton macronutrient physiology since Droop. But, as there isn't a clear description of this experiment, I am not sure. Finally, the ability to calculate a half-saturation from the data in the graph would be very limited because there is no value in an intermediate range of growth (growth rate is either 0 or saturated or

nearly saturated). For this entire paragraph, the study was not designed to address the details of phosphate metabolism, which has already been fairly extensively studied in this species, and their discussion of the previous work is unclear

Response: In **Fig. 1:** We found that growth rates of *E. hulxeyi* were similar between LP and HNHP treatments at LC and high light conditions. This may be due to high affinity for DIP of *E. huxleyi* (Dyhrman and Palenik, 2003). To test this hypothesis, we performed one experiment that examined the response of growth rate of *E. huxleyi* to DIP concentrations at LC and 200 µmol photons m^{-2} s^{-1}.

This text '*Under light saturation condition, relationship of growth rates of E. huxleyi with phosphate concentrations indicated a very high affinity for dissolved inorganic phosphate (DIP) with 0.04 µmol* L^{-1} *half-saturation constant for DIP (Fig. 5).*' was replaced by 'Furthermore, growth rate of *E. huxleyi* is nearly saturated at 0.25 µmol L^{-1} DIP and is saturated at 0.5 µmol L^{-1} DIP and above. This demonstrated that *E. huxleyi* possesses a high affinity for DIP (Fig. 5) which allowed *E. huxleyi* to take up PO_4^{3-} efficiently.' These changes are in **Lines 646–655** on page 30.

Dyhrman, S. T., and Palenik, B.: Characterization of ectoenzyme activity and phosphate-regulated proteins in the coccolithophorid *Emiliania huxleyi*, J Plank. Res., 25, 1215–1225, https://doi.org/10.1093/plankt/fbg086, 2003.

Line 529: Do the authors consider the ballast effect to be completely irrelevant? Also, I have an issue with considering E. huxleyi as representative of the biogeochemically most important coccolithophores. It is the most numerically abundant, most widespread, and most easy to culture in the laboratory. However, E. huxleyi is definitely not the coccolithophore responsible for most sinking inorganic carbon and it may not be an appropriate model for the responses of other principal groups of coccolithophores, as it (and it's close relatives in the Gephyrocapsa genus) is different from most coccolithophores. For example, E. huxleyi does not require Si for calcification while most do.

Response: We agree with this referee that *E. huxleyi* is definitely not the coccolithophore responsible for most sinking inorganic carbon. However, *E. huxleyi* is the most abundant and most widespread coccolithophore species, and it is true that changes in PIC:POC ratios have the potential to affect sinking rate of *E. huxleyi* (Hoffmann et al., 2015)

'In addition, larger PIC:POC ratios have the potential to accelerate sinking rate of *E. huxleyi* cells, facilitating the export of carbon into deeper waters (Hoffmann et al., 2015). ' These changes are in **Lines 752–754** on pages 34 and 35.

In general, I have a hard time following the Discussion. It lacks clarity and focus, and seems to stray into inadequate review of important but peripheral themes. It's difficult for me to provide more detailed comments as I am not convinced that they know what state of nutrient limitation (or not) the cells were in when harvested.

Response: We thank this referee to spent time to review our manuscript and provide useful comments. We refocus on growth, POC and PIC production rates, and the fitted *a* and maximum values of growth, POC and PIC production rates. As mentioned above, low DIN and DIP did not limit growth and POC production in this study.

Interactive effects of seawater carbonate chemistry, light intensity and nutrient availability on physiology and calcification of the coccolithophore *Emiliania huxleyi*

Yong Zhang[1], Feixue Fu[2], David A. Hutchins[2], and Kunshan Gao[1]

[1]State Key Laboratory of Marine Environmental Science, Xiamen University, Xiamen, China

[2]Department of Biological Sciences, University of Southern California, Los Angeles, California

Running head: *carbonate chemistry,* Emiliania huxleyi, *light, nutrients*

Correspondence to: Kunshan Gao (ksgao@xmu.edu.cn)

Keywords: calcification; CO_2; coccolithophore; growth; light; nutrient; photosynthesis

Abstract. Rising atmospheric carbonate dioxide (CO_2) levels lead to increasing CO_2 concentration and declining pH in seawater, as well as ocean warming. This enhances stratification and shoals the upper mixed layer (UML), hindering the transport of nutrients from deeper waters and affecting the light intensity to which phytoplankton are exposed ~~exposing phytoplankton to increased light intensities~~. In ~~the present~~ this study, we investigated combined impacts of CO_2 levels (410 μatm (LC) and 92~~5~~0 μatm (HC)), light intensities (80–480 μmol photons m^{-2} s^{-1}) and nutrient concentrations [101 μmol L^{-1} dissolved inorganic nitrogen (DIN) and 10.5 μmol L^{-1} dissolved inorganic phosphate (DIP) (HNHP); 8.8 μmol L^{-1} DIN and 10.5 μmol L^{-1} DIP (LN); 101 μmol L^{-1} DIN and 0.4 μmol L^{-1} DIP (LP)] on growth, photosynthesis and calcification of the coccolithophore *Emiliania huxleyi*. ~~HC and LN synergistically decreased growth rates of *E. huxleyi* at all light intensities.~~ LN and HC synergistically reduced growth and POC production rates. ~~High light intensities compensated for inhibition of LP on growth rates at LC, but exacerbated inhibition of LP at HC.~~ At high light intensity, LP did not limit growth rate at LC but led to increased high-light inhibition of growth rate at HC. ~~These results indicate that the ability of *E. huxleyi* to compete for nitrate and phosphate may be reduced in future oceans with high CO_2 and high light intensities.~~ These results showed that effects of nutrient concentrations on physiological rates of *E. huxleyi* were modulated by CO_2 level and light intensity. Low nutrient concentrations increased the maximum value and the light-use efficiencies of calcification rate. ~~particulate inorganic carbon quotas and the sensitivity of maximum electron transport rates to light intensity. Light-use~~

~~efficiencies for carbon fixation and calcification rates were significantly larger than that of growth.~~ Our results suggest that interactive effects of multiple environmental factors on coccolithophores need to be considered when predicting their contributions to the biological carbon pump and feedbacks to climate change.

1 Introduction

~~Anthropogenic emission of CO_2 is taken up by the oceans, decreasing pH of seawater and resulting in ocean acidification (OA)~~ Rising atmospheric CO_2 level leads to increasing seawater CO_2 concentration and decreasing pH, which is known as ocean acidification (OA) (Caldeira and Wickett, 2003). On the other hand, rising atmospheric CO_2 also leads to global and ocean warming, which enhances water column stratification and shoals the upper mixed layer (UML) (Wang et al., 2015). ~~This exposes phytoplankton dwelling in the UML to higher light intensities (Gao et al., 2012; Hutchins and Fu, 2017).~~ This affects light exposure of ~~availability exposed by~~ phytoplankton dwelling ~~in the UML~~ therein (Steinacher et al. 2010). In addition, enhanced stratification reduces the transport of nutrients from deep oceans to the UML (Behrenfeld et al., 2006), which reduces the nutrient concentrations in the UML.

Coccolithophores take up CO_2 and/or HCO_3^- from ~~media~~seawater for carboxylation, and use HCO_3^- for calcification which produces coccoliths.

Calcification processes generate CO_2 due to production of protons, ~~which counteracts with photosynthetic CO_2 fixation,~~ and therefore influencing CO_2 influx into the oceans (Rost and Riebesell, 2004). Growth rate, particulate organic (POC) and inorganic carbon (PIC) production rates of *Emiliania huxleyi*, the most abundant calcifying coccolithophore species, usually display optimum responses to a broad range of CO_2 concentration (Bach et al., 2011). ~~with growth, POC and PIC production rates increased, decreased or unaffected by rising CO_2 treatments~~ Growth, POC and PIC production rates could increase, decrease and be unaffected by rising CO_2 treatments across a narrow CO_2 range, which is dependent on the optimal CO_2 levels of these physiological rates and the selected CO_2 range (Langer et al., 2009; Richier et al., 2011; Bach et al., 2015; Jin et al., 2017). ~~Increased light levels could counteract the negative effects of rising CO_2 on calcification in *E. huxleyi* when grown under natural fluctuating sunlight (Jin et al., 2017).~~ Differences in sampling locations, experimental setups, ~~and~~ deviations in the measuring methods and intraspecific differences can generally be responsible for the differential responses of growth, POC and PIC productions to rising CO_2 in *E. huxleyi* (Langer et al., 2009; Meyer and Riebesell, 2015).

POC production as well as growth rates usually increase with elevated light ~~levels~~intensity, level off at saturated light ~~levels~~intensity and decline at inhibited high light ~~levels~~intensity in cocolithophores (Zhang et al., 2015; Jin et al., 2017). Reduction in pigment content and effective photochemical quantum yield (F_v'/F_m') are characteristics of photo-acclimation to high light intensity (Geider et al., 1997;

Gao et al., 2012). At low light intensity, the ratio of light-harvesting protein to photosystem II (PSII) reaction center proteins is large, which facilitates *E. huxleyi* to absorb more energy. At high light intensity, the ratio of photo-protection proteins to PSII reaction center proteins is large, which could protect *E. huxleyi* against damage caused by high light intensities (Mckew et al., 2013).

Nitrogen is required for the biosynthesis of proteins and other macromolecules, including chlorophyll (Riegman et al., 2000). Phosphorus is required for the synthesis of nucleic acids, ATP, and phospholipids in cell membranes (Shemi et al., 2016). Due to source limitation, ~~decreased~~ suboptimal nutrient concentrations usually reduce growth and photosynthetic carbon fixation rates (Cloern et al., 1999; Kim et al., 2007; Harrison et al., 2008). Nevertheless, low nutrient concentrations often enhance the PIC quotas of *E. huxleyi*~~. This is due to the fact that low nutrient concentrations hold the cells in the G1 cell cycle phase where calcification occurs (Müller et al., 2008). A recent proteome study on *E. huxleyi* also shows that nutrient limitation arrests cell cycling (McKew et al., 2015). At molecular levels, nitrate or phosphate limitations down-regulate expression of genes involved in cell cycling, RNA and protein synthesis in *E. huxleyi* (Rokitta et al., 2014, 2016).~~ because low nutrient concentrations arrest cell cycling and lengthen the G1 phase where calcification occurs (Müller et al., 2008; McKew et al., 2015).

Recently, several studies investigated interactive effects of rising CO_2 and light intensity on physiological rates of coccolithophores (Feng et al., 2008; Jin et al., 2017). Zhang et al. (2015) reported that at 50–800 μmol photons m^{-2} s^{-1}, ~~rising~~ 1050

μatm CO_2 ~~levels~~ decreased the maximum growth rate, POC ~~production rate~~ and PIC production rates of *Gephyrocapsa oceanica* ~~.~~compared to 510 μatm. At low light ~~levels~~intensity, coccolithophores increase CO_2 uptake to compensate for inhibition of HCO_3^- uptake on photosynthesis, while at high light intensity they don't increase CO_2 uptake (Kottmeier et al., 2016). Under natural solar radiation, Jin et al. (2017) reported that compared to 395 μatm, ~~rising~~1000 μatm CO_2 ~~levels~~ increased the growth and POC production rates of *E. huxleyi* at high sunlight levels. These indicate that during growth under different experimental conditions, rising CO_2 showed contrasting effects on growth and POC production rates of *E. huxleyi* and *G. oceanica*. ~~Interaction of rising CO_2 with light appears to affect differentially coccolithophores when grown under different experimental setups.~~

Some previous studies have examined the effects of rising CO_2 and nutrient concentrations on the physiology of *E. huxleyi* (Sciandra et al., 2003; Borchard et al., 2011; Engel et al., 2014; Müller et al., 2017). Low nitrate or low phosphate concentrations increased POC and PIC quotas in *E. huxleyi*, and these increases were much less at high CO_2 than at low CO_2 levels (Matthiessen et al., 2012; Rouco et al., 2013). In addition, rising CO_2 levels decreased growth rates at high phosphate concentration, though it did not affect growth rates at low phosphate concentration (Matthiessen et al., 2012). These studies indicate that fitness-relevant traits of *E. huxleyi* may be altered in future high-CO_2 and low-nutrient oceans.

Recently, researchers have paid increasing attentions to combined effects of multiple stressor on marine phytoplankton (Brennan and Collins, 2015; Boyd et al.,

2016; Hutchins and Fu, 2017), considering the fact that phytoplankton cells are simultaneously exposed to physical and chemical factors. In addition, physiological responses of phytoplankton to one environmental factor may be synergistically, antagonistically or neutrally affected by others (Tong et al., 2016; Müller et al., 2017). ~~Even~~And across a broad range of CO_2 concentrations, optimal CO_2 levels and maximal values for growth rate, photosynthetic carbon fixation rate and calcification rate are modulated by temperature and light intensity (Sett et al., 2014; Zhang et al., 2015).

Under chemostat cultures, rising CO_2 levels were found to increase the POC quotas of a non-calcifying strain of *E. huxleyi* (PML 92A) and a calcifying strain of *E. huxleyi* (PML B92/11) at low nutrient concentration and high light intensity (Leonardos and Geider, 2005; Borchard et al., 2011). However, relatively few studies have observed the interactive effects of multiple environmental factors on physiological rates of coccolithophores. To investigate responses of the calcifying *E. huxleyi* strain PMLB92/11 to multiple environmental factors, we employed dilute batch cultures, investigated its growth, POC and PIC ~~quotas~~production rates, maximum (F_v / F_m) and effective photochemical quantum yield (F_v' / F_m') ~~and electron transport rate (*ETR*)~~ at different levels of CO_2, light, dissolved inorganic nitrogen (DIN) and phosphate concentrations (DIP).

2 Materials and methods

2.1 Experimental design

Emiliania huxleyi strain PML B92/11, one of the most commonly used strain in studies of *E. huxleyi*, was obtained from the culture collection at Plymouth. *E. huxleyi* was grown in diluted batch cultures in Aquil (final cell concentrations were 20,000 to 1730,000 cells mL^{-1}) at 20 °C in a GXZ light chamber (Dongnan Instrument Company) under a 12 : 12 h light : dark cycle (light period: 8:00 a.m. to 8:00 p.m.). ~~The synthetic seawater medium Aquil was prepared according to Sunda et al. (2005), added by 2200 µmol L^{-1} bicarbonate (as opposed to 2380 µmol L^{-1} in the original recipe), in order to reflect the alkalinity in the South and East China Seas of about 2200 µmol L^{-1} (Chou et al., 2005; Qu et al., 2017).~~ The Aquil medium was prepared according to Sunda et al. (2005) with the addition of 2200 µmol L^{-1} bicarbonate, resulting in initial concentrations of 2200 µmol L^{-1} total alkalinity (TA). This reflects 2200 µmol L^{-1} alkalinity in the South and East China Seas (Chou et al., 2005; Qu et al., 2017). Initial dissolved inorganic nitrogen (DIN) and phosphate (DIP) concentrations in Aquil were 100 µmol L^{-1} and 10 µmol L^{-1}, respectively (HNHP). For Aquil medium with low DIN concentration (LN), the synthetic seawater contained 8 µmol L^{-1} NO_3^- and 10 µmol L^{-1} PO_4^{3-}, respectively. For low DIP treatment (LP), it had 100 µmol L^{-1} NO_3^- and 0.4 µmol L^{-1} PO_4^{3-}.

Under each nutrient ~~level~~condition, the Aquil media were aerated for 24 h at 20 °C (PVDF 0.22 µm pore size, simplepure, Haining) with air containing 400 µatm or 1000 µatm pCO_2. The dry air/CO_2 mixture was humidified with double distilled water prior to the aeration to minimize evaporation. Then, the Aquil was sterilized by filtration

(0.22 µm pore size, Polycap 75 AS, Whatman) and carefully pumped into autoclaved 500 mL polycarbonate bottles (Nalgene). The bottles were filled with Aquil with no ~~leaving about 10 ml~~ headspace to minimize gas exchange. The volume of the inoculum was calculated (see below) and the same volume of Aquil was taken out from 500 mL bottles before inoculation. Carbonate chemistry parameters (total alkalinity (TA) and pH) were measured at the beginning and end of the experiment.

For each nutrient treatment, 20 bottles at each pCO_2 level were incubated at light intensities of ~~80, 120, 200, 320, and 480~~ 80±5, 120±8, 200±17, 320±16, and 480±30 µmol photons m^{-2} s^{-1} of photosynthetically active radiation (PAR) (4 replicates each) measured using a PAR Detector (PMA 2132, Solar Light Company, Glenside). A flow chart for the experimental treatments is presented in Fig. S1. For the dilute batch cultures, initial cell concentration was 200 cells mL^{-1} and cells were acclimated to the experimental treatments for at least ~~14~~7 generations before starting the experiment (normally 6 days at 80 µmol photons m^{-2} s^{-1}, 5 days at 120 µmol photons m^{-2} s^{-1}, and 4 days at 200–480 µmol photons m^{-2} s^{-1} at all nutrient conditions) (Table S1). ~~Bottles were rotated two times per day at 10:00 a.m. and 6:00 p.m. to make the cells can obtain light homogeniously.~~ Culture bottles were rotated twice at 10:00 a.m. and 6:00 p.m.. To minimize changes in carbonate chemistry, final cell concentrations were lower than 1~~7~~0,000 cells mL^{-1}, and changes in dissolved inorganic carbon (DIC) concentrations were less than 10% (0.5%–9.1%). Langer et al. (2013) found that growth of cells on the fourth to sixth days of batch cultures was in the exponential phase even at 3 µmol L^{-1} NO_3^- or at 0.29 µmol L^{-1} PO_4^{3-} with the same *E. huxleyi*

strain. In this study, all parameters were measured on the fourth to the sixth days, so it is most likely that cells in all treatments were sampled in the exponential growth phase.

2.2 Nutrient concentrations, total alkalinity and pH_T measurements

Sampling started at 10:30 a.m. and finished at 12:00 a.m.. 50 mL samples for determination of inorganic nitrogen and phosphate concentrations were syringe-filtered (0.22 μm pore size, Haining) and measured using a scanning spectrophotometer (Du 800, Beckman Coulter) according to Hansen and Koroleff (1999).

Carbonate chemistry parameters were calculated from total alkalinity (TA) ~~and,~~ pH_T (total scale), phosphate, temperature, and salinity using the ~~CO_2 System~~ CO2SYS (Pierrot et al., 2006). In the final days of incubation, 25 mL samples for TA measurements were filtered (0.22 μm pore size, Syringe Filter) by gentle pressure with 200 mbar in the pump (GM-0.5A, JINTENG) and stored at 4 °C for a maximum of 7 days. TA was measured at 20 °C by potentiometric titration (AS-ALK1+, Apollo SciTech) according to Dickson et al. (2003). Samples for pH_T measurements were syringe-filtered (0.22 μm pore size), and the bottles were filled with overflow and closed immediately. The pH_T was immediately measured at 20 °C with a pH meter (Benchtop pH, Orion 8102BN) calibrated with an ~~equimolal~~ equimolar pH buffer (Tris•HCl, Hanna) for sea water media (Dickson, 1993). Carbonic acid constants K_1 and K_2 were ~~calculated according to~~ taken from Roy et al. (1993).

2.3 Measurements of photochemical parameters

The effective photochemical quantum yield ($F_v^{'}/F_m^{'}$) and maximum photochemical quantum yield (F_v/F_m) of photosystem II (PSII) were assessed using a XE-PAM (Walz, Germany) at 1:00 p.m.. 3 ml samples were taken from the incubation bottles, and $F_v^{'}/F_m^{'}$ values were measured immediately at active light intensities similar to the incubation light levels. 3 mL samples were kept ~~darkly~~ in the dark for 15 min at 20 °C, and F_v/F_m values were determined at a measuring light intensity of 0.3 μmol photons m^{-2} s^{-1} and a saturation pulse of ~~0.8 s at light intensity of~~ 5000 μmol photons m^{-2} s^{-1} with 0.8 s.

~~For electron transport rate (*ETR*) measurements, PAR levels were set between 1 μmol photons m^{-2} s^{-1} and 1600 μmol photons m^{-2} s^{-1} with 9 steps of 45 s each. The *ETR* (mol e^- g^{-1} Chl *a* h^{-1}) was calculated according Dimier et al. (2009), $ETR = (F_v^{'}/F_m^{'}) \times PAR \times 0.5 \times A$, where *A* represent the cellular absorption value normalized to Chl *a*, 0.5 implicits that 50% quanta of the absorbed PAR are distributed to PSII (Dimier et al., 2009). Original *A* value was about 2.47×10^{-7} μmol e^- $cell^{-1}$ s^{-1} and normalized *A* value was about 8.40×10^{-3} mol e^- g^{-1} Chl *a* h^{-1}. Photosynthetic response to irradiance (P-I curves) were analyzed according to Jasby and Platt (1976): $ETR = ETR_{max} \times \tanh(alpha \times PAR / ETR_{max})$, where ETR_{max} represents light-saturated *ETR*, and *alpha* is the slop of the P-I curve at limiting irradiance, I_k calculated from the expression $ETR_{max} / alpha$ and represents the onset of light saturation.~~

2.4 Cell density measurements

At the end of the incubation, about 25 ml samples were taken from the incubation bottles at about 2:30 p.m.. Cell densities were measured ~~by~~ using a Z2 Coulter Particle Count~~er~~ and Size Analyzer (Beckman Coulter). The diameter of detected particles was set to 3 to 7 μm in the instrument, which excludes detached coccoliths because the diameter of coccolith is less than 3 μm (Müller et al., 2012). Cell concentration was also measured by a Cell Lab Quanta SC flow cytometer (Beckman Coulter), and variation in measured cell concentration between two methods was about 4.3%. Growth rate (μ) was calculated according to the equation: $\mu = (\ln N_1 - \ln N_0) / d$, where N_0 is 200 cells mL^{-1} and N_1 is the cell concentration in the final days of experiment, and d is the growth time span in days.

2.5 Particulate organic (POC) and inorganic carbon (PIC) measurements

GF/F filters, pre-combusted at 450 °C for 8 h, were used to filter the samples of total particulate carbon (TPC) and particulate organic carbon (POC). TPC and POC samples were stored darkly at –20°C. For POC measurements, samples were fumed with HCl for 12 h to remove inorganic carbon, and samples for TPC measurements were not treated with HCl. All samples were dried at 60 °C for 12 h, and analyzed using a Perkin Elmer Series II CHNS/O Analyzer 2400 instrument (Perkin Elmer Waltham, MA). Particulate inorganic carbon (PIC) quota was calculated as the ~~variance~~ difference between TPC quota and POC quota. POC and PIC production

rates were calculated by multiplying ~~their~~ cellular contents with μ (d^{-1}), respectively. Variations in measured carbon content between the four replicates were calculated to be 1–13% in this study.

2.6 Response of growth rate of *E. huxleyi* to different dissolved inorganic phosphate (DIP) concentrations

5 L Aquil media were enriched with 100 μmol L^{-1} DIN, aerated for 24 h at 20 °C with air containing 400 μatm pCO_2, sterilized by filtration (0.22 μm pore size, Polycap 75 AS, Whatman) and then pumped into autoclaved 250 mL PC bottles. 10 μmol L^{-1}, 3 μmol L^{-1}, 1.5 μmol L^{-1}, 0.5 μmol L^{-1}, 0.25 μmol L^{-1} DIP (finial concentration) were respectively added into Aquil media with three replicates at each DIP concentration. 200 cells mL^{-1} was inoculated to Aquil media and all samples were cultured at 200 μmol photons m^{-2} s^{-1} for 4 days before starting the experiment. Finial cell concentration was measured by using a Z2 Coulter Particle Count and Size Analyzer (Beckman Coulter).

2.~~6~~7 Data analysis

Responses of growth rates, POC and PIC ~~quotas~~ production rates, PIC:POC ratio~~,~~ ~~POC and PIC production rates~~ to incubation light intensities were fitted using the model provided by Eilers and Peeters (1988): $y = \frac{PAR}{a \times PAR^2 + b \times PAR + c}$, where the parameters a, b and c are fitted in a least square manner. The apparent light use efficiency, the slope (α), for each light response curve was estimated as $\alpha = 1/c$. The

maximum values (V_{max}) of growth, POC and PIC production rates were calculated according to $V_{max} = \frac{1}{b + 2\sqrt{ac}}$.

A three-way ANOVA was used to determine the main effect of dissolved inorganic ~~nitrate (or phosphate)~~ nutrient concentration, pCO_2, light intensity and their interactions for these variables. ~~A three-way ANOVA was performed to compare the fitted α between growth, POC and PIC production rates at low and high CO_2 levels under different nutrient conditions.~~ A two-way ANOVA was performed to test the main effect of dissolved inorganic nutrient concentration, pCO_2 and their interactions on fitted a and V_{max} of growth, POC and PIC production rates. When necessary, a Tukey Post hoc (Tukey HSD) test was used to identify the differences between two CO_2 levels, ~~nitrate (or phosphate)~~ nutrient concentrations or light ~~levels~~intensities. A Shapiro-Wilk's test was conducted to test residual normality and a Levene test was used to test for variance homogeneity of significant data. Statistical analysis was conducted by using R and significant level was set at $p < 0.05$.

3 Results

3.1 Dissolved inorganic nitrogen and phosphate concentrations, and carbonate chemistry paremeters

At the HNHP condition, dissolved inorganic nitrogen (DIN) and phosphate (DIP) concentrations were 101 ± 1.1 μmol L^{-1} and 10.5 ± 0.2 μmol L^{-1}, respectively, at the beginning of the experiments, and were 92.8 ± 1.6 μmol L^{-1} and 9.7 ± 0.2 μmol L^{-1} in

the final days of the experiment (Table S~~1~~2). At the LN condition, DIN concentrations were 8.8 ± 0.1 μmol L^{-1} at the beginning of the experiment and were 1.0 ± 0.4 μmol L^{-1} at the end of the experiment. In the LP treatment, DIP concentrations were 0.4 ± 0.1 μmol L^{-1} at the beginning of the experiment, and were below the detection limit (< 0.04 μmol L^{-1}) at the end of the experiment.

The carbonate system parameters of the seawater at the beginning and end of the incubation ~~(mean values for the beginning and end of incubations)~~ are shown in Table 1. ~~For low CO_2 (LC) condition, the pCO_2 levels of the media were about 435 μatm at HNHP, 410 μatm at LN and 370 μatm under LP conditions, and the pH_T values (reported on the total scale) were about 8.10 at HNHP, 8.11 at LN and 8.16 at LP. For high CO_2 (HC) condition, the pCO_2 levels of the media were about 970 μatm at HNHP, 935 μatm at LN and 850 μatm at LP, and the pH_T values were about 7.80 at HNHP, 7.80 at LN, and 7.85 at LP conditions. .~~ Within the low CO_2 (LC) treatment, pCO_2 levels of the seawater declined by 16% at HNHP, 19% at LN and 8% at LP, and pH values increased by 0.07 at HNHP, 0.06 at LN and 0.02 at LP (Tukey HSD, all $p < 0.05$). Within the high CO_2 (HC) treatment, pCO_2 levels of the seawater declined by 23% at HNHP, 21% at LN and 32% at LP, and pH values increased by 0.1 at HNHP, 0.09 at LN and 0.15 at LP (Tukey HSD, all $p < 0.05$). Average pCO_2 levels were 410 μatm for all LC conditions, and were 920 μatm for all HC conditions.

3.2 Growth rate

Under each nutrient condition, at both LC and HC, growth rates of *E. huxleyi*

increased with elevated light intensity up to 200 μmol photons m^{-2} s^{-1} and significantly declined thereafter (~~Three-way ANOVA;~~ Tukey ~~Post hoc~~ HSD, all df = 2, all $p < 0.001$) (Fig. 1; Table 2). Compared with LC, growth rates at HC were 2%–7% lower at HNHP (Tukey HSD, $p < 0.05$), 5%–9% lower at LN (Tukey HSD, $p < 0.01$) and 3%–24% lower at LP (Tukey HSD, $p < 0.01$), respectively (Table 3). Under LP treatment, HC-induced reduction of growth rate was large~~r~~ at high~~er~~ light ~~levels~~intensity (Fig. 1c).

At LC, growth rate at LN was similar with that at HNHP under limited light intensity with 80 μmol photons m^{-2} s^{-1} (Tukey HSD, df = 1, $p = 0.82$), and was significantly lower than at HNHP under optimal and supra-optimal light intensities (Tukey HSD, both df = 1, $p < 0.01$ for 200 μmol photons m^{-2} s^{-1} ~~treatment~~; $p = 0.005$ for 480 μmol photons m^{-2} s^{-1} ~~treatment~~). At HC, growth rates at LN were significantly lower than those at HNHP under limited, optimal and supra-optimal light intensities (Tukey HSD, all df = 1, $p < 0.01$ for 80, 200, 480 μmol photons m^{-2} s^{-1} ~~treatments~~).

At LC and at 80 μmol photons m^{-2} s^{-1}, growth rate at LP was lower than at HNHP (Tukey HSD, df = 1, $p < 0.001$); while at 120–480 μmol photons m^{-2} s^{-1}, growth rates were no significant differences between LP and HNHP (Tukey HSD, all df = 1, all $p > 0.1$) (Fig. 1; Table 3). At HC and at 80, 120 and 480 μmol photons m^{-2} s^{-1}, growth rates were significantly lower at LP than at HNHP; at 200 and 320 μmol photons m^{-2} s^{-1}, growth rates were not significantly different between LP and HNHP (Tukey HSD, both df = 1, both $p > 0.05$).

3.3 POC quota

Under HNHP or LP conditions, at LC, POC quotas were not significantly different among 80, 120 and 200 μmol photons m^{-2} s^{-1} and increased with increased light intensity from 200 to 480 μmol photons m^{-2} s^{-1} (Three-way ANOVA; Tukey Post hoc, both df = 1, both $p < 0.01$); while at HC, POC quotas increased with elevated light intensity up to 480 μmol photons m^{-2} s^{-1} (Fig. 2a,c; Tables 2; 3). At LN, at both LC and HC, POC quotas at 320 μmol photons m^{-2} s^{-1} were significantly larger than at other light intensities (Fig. 2b).

At HNHP or at LN, POC quotas did not show significant differences between HC and LC (Fig. 2a,b). At LP, at 80 μmol photons m^{-2} s^{-1}, POC quotas were significantly larger at LC than at HC (df = 1, $p = 0.003$), while at 480 μmol photons m^{-2} s^{-1}, they were lower (df = 1, $p = 0.001$).

At both LC and HC, POC quotas were not significantly different between LN and HNHP at 80–320 μmol photons m^{-2} s^{-1}, while they were lower at LN than at HNHP at 480 μmol photons m^{-2} s^{-1} ($p < 0.01$). At both LC and HC, POC quotas were not significantly different between LP and HNHP at 80–480 μmol photons m^{-2} s^{-1} (all df = 1, all $p > 0.05$).

3.4 PIC quota

At HNHP or at LN, under either LC or HC, PIC quotas increased with increasing light intensity until 320 μmol photons m^{-2} s^{-1} (Three-way ANOVA; Tukey Post hoc, all df = 1, all $p < 0.001$) and then leveled off with further increasing light intensity (Fig.

2d,e; Tables 2; 3). At LP under LC conditions, PIC quotas increased significantly when light intensity increased from 80 to 200 μmol photons m^{-2} s^{-1} and significantly declined thereafter (both df = 1, both $p < 0.001$) (Fig. 2f), while at LP and HC, there were no significant differences among the light levels (all $p > 0.05$).

At HNHP or at LN, PIC quotas were larger at LC than at HC (all df = 1, all $p > 0.05$ at 80, 120, 200 treatments; both $p < 0.01$ at 320 and 480 treatments) (Fig. 2d,e). Under LP conditions at 200 and 320 μmol photons m^{-2} s^{-1}, PIC quotas were larger at LC than at HC (both df = 1, both $p < 0.05$) (Fig. 2f).

At both LC and HC, PIC quotas were larger at LN than at HNHP (all df = 1, all $p > 0.05$ at 80 treatment; $p < 0.05$ at 120–480 treatments) (Fig. 2d,e). For both LC and HC conditions at 80–200 μmol photons m^{-2} s^{-1}, PIC quotas were larger at LP than at HNHP (all df = 1, all $p < 0.05$), while at 320 and 480 μmol photons m^{-2} s^{-1}, they were not significantly different between LP and HNHP (Fig. 2f).

3.5 PIC:POC ratio

At HNHP under LC, PIC:POC ratio increased with elevated light intensity until 320 μmol photons m^{-2} s^{-1} and significantly declined thereafter (Three way ANOVA, Tukey Post hoc, df = 1, $p < 0.05$) (Fig. 2g; Tables 2; 3), while at HC, they were not significantly different between light treatments (all $p > 0.05$). At LN in both LC and HC treatments, PIC:POC ratio increased when light intensity increased from 80 to 200 μmol photons m^{-2} s^{-1} and were not significantly different between 200, 320 and 480 μmol photons m^{-2} s^{-1} (Fig. 2h). At LP under LC conditions, PIC:POC ratio

~~increased with increasing light intensity until 200 µmol photons m^{-2} s^{-1}, and declined with further increasing light intensity (both df = 1, both $p < 0.05$) (Fig. 2i), while at HC, they were not significantly different between light treatments (df = 4, $p > 0.05$).~~

~~At either HNHP or at LP, at light levels of 80–480 µmol photons m^{-2} s^{-1}, PIC:POC ratio were not significantly different between LC and HC (all df = 1, all $p > 0.05$) (Fig. 2g,i). At LN under 320 and 480 µmol photons m^{-2} s^{-1}, PIC:POC ratios were larger at LC than at HC (both df = 1, both $p < 0.05$) (Fig. 2h).~~

~~At both LC and HC, under 80–480 µmol photons m^{-2} s^{-1} PIC:POC ratios were larger at LN than at HNHP (all df = 1, $p > 0.05$ at the 80 treatment; $p < 0.05$ at the 120 to 480 treatments) (Fig. 2g,h). In both LC and HC conditions, at 80–200 µmol photons m^{-2} s^{-1} PIC:POC ratios were larger at LP than at HNHP (all df = 1, all $p <$ 0.05) (Fig 2g,i), while at 320 and 480 µmol photons m^{-2} s^{-1}, they were not significantly different between LP and HNHP.~~

3.~~6~~ 3 F_v/F_m and F_v'/F_m'

F_v/F_m and F_v'/F_m' showed the same patterns (Fig. ~~3~~2). ~~At each nutrient condition, at both LC and at HC~~ At all nutrient and CO_2 levels, F_v/F_m and F_v'/F_m' decreased with elevated light intensity until 480 µmol photons m^{-2} s^{-1} (~~Three way ANOVA;~~ Tukey HSD~~Post hoc~~, all df = ~~1~~4, all $p < 0.01$) (Fig. 2~~3a–f~~; Tables 2; 3).

~~At either HNHP or LP, o~~Only at LP and at 480 µmol photons m^{-2} s^{-1} F_v/F_m value~~s were~~was significantly larger at LC than at HC (~~both~~ Tukey HSD, df = 1, ~~both~~ $p < 0.01$) (Fig. 2~~3a,~~c). At LN in the light ~~range~~intensities of 80–480 µmol photons m^{-2} s^{-1}, F_v/F_m values were not significantly different between LC and HC (Tukey HSD, all df

= 1, all $p > 0.05$) (Fig. 2~~3~~b).

At both LC and HC, ~~from~~at 80–480 µmol photons m^{-2} s^{-1} F_v/F_m did not show significant differences between LN and HNHP (Tukey HSD, all df = 1, all $p > 0.05$), and at 480 µmol photons m^{-2} s^{-1}, they were lower at LP than at HNHP at both LC and HC (Tukey HSD, both df = 1, both $p < 0.05$) (Fig. 2~~3~~a,c).

At HNHP ~~from~~ and at 80 to 480 µmol photons m^{-2} s^{-1}, F_v' / F_m' values were similar between LC and HC (Tukey HSD, all df = 1, all $p > 0.05$) (Fig. 2~~3~~d). At LN under 200 µmol photons m^{-2} s^{-1}, and at LP under 480 µmol photons m^{-2} s^{-1}, F_v' / F_m' values were larger at LC than at HC (Tukey HSD, both df = 1, both $p < 0.01$) (Fig. 2~~3~~e,f).

At LC under 200 µmol photons m^{-2} s^{-1}, F_v' / F_m' values were significantly larger at LN than at HNHP, as well as at LP ~~compared to~~ than at HNHP (Tukey HSD, both df = 1, both $p < 0.05$) (Fig. 2~~3~~d,e,f). At HC under 480 µmol photons m^{-2} s^{-1} F_v' / F_m' values were significantly lower at LP than at HNHP (Tukey HSD, df = 1, $p < 0.01$) (Fig. 2~~3~~d,f).

3.4 POC production rate

At HNHP or LP conditions, at both LC and HC, POC production rates increased significantly with increasing light intensity until 480 µmol photons m^{-2} s^{-1} (Tukey HSD, all df = 4, $p < 0.01$) (Fig. 3a,c; Tables 2; 3). At LN, at both LC and HC, POC production rate increased when light intensity increased from 80 to 320 µmol photons m^{-2} s^{-1} (Tukey HSD, both df = 3, $p < 0.01$) and significantly declined thereafter (Fig. 3b).

At HNHP or LN conditions, at all light intensities, POC production rates did not show significant differences between HC and LC treatments (Tukey HSD, all df = 1, all $p > 0.05$) (Fig. 3a,b). At LP, at 80 and 320 µmol photons m^{-2} s^{-1}, POC production rates were significantly larger at LC than at HC (Tukey HSD, both df = 1, both $p < 0.01$) (Fig. 3c).

At both LC and HC, at 80–320 µmol photons m^{-2} s^{-1}, POC production rates were not significantly different between LN and HNHP, and between LP and HNHP (Tukey HSD, all df = 1, all $p > 0.05$); while at 480 µmol photons m^{-2} s^{-1}, they were lower at LN or LP than at HNHP conditions (Tukey HSD, df = 1, $p < 0.05$) (Fig. 3a,b,c).

3.5 PIC production rate

At HNHP or LN conditions, at both LC and HC, PIC production rates increased significantly when light intensity increased from 80–320 µmol photons m^{-2} s^{-1} (Tukey HSD, all df = 3, all $p < 0.05$) (Fig. 3d,e; Tables 2; 3), and declined thereafter (Tukey HSD, df = 1, $p < 0.05$ at LC; $p > 0.1$ at HC). At LP condition, at both LC and HC, PIC production rates increased significantly until 200 µmol photons m^{-2} s^{-1} (Tukey HSD, both df = 2, both $p < 0.05$) (Fig. 3f), and declined with further increases in light intensity (Tukey HSD, df = 2, $p < 0.05$ at LC; $p > 0.1$ at HC) (Fig. 3f).

At HNHP or LN conditions, at 320 µmol photons m^{-2} s^{-1}, PIC production rates were larger at LC than at HC (Tukey HSD, df = 1, $p < 0.05$) (Fig. 3d,e). At LP, at all light intensities, PIC production rates were no significant differences between LC and HC treatments (Tukey HSD, all df = 1, all $p > 0.05$) (Fig. 3f).

At both LC and HC, at all light intensities, PIC production rates were larger at LN than at HNHP (Tukey HSD, all df = 1, $p > 0.05$ at 80 µmol photons m^{-2} s^{-1}; all $p < 0.05$ at 120–480 µmol photons m^{-2} s^{-1}) (Fig. 3d,e). At LC, at 120 and 200 µmol photons m^{-2} s^{-1}, PIC production rates were significantly larger at LP than at HNHP (Tukey HSD, both df = 1, both $p < 0.05$). At HC, at all light intensities, PIC production rates were not significantly different between LP and HNHP conditions (Tukey HSD, all df = 1, all $p > 0.05$) (Fig. 3d,f).

3.6 PIC:POC ratio

At HNHP and at LC, PIC:POC ratio increased with increasing light intensity until 320 µmol photons m^{-2} s^{-1} (Tukey HSD, df = 3, $p < 0.01$) and slightly declined thereafter (Tukey HSD, df = 1, $p = 0.13$) (Fig. 3g; Tables 2; 3). At HNHP and at HC, they were not significantly different between light treatments (Tukey HSD, df = 4, $p > 0.05$). At LN, at both LC and HC, PIC:POC ratio increased significantly when light intensity increased from 80 to 200 µmol photons m^{-2} s^{-1} (Tukey HSD, both df = 2, $p < 0.01$) and did not show significant differences at 200–480 µmol photons m^{-2} s^{-1} (Tukey HSD, both df = 2, $p > 0.1$) (Fig. 3h). At LP and at LC, PIC:POC ratio increased with increasing light intensity until 200 µmol photons m^{-2} s^{-1}, and declined with further increasing light intensity (Tukey HSD, df = 2, $p < 0.05$) (Fig. 3i). At LP and at HC, they were not significantly different between light intensities (Tukey HSD, df = 4, $p > 0.05$) (Fig. 3i).

At HNHP or at LP, at 80–480 µmol photons m^{-2} s^{-1}, PIC:POC ratio were not

significantly different between LC and HC treatments (Tukey HSD, all df = 1, all $p >$ 0.05) (Fig. 3g,i). At LN, at 320 and 480 µmol photons m^{-2} s^{-1}, PIC:POC ratios were larger at LC than at HC (Tukey HSD, both df = 1, both $p < 0.05$) (Fig. 3h).

At both LC and HC, at 80–480 µmol photons m^{-2} s^{-1}, PIC:POC ratios were larger at LN than at HNHP (Tukey HSD, all df = 1, $p > 0.05$ at 80 µmol photons m^{-2} s^{-1}; $p <$ 0.05 at 120–480 µmol photons m^{-2} s^{-1}) (Fig. 3g,h). At both LC and HC, at 80–200 µmol photons m^{-2} s^{-1} PIC:POC ratios were larger at LP than at HNHP (Tukey HSD, all df = 1, all $p < 0.05$) (Fig 3g,i), while at 320 and 480 µmol photons m^{-2} s^{-1}, they were not significantly different between LP and HNHP (Tukey HSD, both df = 1, both $p > 0.05$) (Fig 3g,i).

~~***3.7 ETR_{max}***~~

~~At HNHP and at LC, ETR_{max} increased significantly with increasing light intensities until 200 µmol photons m^{-2} s^{-1} (df = 1, $p < 0.01$), and leveled off with further increasing light intensities (Fig. 3g; Tables 2; 3). At HNHP and at HC, with light intensities increasing from 80 to 120 µmol photons m^{-2} s^{-1}, ETR_{max} increased remarkably (df = 1, $p < 0.01$), and declined significantly when light intensities further increased to 480 µmol photons m^{-2} s^{-1} (df = 1, $p < 0.05$). At LN or at LP, under both LC and HC, ETR_{max} increased with increasing light intensities until 200 µmol photons m^{-2} s^{-1} and declined thereafter (all df = 1, all $p < 0.01$) (Fig. 3h,i).~~

~~At HNHP and only at 480 µmol photons m^{-2} s^{-1}, ETR_{max} was lower at HC than at LC (df =1, $p < 0.01$) (Fig. 3g; Table 3). At LN across the light range of 80–480 µmol~~

~~photons m^{-2} s^{-1}, ETR_{max} values were similar between HC and LC (Fig. 3h). At LP under 320 µmol photons m^{-2} s^{-1}, ETR_{max} was larger at HC than at LC; while at 480 µmol photons m^{-2} s^{-1}, they were lower (both df =1, both $p < 0.05$) (Fig. 3i).~~

~~At both LC and HC from 80–480 µmol photons m^{-2} s^{-1}, ETR_{max} values were larger at LN than at HNHP (Tukey Post hoc, all df = 1, $p < 0.01$ for the 120, 200 and 320 treatments at LC; $p > 0.05$ for the 80 and 480 treatments at LC; $p < 0.01$ for the 80, 200, 320 and 480 treatments at HC; $p > 0.05$ for the 120 treatment at HC) (Fig. 3g,h). At LC under 80 µmol photons m^{-2} s^{-1}, ETR_{max} was lower at LP than at HNHP (df = 1, $p > 0.1$); while at 120–480 µmol photons m^{-2} s^{-1}, ETR_{max} values were larger (Tukey Post hoc, all df = 1, $p > 0.05$ for 120, 320 and 480 treatments; $p < 0.01$ for 200 treatment) (Fig. 3g,h). At HC under 80 and 120 µmol photons m^{-2} s^{-1}, ETR_{max} values were lower at LP than at HNHP (Tukey Post hoc, both df = 1, $p > 0.1$ for the 80 treatment; $p < 0.01$ for the 120 treatment), while at 200–480 µmol photons m^{-2} s^{-1}, they were larger (Tukey Post hoc, all df = 1, $p < 0.01$ for 200 and 320 treatments; $p > 0.1$ for 480 treatment).~~

3.~~8~~7 Apparent light use efficiency (α) and maximum value ~~for~~of growth, POC and PIC production rates

At each nutrient condition, α values of fitted curves of growth, POC and PIC production rates were not significantly different between LC and HC, with the exception of α of PIC production rate at LP (Tukey HSD, df = 1, $p < 0.05$) (Fig. 4). At both LC and HC, α values of fitted curves of growth and POC production rates did not

show significant differences between HNHP, LN and LP conditions, with the exception of a of POC production rate between HNHP-LC and LP-HC conditions (Tukey HSD, df = 1, $p < 0.05$) (Fig. 4c). At LN under both LC and HC, and at LP under LC, α values of PIC production rates were larger than those of POC production rates, which were larger than those of growth rates (Tukey HSD, all df = 1, all $p < 0.01$) (Fig. 4a,c,e).

~~At HNHP under both LC and HC, α values of fitted curves for POC and PIC production rates were not significantly different, and they were significantly larger than those for growth rates (both df = 1, both $p < 0.01$) (Fig. 4a). At LN under both LC and HC, and at LP under LC, α values for PIC production rates were larger than those for POC production rates, which were larger than those for growth rates (all df = 1, all $p < 0.01$) (Fig. 4b,c). At LP and HC, α values for POC and PIC production rates did not show significant differences and they were larger than that for growth rates (Fig. 4c).~~

~~At both LC and at HC, α values of fitted curves of growth rates or POC production rates were not significantly different between LN and HNHP, and between LP and HNHP (Fig. 4). At LC, α values for PIC production rates were lower at HNHP than at LN or at LP (both df = 1, both $p < 0.01$); at HC, they were not significantly different between HNHP and LP (Fig. 4).~~

At HNHP, LN or LP condition, maximum growth rates were significantly larger at LC than at HC (Tukey HSD, all df = 1, all $p < 0.05$) (Fig. 4b). At both LC and HC, maximum growth rates were larger at HNHP than at LN (Tukey HSD, both df = 1,

both $p < 0.05$), and they were similar between HNHP and LP (Tukey HSD, both df = 1, both $p > 0.05$) (Fig. 4b).

At each nutrient condition, maximum POC production rates were slightly larger at LC than at HC (Tukey HSD, all df = 1, all $p > 0.05$) (Fig. 4d). At LC, maximum POC production rate was lower at LN than at HNHP and LP (Tukey HSD, df = 1, $p < 0.05$ between LN and HNHP; $p > 0.05$ between LN and LP). At HC, they did not show significant differences between HNHP, LN and LP conditions (Tukey HSD, df = 2, $p > 0.05$) (Fig. 4d).

At HNHP, LN or LP condition, maximum PIC production rates were significantly larger at LC than at HC (Tukey HSD, all df = 1, all $p < 0.05$) (Fig. 4f). At both LC and HC, maximum PIC production rates were larger at LN than at HNHP or LP (Tukey HSD, df = 2, $p < 0.05$) (Fig. 4f).

4 Discussion

In this study, growth rates of *E. huxleyi* were larger than 1 in almost all treatments, and cells divided 1–2 times per day (Fig .1), which indicates non-limiting nutrient conditions during the incubation. Based on measured PON quota and cell concentration in this study (Figs. 1 and S6), PON concentrations at the end of incubations were estimated to be 7.8–9.3 μmol L^{-1} at different nutrient conditions (Table S2). These data were closely correlated with molar drawdown of dissolved inorganic nitrogen (DIN) during the incubations. Furthermore, residual 1 μmol L^{-1}

DIN in the final day of the incubation showed non-limitation of growth and POC production rates by nitrogen. On the other hand, Rokitta et al. (2016) reported that F_v/F_m of *E. huxleyi* was 50% lower at P-depleted than at P-replete conditions. In this study, F_v/F_m and POC quota were very similar between LP and HNHP treatments (Figs. 2 and S3), which suggest that LP did not limit growth and carbon fixation.

~~In this study, we investigated synergistic negative effects of low nutrient concentrations and rising pCO_2 on growth rates, especially at limiting low and inhibiting high light intensities. Notably, high light intensities compensated for inhibition of LP on growth rates at LC. LN reduced POC quota and its sensitivity to light intensity. Both LN and LP increased PIC quotas, PIC:POC ratio, and *ETR* efficiency.~~

4.1 Low ~~nutrient~~ dissolved inorganic nitrogen concentration~~s~~ and high pCO_2 level synergistically reduced growth rate.

~~Langer et al. (2013) detected that cell numbers on the fourth to sixth days during cultures were in the exponential growth phase even at 3 μmol L^{-1} NO_3^- or at 0.29 μmol L^{-1} PO_4^{3-} with the same *E. huxleyi* strain. In addition, other *E. huxleyi* strains were in the exponential phase of growth on the fourth to the seventh days in the cultures with 2.5–8 μmol L^{-1} NO_3^- or at 0.4–0.55 μmol L^{-1} PO_4^{3-} (Perrin et al., 2016; Rokitta et al., 2016). All parameters were measured on the fourth to the sixth days, and it is most likely that cells at all treatments were sampled in the exponential growth phase in this study.~~

Less energy availability limited growth rates of *E. huxleyi* at lower light intensities, while reduction in growth rates at high light intensities could be related to photooxidative damage or photoinhibition (Fig. 1), because high light intensity can constantly damage the reaction centers of photosystem II (PSII) of *E. huxleyi* (Fig. 2) and maximize electron turnover rate through PSII centers ~~of *E. huxleyi* (Fig. 3a–f)~~ (Behrenfeld et al. 1998; Ragni et al., 2008). ~~Nevertheless, photoinhibition was not observed in electron transport rate (*ETR*) of the cells grown at 480 µmol photons m^{-2} s^{-1} even exposed to light intensity of 1600 µmol photons m^{-2} s^{-1} (Figs. 1 and S3). This implies that the photochemical performance during a short time exposure can hardly reflect the growth response. At HC, the negative effect of high $[H^+]$ on growth rate was larger than positive effects of increased CO_2 and HCO_3^- concentrations, which could be attributed to lower growth rates at HC than at LC (Fig. 1) (Bach et al., 2011).~~ Lower growth rates at HC than at LC are due to the fact that at HC the negative effect of high $[H^+]$ on growth rate was larger than positive effects of increased CO_2 and HCO_3^- concentrations (Bach et al., 2011).

~~Based on measured PON quota and cell concentration in this study (Figs. 1 and S6), PON concentrations at the end of incubations were estimated to be 7.8–9.3 µmol L^{-1} at different nutrient conditions (Table S1). These were closely correlated with molar drawdown of dissolved inorganic nitrate (DIN) in the cultures. *E. huxleyi* appeared to be a poor competitor for inorganic nitrate under low levels of nitrate availability (Fig. 1). Reduced levels of gene expressions and nitrate reductase (NRase) activity in *E. huxleyi* cells grown under low nitrate could be responsible (Bruhn et al., 2010; Rouco~~

~~et al., 2013), thus resulting in reduced nitrate assimilation. In addition,~~ LN concentration was shown to down-regulate transcripts of genes related to nitrate reductase (NRase) activity, synthesis of amino acids, RNA polymerases and nitrogen metabolism in *E. huxleyi* (Bruhn et al., 2010; Rouco et al., 2013; Rokitta et al., 2014), which led to lower overall biosynthetic activity and decreased the growth rates (Fig. 1). Synergistic effects of LN and HC on growth rates indicate that these conditions may inhibit cellular metabolic activity simultaneously (Fig. 1) (Sciandra et al., 2003). In fact, intracellular [H^+] have been reported to be higher in HC-grown than in LC-grown *E. huxleyi* cells (Suffrian et al., 2011). To transport extra H^+ out of cells, *E. huxleyi* at HC need more transporters and energy, but LN is likely to limit the synthesis of these transporters and energy supply (Fig. S6), therefore, it exacerbated the negative effects of high [H^+] on growth of *E. huxleyi* ~~(Fig. S6)~~ (Bruhn et al., 2010).

4.2 Effect of low dissolved inorganic phosphate concentration on growth rate was modulated by light intensity and CO_2 level.

E. huxleyi possesses an exceptional phosphorus acquisition capacity, which could allow it to dominate in phosphate-limiting environments (Dyhrman and Palenik, 2003). ~~In this study, at low levels of light intensity, uptake of phosphate could be energy limited, thus their growth was more inhibited at LP (Fig. 1c).~~ In this study, low light intensity not only limited cell growth but also was suggested to limit phosphate uptake rates (Nalewajko and Lee, 1983). In this case, compared to the HNHP

condition, growth rates of *E. huxleyi* at LP condition were more likely to be limited by low-light intensity (Fig. 1a,c). High light intensity provided energy for cells to take up P, and cells at LP condition need to consume more energy to up-regulate P uptake (Nalewajko and Lee, 1983) which may lead to decreased high-light inhibition of growth rate at LP than at HNHP condition under LC. ~~Under light saturation condition, relationship of growth rates of *E. huxleyi* with phosphate concentrations indicated a very high affinity for dissolved inorganic phosphate (DIP) with 0.04 µmol L^{-1} half-saturation constant for DIP~~ Furthermore, growth rate of *E. huxleyi* was nearly saturated at 0.25 µmol L^{-1} DIP and was saturated at 0.5 µmol L^{-1} DIP and above. This demonstrated that *E. huxleyi* possesses a high affinity for DIP (Fig. 5)~~(Fig. 5). Since LP was reported to enhance expression of gene with a role in phosphorus assimilation or metabolism and synthesis of inorganic PO_4^{3-} transporters (Dyhrman et al., 2006; McKew et al., 2015; Rokitta et al., 2016),~~ which allowed *E. huxleyi* to take up PO_4^{3-} efficiently ~~enough., so that LP did not result in reduced growth rate at LC in this study (Fig. 1)~~. Rokitta et al. (2016) showed that even though PO_4^{3-} concentration in the culture media declined to zero (undetectable), cell number sustained to increase for 4 days, which indicates~~ing~~ that *E. huxleyi* cells could store phosphorus ~~PO_4^{3-} and use them later~~ for later use. Consequently, high energy consumption mechanism, ~~high affinity,~~ efficient uptake and storage capacity for phosphorus ~~PO_4^{3-}~~ in *E. huxleyi* could account for there being no significant differences in growth rates between LP and HNHP ~~under~~at LC and ~~saturating and supra-optimal~~ high light intensities. In fact, as reported previously, higher growth rates of *E. huxleyi* at LP in comparison to

HP were found during exponential growth phase in batch cultures (Rokitta et al., 2016). In natural seawaters, *E. huxleyi* usually starts to bloom following diatom blooms (Tyrrell and Merico, 2004). which may be related to a high growth rate of *E. huxleyi* at low nutrient concentrations. Therefore, our results also indicate that high growth rate of *E. huxleyi* at low nutrients concentrations may drive the succession of diatom to *E. huxleyi*.

Rising CO_2 was found to lead to higher phosphorous requirements for growth, carbon fixation and nitrogen uptake, and to decrease alkaline phosphate (APase) activity in *E. huxleyi* (Matthiessen et al., 2012; Rouce et al., 2013). At HC, higher phosphorous requirements may lead to lower growth rates at LP in comparison to HNHP (Fig. 1a,c). In addition, elevated CO_2 concentrations can down-regulate the uptake capacity of the cells for CO_2 and/or HCO_3^- (CO_2 concentration mechanisms), which could lead to less energy cost for maintaining active uptake mechanisms (Gao et al., 2012), and the save energy in the HC-grown cells, consequently, might have exacerbates photo-inhibition, leading to higher inhibition of the growth under LP and high light intensities (Fig. 1c). In addition, at LP, cell volume was 17% larger at HC than at LC under the highest light intensity (Table S1). Large cell volume can directly lead to lower growth rates and reduce nutrient uptake by cells, thereby limiting growth. Another possible reason for low tolerance to high-light intensity in growth rate at LP and HC might be a combined effect of LP and HC on the carbon concentrating mechanism (CCM) of *E. huxleyi*. LP or HC is hypothesized to down-regulate the activity of CCM in the green algae *Chlorella emersonii* and in *E.*

huxleyi, respectively (Rost and Riebesell, 2004; Beardall et al. 2005). When grown at HC, LP may minimize the activity of CCM of *E. hulxeyi*, which could lead to less energy cost for maintaining high efficient CCM. The saved energy in the HC- and LP-grown cells might have exacerbated photo-inhibition. In summary, high phosphorous requirement, large cell volume and less energy consumption at LP and HC conditions may lead to increased high-light inhibition of growth rates of *E. huxleyi* (Fig. 1).

4.~~2~~3 Low dissolved inorganic ~~nitrogen~~nutrient concentration and high pCO_2 level synergistically reduced POC ~~quota~~production rate.

~~At LC, *E. huxleyi* mainly uses external HCO_3^- as an inorganic carbon source for photosynthesis and calcification, and increasing light intensities are able to increase HCO_3^- uptake rates (Kottmeier et al., 2016). This may explain why POC and PIC quotas and production rates increased with increasing light intensity (Figs. 2 and S5). HC down-regulates gene expression related to the HCO_3^- transporter (Rokitta et al., 2012) and decreases the HCO_3^- uptake rate in *E. huxleyi* (Kottmeier et al. 2016), leading to lower PIC quotas at HC than at LC (Fig. 2).~~ At LC, *E. huxleyi* mainly uses external HCO_3^- as an inorganic carbon source to synthesize POC and PIC, and increasing light intensity increases the HCO_3^- uptake rate (Kottmeier et al., 2016) which results in large POC and PIC production rates at high light intensity (Fig. 3). However, at HC, expression of gene related to the HCO_3^- transporter was down-regulated and the HCO_3^- uptake rate was reduced (Rokitta et al., 2012;

Kottmeier et al. 2016), which lead to lower PIC production rates at HC than at LC. Meanwhile, cells at HC can increase CO_2 uptake to compensate for low HCO_3^- -uptake for photosynthetic C fixation (Kottmeier et al., 2016), which explains~~ing~~ the similar POC quotas between HC and LC (Fig. ~~2a~~ S3).

~~LN down regulates expression of the *rbc*L gene coding for the large subunit of the ribulose-1,5-biphosphate carboxylase/oxygenase (RUBISCO) (Bruhn et al., 2010; Rokitta et al., 2014). To conserve nitrogen, cells at LN prefer to shut down the synthesis of RUBISCO and then reduce carbon fixation (Falkowski et al., 1989) (Fig. 2b).~~LN was found to reduce the enzymatic function and cellular metabolic rates such as reduce synthesis and activity of ribulose-1,5-biphosphate carboxylase/oxygenase (RUBISCO), which decreases POC quota at both LC and HC (Falkowski et al., 1989; Rokitta et al., 2014) (Fig. S3 and S6). Furthermore, in comparison to LC, lower cell division rates at HC further reduce POC production rates at LN. ~~At HC, lower cell division rates resulted in lower POC and PIC production rates than at LC (Fig. S5).~~ On the other hand, large cell volume at LP and HC condition was responsible for low cell division rate and low POC production rate in comparison to HNHP (Figs 1, 3 and S3).

4.~~3~~4 Low dissolved inorganic nutrient concentrations facilitated calcification rate ~~and maximum electron transport rates (ETR_{max})~~.

~~Müller et al. (2008) found that calcification (PIC production) occurred only in the G1 cell cycle phase, and that LN or LP held cells in the G1 phase longer, which led to larger PIC quotas and calcification rates at LN or at LP than at HNHP (Figs. 2 and S5).~~

~~LC and LP treatment decreased cell division rates, elongated cell cycle, and increased coccolith production of *E. huxleyi* in the darkness (Paasche and Brubak, 1994). In the present work, however, we found slightly faster cell division (growth) and identical calcification rates at LP and high light intensities (Figs. 1c, 2f and S5). LP has been shown to up-regulate the genes involved in calcium binding proteins such as the glutamic acid related to synthesize of coccolith, calcium homeostasis and transcription factor (*cmyb*) (Wahlund et al., 2004; Dyhrman et al., 2006), and facilitates the formation of cytoplasmic membrane bodies (Shemi et al., 2016). These are related to the pathways associated with production of coccoliths (Young and Henriksen, 2003) and may also be responsible for larger PIC quotas at LP.~~ Nimer and Merrett (1993) reported that decreased DIN concentration facilitates calcification rate of *E. huxleyi*. This is consistent with our result. Due to lower photosynthetic carbon fixation rate and larger calcification rate at LN in comparison to HNHP (Fig. 3), we could expect that at LN, a high proportion of intracellular HCO_3^- or CO_2 was reallocated to synthesize particulate inorganic carbon. On the other hand, at LP, slightly larger PIC production rate is likely due to larger cell volume in comparison to HNHP (Fig. 3).

Calcification of coccolithophores makes an important contribution to marine carbonate counter pumps in the pelagic ocean (Rost and Riebesell, 2004). Enhanced calcification of *E. huxleyi* at low nutrient concentrations implies that blooms of calcifying *E. huxleyi* diminish the potential of the oceanic CO_2 uptake compared to non-calcifying phytoplankton blooms. ~~On the other hand~~ In addition, larger PIC:POC

ratios ~~imply~~have the potential to accelerate~~ faster~~ sinking rate of *E. huxleyi* cells, facilitating the export of carbon into deeper waters (Hoffmann et al., 2015).

~~At low light intensities, the ETR_{max} values were severely limited by low energy input. Supraoptimal light intensities have been found to significantly reduce the abundance of several proteins involved in repair and assembly of PSII, such as repair of photodamaged Psb D1 proteins in the reaction center of PSII of *E. huxleyi* (McKew et al., 2013). These suggest that high light intensity is likely to do great damage to the PSII structure and then reduce the ETR_{max}. Especially at HC, supraoptimal light intensity and saved energy from down-regulation of CCM activity synergistically decreased ETR_{max} (Fig. 3).~~

~~A previous study found that calcification can be an additional sink for electrons in *E. huxleyi* (Xu and Gao 2012). Compared with HNHP, larger ETR_{max} at LN or at LP and at saturating light intensities likely resulted from larger calcification rates (Figs. 2 and 3). On the other hand, growth, photosynthetic carbon fixation and nitrogen uptake need energy originating from electron transport (Zhang et al., 2015). At LP and at limiting levels of light intensity, lower growth, photosynthetic carbon and nitrate assimilation rates coincided with lower ETR_{max} (Figs. 1–3), implying correlations of these physiological processes.~~

To provide organic carbon fixed by photosynthesis to support growth and other metabolic processes, cells need to maintain larger light-use efficiency (α) for POC production rates (Fig. 4). ~~Calcification is an energy-dependent process (Riebesell and Tortell, 2011), and increased calcification rates at low nutrient concentrations could be~~

~~aided by higher light-use efficiencies (Fig. 4). In addition, besides taking up inorganic carbon sources and Ca^{2+} from the seawater to calcify, cells need extra energy to expel H^+ generated during calcification from the cells (Jin et al., 2017), these may also account for higher light-use efficiencies for PIC production rates.~~ To calcify, *E. hulxeyi* cells need to take up HCO_3^- and Ca^{2+} from the seawater, which consumes energy. Besides that, they also need to extrude H^+ generated during calcification into the cytosol to favour the conversion of HCO_3^- to CO_3^-, which also needs some energy (Paasche 2002). Thus, calcification is an energy consuming process. To maintain large calcification rate at low nutrient concentration, cells possess high light-use efficiencies and can then obtain more energy to take up HCO_3^- and Ca^{2+}, and extrude H^+ into the cytosol.

Using a chemostat culture, Müller et al. (2017) reported that DIN or DIP limitation decreased the POC and PIC production rates (in pg C $cell^{-1}$ d^{-1}) by 50% and rising pCO_2 levels did not affect POC production rates. However, when normalized to cell volume, nutrient limitation did not affect POC and PIC production rates (in pg C $cellV^{-1}$ d^{-1}), and rising pCO_2 levels reduced POC and PIC production rates. In our study, decreased DIN or DIP concentration reduced the normalized POC production rates (in pg C $cellV^{-1}$ d^{-1}), and increased the normalized PIC production rates at both LC and HC (Fig. S5). Differing results between the study of Müller et al. (2017) and ours may result from different experimental setups. Growth was really limited by N or P, cells were cultured in a continuous photon flux, and cell growth was in the stable phase when POC and PIC samples were taken in the study of Müller et al. (2017).

While we took POC and PIC samples in the exponential growth phase, and LN or LP did not really limit growth of *E. huxleyi* in our study.

Nutrient availability, CO_2 level and light intensity significantly interacted to affect growth rate, POC and PIC ~~quotas~~ production rates, F_v / F_m, and F_v' / F_m' ~~and ETR_{max}~~ (Table 2). Obviously, the question how growth, carbon fixation and calcification rates of *E. huxleyi* would respond to ocean global changes needs to be examined under multiple stressors and under natural environmental variations (Feng et al., 2008, 2017). ~~In comparison to the current ocean environment, under HC and HL conditions as expected in future oceans, effects of LN and LP on carbon fixation of *E. huxleyi* may partly negate each other (Fig.2, Table 3).~~ Although both HC and HL reduced calcification rates of *E. huxleyi*, low nutrient concentrations showed dominant positive effects on PIC quota or calcification rate (Fig. ~~2~~3d−f), suggesting that calcification of *E. huxleyi* may increase in the future pelagic oceans. Our study demonstrates that complex effects of multiple environmental drivers on phytoplankton require us to investigate the underlying mechanisms of these interactions, in order to comprehend how ecological and biogeochemical functions of key phytoplankton groups may respond to ocean global changes.

References

Bach, L. T., Riebesell, U., and Schulz, K. G.: Distinguishing between the effects of ocean acidification and ocean carbonation in the coccolithophore *Emiliania huxleyi*, Limnol. Oceanogr., 56, 2040–2050, https://doi.org/10.4319/lo.2011.56.6.2040, 2011.

Bach, L. T., Riebesell, U., Gutowska, M. A., Federwisch, L., and Schulz, K. G.: A unifying concept of coccolithophore sensitivity to changing carbonate chemistry embedded in an ecological framework, Prog. Oceanogr., 135, 125–138, doi: 10.1016/j.pocean.2015.04.012, 2015.

Beardall, J., Roberts, S., Raven, J. A. : Regulation of inorganic carbon acquisition by phosphorus limitation in the green alga *Chlorella emersonii*, Can. J. Bot., 83, 859–864, https://doi.org/10.1139/b05-070, 2005.

Behrenfeld, M., O'Malley, R., Siegel, D., McClain, C., Sarmiento, J., Feldman, G., Milligan, A., Falkowski, P., Letelier, R., and Boss, E.: Climate-driven trends in contemporary ocean productivity, Nature, 444, 752–755, https://doi.org/10.1038/nature05317, 2006.

Behrenfeld, M. J., Prasil, O., Kolber, Z. S., Babin, M., Falkowski, P. G. : Compensatory changes in photosystem II electron turnover rates protect photosynthesis from photoinhibition, Photosynth. Res., 58, 259–268, http://doi,org/10.1023/A:1006138630573, 1998.

Borchard, C., Borges, A. V., Händel, N., and Engel, A.: Biogeochemical response of Emiliania huxleyi (PML B92/11) to elevated CO_2 and temperature under

841 phosphorous limitation: A chemostat study, J. Exp. Mar. Biol. Ecol., 410, 61–71,
842 https://doi.org/10.1016/j.jembe.2011.10.004, 2011.
843 Boyd, P. W., Dillingham, P. W., McGraw, C. M., Armstrong, E. A., Cornwall, C. E.,
844 Feng, Y. Y., Hurd, C. L., Gault-Ringold, M., Roleda, M. Y., Timmins-Schiffman,
845 E., and Nunn, B. L.: Physiological responses of a Southern Ocean diatom to
846 complex future ocean conditions, Nat. Clim. Change, 6, 207–213,
847 https://doi.org/10.1038/NCLIMATE2811, 2016.
848 Brennan, G., and Colllins, S.: Growth responses of a green alga to multiple
849 environmental drivers, Nat. Clim. Change, 5, 892–897,
850 https://doi.org/10.1038/nclimate2682, 2015.
851 Bruhn, A., LaRoche, J., and Richardson, K.: *Emiliania huxleyi* (Prymnesiophyceae):
852 nitrogen-metabolism genes and their expression in response to external nitrogen
853 sources, J. Phycol., 46, 266–277, https://doi.org/10.1111/j.1529-8817.2010.00809,
854 2010.
855 Caldeira, K., and Wickett, M. E.: Oceanography: anthropogenic carbon and ocean pH,
856 Nature, 425, 365–365, https://doi.org/10.1038/425365a, 2003.
857 Chou, W. C., Sheu, D. D., Chen, C. A., Wang, S. L., and Tseng, C. M.: Seasonal
858 variability of carbon chemistry at the SEATS time-series site, Northern South
859 China Sea betweeen 2002 and 2003, Terr. Atmos. Ocean. Sci., 16, 445–465,
860 https://doi.org/10.3319/TAO.2005.16.2.445, 2005.
861 Cloern, J. E.: The relative importance of light and nutrient limitation of phytoplankton
862 growth: a simple index of coastal ecosystem sensitivity to nutrient enrichment,

Aquat. Ecol., 33, 3–16, https://doi.org/10.1023/A:1009952125558, 1999.

Dickson, A. G.: pH buffers for sea water media based on the total hydrogen ion concentration scale, Deep Sea Res., 40, 107–118, 1993.

Dickson, A. G., Afghan, J. D., and Anderson, G. C.: Reference materials for oceanic CO_2 analysis: a method for the certification of total alkalinity, Mar. Chem., 80, 185–197, https://doi.org/10.1016/S0304-4203(02)00133-0, 2003.

~~Dimier, C., Brunet, C., Geider, R., and Raven, J.: Growth and photoregulation dynamics of the picoeukaryote *Pelagomonas calceolata* in fluctuating light, Limnol. Oceanog., 54, 823–836, https://doi.org/10.4319/lo.2009.54.3.0823, 2009.~~

~~Dyhrman, S. T., Haley, S. T., Birkeland, S. R., Wurch, L. L., Cipriano, M. J., and McArthur, A. G.: Long serial analysis of gene expression for gene discovery and transcriptome profiling in the widespread marine coccolithophore *Emiliania huxleyi*, Appl. Environ. Microb., 72, 252–260, https://doi.org/10.1128/AEM.72.1.252-260.2006, 2006.~~

Dyhrman, S. T., and Palenik, B.: Characterization of ectoenzyme activity and phosphate-regulated proteins in the coccolithophorid *Emiliania huxleyi*, J Plank. Res., 25, 1215–1225, https://doi.org/10.1093/plankt/fbg086, 2003.

Eilers, P., and Peeters, J.: A model for the relationship between light intensity and the rate of photosynthesis in phytoplankton, Ecol. Model., 42, 199–215, https://doi.org/10.1016/0304-3800(88)90057-9, 1988.

Engel, A., Novoa C. C., Wurst, M., Endres, S., Tang, T., Schartau, M., Lee, C.: No detectable effect of CO_2 on elemental stoichiometry of *Emiliania huxleyi* in

885 nutrient-limited, acclimated continuous cultures, Mar. Ecol. Prog. Ser., 507, 15–30,
886 https://doi.org/10.3354/meps10824, 2014.
887 Falkowski, P. G., Sukenik, A., and Herzig, R.: Nitrogen limitation in *Isochrysis*
888 *galbana* (Haptophyceae). II. Relative abundance of chloroplast proteins, J. Phycol.,
889 25, 471–478, https://doi.org/10.1111/j.1529-8817.1989.tb00252.x, 1989.
890 Feng, Y. Y., Roleda, M. Y., Armstrong, E., Boyd, P. W., and Hurd, C. L.:
891 Environmental controls on the growth, photosynthetic and calcification rates of a
892 Southern Hemisphere strain of the coccolithophore *Emiliania huxleyi*, Limnol.
893 Oceanogr, 62, 519–540, https://doi.org/10.1002/lno.10442, 2017.
894 Feng, Y. Y., Warner, M. E., Zhang, Y. H., Sun, J., Fu, F. X., Rose, J. M., and Hutchins,
895 D. A.: Interactive effects of increased pCO_2, temperature and irradiance on the
896 marine coccolithophore *Emiliania huxleyi* (Prymnesiophyceae), Europ. J. Phycol.,
897 43, 87–98, https://doi.org/10.1080/09670260701664674, 2008.
898 Gao, K. S., Xu, J. T., Gao, G., Li, Y. H., Hutchins, D. A., Huang, B. Q., Wang, L.,
899 Zheng, Y., Jin, P., Cai, X. N., Häder, D. P., Li, W., Xu, K., Liu, N. N., and
900 Riebesell, U.: Rising CO_2 and increased light exposure synergistically reduce
901 marine primary productivity, Nat. Clim. Change, 2, 519–523,
902 https://doi.org/10.1038/nclimate1507, 2012.
903 Geider, R. J., MacIntyre, H. L., and Kana, T. M.: A dynamic model of phytoplankton
904 growth and acclimation: responses of the balanced growth rate and chlorophyll *a* :
905 carbon ratio to light, nutrient-limitation and temperature, Mar. Ecol. Prog. Ser.,
906 148, 187–200, https://doi.org/10.3354/meps148187, 1997.

907 Hansen, H. P., and Koroleff, F.: Determination of nutrients, in: Methods of seawater
908 analysis, edited by: Grasshoff, K., Kremling, K., and Ehrhardt, M., WILEY-VCH
909 Publishers, 159–228, 1999.
910 Harrison, W. G., and Li, W. K. W: Phytoplankton growth and regulation in the
911 Labrador Sea: light and nutrient limitation, J. Northw. Atl. Fish. Sci., 39, 71–82,
912 https://doi.org/10.2960/J.v39.m592, 2008.
913 Hoffmann, R., Kirchlechner, C., Langer, G., Wochnik, A. S., Griesshaber, E., Schmah,
914 W. W., and Scheu, C.: Insight into *Emiliania huxleyi* coccospheres by focused ion
915 beam sectioning, Biogeosciences, 12, 825–834,
916 https://doi.org/10.5194/bg-12-825-2015, 2015.
917 Hutchins, D. A., and Fu, F. X.: Microorganisms and ocean global change, Nat.
918 Microbiol., 2, 17058, https://doi.org/10.1038/nmicrobiol.2017.58, 2017.
919 ~~Jasby, A. D., and Platt, T.: Mathematical formulation of the relationship between~~
920 ~~photosynthesis and light for phytoplankton, Limnol. Oceanogr., 21, 540–547,~~
921 ~~https://doi.org/10.4319/lo.1976.21.4.0540, 1976.~~
922 Jin, P., Ding, J., Xing, T., Riebesell, U., and Gao, K.: High levels of solar radiation
923 offset impacts of ocean acidification on calcifying and non-calcifying strains of
924 *Emiliania huxleyi*, Mar. Ecol. Prog. Ser., 568, 47–58,
925 https://doi.org/10.3354/meps12042, 2017.
926 Kim, H. S., Hwang, S. J., Shin, J. K., An, K. G., and Yoon, C. G.: Effects of limiting
927 nutrients and N:P ratios on the phytoplankton growth in a shallow hypertrophic
928 reservoir, Hydrobiologia, 581, 255–267,

https://doi.org/10.1007/s10750-007-0727-1, 2007.

Kottmeier, D. M., Rokitta, S. D., and Rost, B.: Acidification, not carbonation, is the major regulator of carbon fluxes in the coccolithophore *Emiliania huxleyi*, New Phytol., 211, 126–137, https://doi.org/10.1111/nph.13885, 2016.

Langer, G., Nehrke, G., Probert, I., Ly, J., and Ziveri, P.: Strain-specific responses of *Emiliania huxleyi* to changing seawater carbonate chemistry, Biogeosciences, 6, 2637–2646, https://doi.org/10.5194/bg-6-2637-2009, 2009.

Langer, G., Oetjen, K., and Brenneis, T.: Coccolithophores do not increase particulate carbon production under nutrient limitation: A case study using *Emiliania huxleyi* (PML92/11), J. Exp. Mar. Biol. Ecol., 443, 155–161, 2013

Leonardos, N., and Geider, R. J.: Eleveted atmospheric carbon dioxide increases organic carbon fixation by *Emiliania huxleyi* (Haptophyta), under nutrient-limited high-light conditions, J. Phycol., 41, 1196–1203, https://doi.org/10.1111/j.1529-8817.2005.00152.x, 2005.

Matthiessen, B., Eggers, S. L., and Krug, S. A.: High nitrate to phosphorus regime attenuates negative effects of rising pCO_2 on total population carbon accumulation, Biogeosciences, 9, 1195–1203, https://doi.org/10.5194/bg-9-1195-2012, 2012.

McKew, B. A., Davey, P., Finch, S. J., Hopkins, J., Lefebvre, S. C., Metodiev, M. V., Oxborough, K., Raines, C. A., Lawso, T., and Geider, R. J.: The trade-off between the light-harvesting and photoprotective functions of fucoxanthin-chlorophyll proteins dominates light acclimation in *Emiliania huxleyi* (clone CCMP 1516), New Phytol., 200, 74–85, https://doi.org/10.1111/nph.12373, 2013.

McKew, B. A., Metodieva, G., Raines, C. A., Metodier, M. V., and Geider, R. J.: Acclimation of *Emiliania huxleyi* (1516) to nutrient limitation involves precise modification of the proteome to scavenge alternative sources of N and P, Environ. Microbiol., 17, 4050–4062, https://doi.org/10.1111/1462-2920.12957, 2015.

Meyer, J., and Riebesell, U.: Reviews and syntheses: Responses of coccolithophores to ocean acidification: a meta-analysis, Biogeosciences, 12, 1671–1682, https://doi.org/10.5194/bg-12-1671-2015, 2015.

Müller, M. N., Antia, A. N., and LaRoche, J.: Influence of cell cycle phase on calcification in the coccolithophore *Emiliania huxleyi*, Limnol. Oceanogr., 53, 506–512, https://doi.org/10.4319/lo.2008.53.2.0506, 2008.

Müller, M. N., Beaufort, L., Bernard, O., Pedrotti, M. L., Talec, A., Sciandra, A. : Influence of CO_2 and nitrogen limitation on the coccolith volume of *Emiliania huxleyi* (Haptophyta), Biogeosciences, 9, 4155–4167, https://doi.org/10.5194/bg-9-4155-2012, 2012.

Müller, M. N., Trull, T. W., and Hallegraeff, G. M.: Independence of nutrient limitation and carbon dioxide impacts on the Southern Ocean coccolithophore *Emiliania huxleyi*, ISME J., 11, 1777–1787, https://doi.org/10.1038/ismej.2017.53, 2017.

Nalewajko, C., Lee, K. : Light stimulation of phosphate uptake in marine phytoplankton, Mar. Bio., 74, 9–15, https://doi.org./10.1007/BF00394269, 1983.

Nimer, N. A., Merrett, M. J. : Calcification rate in *Emiliania huxleyi* Lohmann in response to light, nitrate and availability of inorganic carbon, New Phytol., 123,

673–677, http://www.jstor.org/stable/2557879, 1993.

Paasche, E. : A review of the coccolithophorid *Emiliania huxleyi* (Prymnesiophyceae), with particular reference to growth, coccolith formation, and calcification-photosynthesis interactions, Phycologia, 40, 503–529, https://doi.org/10.2216/i0031-8884-40-6-503.1, 2002.

~~Paasche, E., and Brubak, S.: Enhanced calcification in the coccolithophorid *Emiliania huxleyi* (Haptophyceae) under phosphorus limitation, Phycologia, 33, 324–330, 1994.~~

~~Perrin, L., Probert, I., Langer, G., and Aloisi, G.: Growth of the coccolithophore *Emiliania huxleyi* in light- and nutrient-limited batch reactors: relevance for the BIOSOPE deep ecological niche of coccolithophores, Biogeosciences, 13, 5983–6001, https://doi,org/10.5194/bg-13-5983-2016, 2016.~~

Pierrot, D., Lewis, E., and Wallace, D. W. R.: MS Excel program developed for CO_2 system calculations, ORNL/CDIAC-105, Carbon Dioxide Information Analysis Centre, Oak Ridge National Laboratory, U.S. Department of Energy. https://doi.org/10.3334/CDIAC/otg.CO2SYS_XLS_CDIAC105a, 2006.

Qu, B., Song, J., Yuan, H., Li, X., Li, N., and Duan, L.: Comparison of carbonate parameters and air-sea CO2 flux in the southern Yellow Sea and East China Sea during spring and summer of 2011, J. Oceanogr., 73, 365–382, https://doi.org/10.1007/s10872-016-0409-6, 2017.

Ragni, M., Airs, R. L., Leonardos, N., and Geider, R. J.: Photoinhibition of PSII in *Emiliania huxleyi* (Haptophyta) under high light stress: the roles of

photoacclimation, photoprotection, and photorepair, J. Phycol., 44, 670–683, https://doi.org/10.1111/j.1529-8817.2008.00524.x, 2008.

Richier, S., Fiorini, S., Kerros, M. E., Von Dassow, P., and Gattuso, J. P.: Response of the calcifying coccolithophore *Emiliania huxleyi* to low pH/high pCO_2: from physiology to molecular level, Mar. Biol., 158, 551–560, https://doi.org/10.1007/s00227-010-1580-8, 2011.

~~Riebesell, U., and Tortell, P. D.: Effects of ocean acidification on pelagic organisms and ecosystems, in: Ocean acidification, edited by: Gattuso, J. P., and Hansson, L., Oxford University Press, 99–121, 2011.~~

Riegman, R., Stolte, W., Noordeloos, A. A. M., and Slezak, D.: Nutrient uptake and alkaline phosphatase (EC3:1:3:1) activity of *Emiliania huxleyi* (Prymnesiophyceae) during growth under N and P limitation in continuous cultures, J. Phycol., 36, 87–96, https://doi.org/10.1046/j.1529-8817.2000.99023.x, 2000.

~~Rokitta, S. D., John, U., and Rost, B.: Ocean acidification affects redox-balance and ion-homeostasis in the life-cycle stages of *Emiliania huxleyi*, PLOS ONE, 7, e52212, https://doi.org/10.1317/journal.pone.0052212, 2012.~~

Rokitta, S. D., von Dassow, P., Rost, B., and John, U.: *Emiliania huxleyi* endures N-limitation with an efficient metabolic budgeting and effective ATP synthesis, BMC Genomics, 15, 1051–1064, https://doi.org/10.1186/1471-2164-15-1051, 2014.

Rokitta, S. D., von Dassow, P., Rost, B., and John, U.: P- and N-depletion trigger

similar cellular responses to promote senescence in eukaryotic phytoplankton, Front. Mar. Sci., 3, 109, https://doi.org/10.3389/fmars.2016.00109, 2016.

Rost, B., and Riebesell, U.: Coccolithophores and the biological pump: responses to environmental changes, in: Coccolithophores – From Molecular Biology to Global Impact, edited by: Thierstein, H. R. and Young, J. R., Springer, Berlin, 99–125, https://doi.org/10.1007/978-3-662-06278-4_52004, 2004.

Rouco, M., Branson, O., Lebrato, M., and Iglesias-Rodríguez, M. D.: The effect of nitrate and phosphate availability on *Emiliania huxleyi* (NZEH) physiology under different CO_2 scenarios, Front. Microbiol., 4, 155, https://doi.org/10.3389/fmicb.2013.00155, 2013.

Roy, R. N., Roy, L. N., Vogel, K. M., Porter-Moore, C., Pearson, T., Good, C. E., Millero, F. J., and Campbell, D. C.: Thermodynamics of the dissociation of boric acid in seawater at S 5 35 from 0 degrees C to 55 degrees C, Mar. Chem., 44, 243–248, 1993.

Sciandra, A., Harlay, J., Lefévre, D., Lemée, R., Rimmelin, P., Denis, M., and Gattuso, J. P.: Response of coccolithophorid *Emiliania huxleyi* to elevated partial pressure of CO_2 under nitrogen limitation, Mar. Ecol. Prog. Ser., 261, 111–122, https://doi.org/10.3354/meps261111, 2003.

Sett, S., Bach, L. T., Schulz, K. G., Koch-Klavsen, S., Lebrato, M., and Riebesell, U.: Temperature modulates coccolithophorid sensitivity of growth, photosynthesis and calcification to increasing seawater pCO_2, PLOS ONE, 9, e88308, https://doi.org/10.1371/journal.pone.0088308, 2014.

1039 Shemi, A., Schatz, D., Fredricks, H. F., Van Mooy, B. A. S., Porat, Z., and Vardi, A:
1040 Phosphorus starvation induces membrane remodeling and recycling in *Emiliania*
1041 *huxleyi*, New Phytol., 211, 886–898, https://doi.org/10.1111/nph.13940, 2016.
1042 Steinacher, M., Joos, F., Frölicher, T. L., Bopp, L., Cadule, P., Cocco, V., Doney, S. C.,
1043 Gehlen, M., Lindsay, K., Moore, J. K., Schneider, B., Segschneider, J. : Projected
1044 21st century decrease in marine productivity: a multi-model analysis,
1045 Biogeosciences, 7, 979–1005, https://doi.org/10.5194/bg-7-979-2010, 2010.
1046 Suffrian, K., Schulz, K. G., Gutowska, M., Riebesell, U., and Bleich, M.: Cellular pH
1047 measurements in *Emiliania huxleyi* reveal pronounced membrane proton
1048 permeability, New Phytol, 190, 595–608,
1049 https://doi.org/10.1111/j.1469-8137.2010.03633.x, 2011.
1050 Sunda, W. G., Price, N. M., and Morel, F. M. M.: Trace metal ion buffers and their use
1051 in culture studies, in: Algal culturing techniques, edited by: Andersen R. A.,
1052 Elsevier Academic Press, London, 53–59, 2005
1053 Tong, S. Y., Hutchins, D. A., Fu, F. X., and Gao, K. S.: Effects of varying growth
1054 irradiance and nitrogen sources on calcification and physiological performance of
1055 the coccolithophore *Gephyrocapsa oceanica* grown under nitrogen limitation,
1056 Limnol. Oceanogr., 61, 2234–2242, https://doi.org/10.1002/lno.10371, 2016.
1057 Tyrrell, T., and Merico, A.: *Emiliania huxleyi*: bloom observations and the conditions
1058 that induce them, in: Coccolithophores: From molecular biology to global impact,
1059 edited by: Thierstein, H. R., and Young, J. R., Springer, Berlin, 75–97,
1060 https://doi.org/10.1007/978-3-662-06278-4_4, 2004.

~~Wahlund, T. M., Hadaegh, A. R., Clark, R., Nguyen, B., Fanelli, M., and Read, B. A.: Analysis of expressed sequence tags from calcifying cells of marine coccolithophorid (*Emiliania huxleyi*), Mar. Biotechnol., 6, 278–290, https://doi.org/10.1007/s10126-003-0035-3, 2004.~~

Wang, G., Xie, S. P., Huang, R. X., and Chen, C.: Robust warming pattern of global subtropical oceans and its mechanism, J. Clim., 28, 8574–8584, https://doi.org/10.1175/JCLI-D-14-00809.1, 2015.

~~Xu, K., and Gao, K. S.: Reduced calcification decreases photoprotective capability in the coccolithophorid *Emiliania huxleyi*, Plant Cell Physiol., 53, 1267–1274, https://doi.org/10.1093/pcp/pcs066, 2012.~~

~~Young, J. R., and Henrikse, K.: Biomineralization within vesicles: the calcite of coccoliths, Rev. Mineral. Geochem., 54, 189–215, https://doi.org/10.2113/0540189, 2003.~~

Zhang, Y., Bach, L. T., Schulz, K. G., and Riebesell, U.: The modulating effect of light intensity on the response of the coccolithophore *Gephyrocapsa oceanica* to ocean acidification, Limnol. Oceanogr., 60, 2145–2157, https://doi.org/10.1002/lno.10161, 2015.

Competing interests: The authors declare that they have no conflict of interest.

Acknowledgements.

This study was supported by National Natural Science Foundation (41720104005, 41430967), and Joint project of National Natural Science Foundation of China and Shandong province (No. U1606404), China Postdoctoral Science Foundation (2017M612129) and the outstanding postdoctoral program of State Key Laboratory of Marine Environmental Science (Xiamen University). FF and DH's visits to Xiamen was supported by MEL's visiting scientists program.

Figure Legends

Figure 1. Growth rate of *Emiliania huxleyi* as a function of light intensities at low pCO_2 (LC) and high pCO_2 levels (HC) at high dissolved inorganic nitrogen (DIN) and phosphate (DIP) concentrations (HNHP)(**a**), low DIN and high DIP concentrations (LN) (**b**), or high DIN and low DIP concentrations (LP) (**c**). The ~~solid~~ lines in each panel were fitted using the model provided by Eilers and Peeters (1988). The values represent the mean ± standard deviation for four replicates.

Figure 2. At both LC and HC, maximum photochemical quantum yield (F_v/F_m) of *E. huxleyi* as a function of light intensity at HNHP (**a**), LN (**b**) and LP (**c**) conditions. At both LC and HC, light response of effective photochemical quantum yield (F_v'/F_m') of *E. huxleyi* at HNHP (**d**), LN (**e**) and LP (**f**) conditions. The values represent the mean ± standard deviation for four replicates.

Figure ~~2~~3. At both LC and HC, POC ~~quotas~~production rate of *E. huxleyi* as a function of light intensity~~ies~~ at HNHP (**a**), LN (**b**) and LP (**c**) conditions. At both LC and HC, light response~~s~~ of PIC ~~quotas~~production rate of *E. huxleyi* at HNHP (**d**), LN (**e**) and LP (**f**) conditions. At both LC and HC, light response~~s~~ of PIC:POC ratio~~s~~ of *E. huxleyi* at HNHP (**g**), LN (**h**) and LP (**i**) conditions. The ~~solid~~ lines in each panel were fitted using the model provided by Eilers and Peeters (1988). The values represent the mean ± standard deviation for four replicates.

~~**Figure 3.** At both LC and HC, maximum photochemical quantum yields (F_v/F_m) of *E. huxleyi* as a function of light intensities at HNHP (**a**), LN (**b**) and LP (**c**) conditions. At both LC and HC, light responses of effective photochemical quantum yields (F_v'/F_m') of *E. huxleyi* at HNHP (**d**), LN (**e**) and LP (**f**) conditions. At both LC and HC, light responses of fitted maximum electron transport rate (ETR_{max}) of *E. huxleyi* at HNHP (**g**), LN (**h**) and LP (**i**) conditions. The values represent the mean ± standard deviation for four replicates.~~

Figure 4. At both LC and HC, fitted *a* (**a**) and maximum (**b**) of growth rate at HNHP, LN and LP conditions. At both LC and HC, fitted *a* (**c**) and maximum (**d**) of POC prodution rate at HNHP, LN and LP conditions. At both LC and HC, fitted *a* (**e**) and maximum (**f**) of PIC production rate at HNHP, LN and LP conditions. α was the slope of fitted lines for growth, POC and PIC production rates. Different letters showed statistical differences based on the Tukey post hoc test. The values represent the mean ± standard deviation for four replicates.

~~**Figure 4.** At both LC and HC, apparent light-use efficiency (α) for growth, POC and PIC production rates of *E. huxleyi* at HNHP (**a**), LN (**b**) and LP (**c**) conditions. α was the slope of fitted lines for growth, POC and PIC production rates. μ represents growth rate, POCpro represents POC production rate and PICpro represents PIC production rate. Different letters showed statistically difference. The values represent the mean ± standard deviation for four replicates.~~

Figure 5. Growth rate of *E. huxleyi* as a function of dissolved inorganic phosphate (DIP) concentrations at LC under 200 µmol photons m^{-2} s^{-1}. DIN concentration was 100 µmol L^{-1} in all culture media, and DIP concentrations were set up to 0 µmol L^{-1}, 0.25 µmol L^{-1}, 0.5 µmol L^{-1}, 1.5 µmol L^{-1}, 3 µmol L^{-1} and 10 µmol L^{-1} in the culture media. All samples were incubated at 200 µmol photons m^{-2} s^{-1} and at LC410 µatm pCO_2 for 4 days. Solid line was fitted using the Michaelis-Menten equation. , and tThe values represent the mean ± standard deviation for threefour replicates.

Table 1. Carbonate chemistry parameters ~~(mean values for the beginning and end of incubations)~~ of the ~~media~~ seawater at the beginning and end of the incubations at different nutrient conditions and pCO_2 levels. TA and pH samples were collected and measured before and in the final days of the experiment.

			pCO_2 (μatm)	pH (total scale)	TA (μmol L^{-1})	DIC (μmol L^{-1})	HCO_3^- (μmol L^{-1})	CO_3^{2-} (μmol L^{-1})	CO_2 (μmol L^{-1})	Ω calcite
HNHP			435±56[a]	8.10±0.05[a]	2225±22[a]	1970±26[a]	1778±37[a]	178±17[a]	14±2[a]	4.3±0.4[a]
			970±157[b]	7.80±0.06[b]	2223±22[a]	2100±24[b]	1970±29[b]	99±14[b]	31±5[b]	2.4±0.3[b]
LN			410±52[a]	8.11±0.04[a]	2139±47[a]	1888±60[a]	1700±65[a]	172±10[a]	13±2[a]	4.1±0.2[a]
			936±143[b]	7.80±0.05[b]	2154±41[a]	2034±55[b]	1908±58[b]	96±10[b]	30±5[b]	2.3±0.2[b]
LP			372±26[a]	8.16±0.02[a]	2225±25[a]	1950±27[a]	1740±30[a]	198±8[a]	12±1[a]	4.7±0.2[a]
			852±158[b]	7.85±0.06[b]	2226±21[a]	2092±28[b]	1954±34[b]	110±15[b]	28±5[b]	2.7±0.4[b]

			pCO_2 (μatm)	pH (total scale)	TA (μmol L^{-1})	DIC (μmol L^{-1})	HCO_3^- (μmol L^{-1})	CO_3^{2-} (μmol L^{-1})	CO_2 (μmol L^{-1})	Ω calcite
HNHP	LC	Before	510±17[a]	8.04±0.01[a]	2228±17[a]	2004±20[a]	1829±21[a]	159±2[a]	16±1[a]	3.8±0.1[a]
		End	428±57[b]	8.11±0.05[b]	2225±24[a]	1967±22[b]	1773±34[b]	180±18[a]	14±2[b]	4.3±0.5[a]
	HC	Before	1210±53[a]	7.71±0.02[a]	2219±19[a]	2131±22[a]	2010±22[a]	81±2[a]	39±2[a]	1.9±0.1[a]
		End	935±139[b]	7.81±0.06[b]	2225±24[a]	2098±12[b]	1966±17[b]	102±14[b]	30±4[b]	2.4±0.3[b]
LN	LC	Before	483±23[a]	8.06±0.02[a]	2204±10[a]	1973±10[a]	1796±13[a]	162±6[a]	16±1[a]	3.9±0.1[a]
		End	391±39[b]	8.12±0.03[b]	2123±38[b]	1866±45[b]	1679±48[b]	175±9[b]	13±1[b]	4.2±0.2[b]
	HC	Before	1126±66[a]	7.73±0.02[a]	2201±3[a]	2105±7[a]	1983±9[a]	85±4[a]	36±2[a]	2.02±0.1[a]
		End	888±114[b]	7.82±0.05[b]	2142±38[b]	2016±47[b]	1890±49[b]	98±8[b]	29±4[b]	2.4±0.2[b]
LP	LC	Before	397±16[a]	8.14±0.02[a]	2248±30[a]	1982±22[a]	1777±17[a]	192±8[a]	13±1[a]	4.6±0.2[a]
		End	365±24[b]	8.16±0.02[a]	2219±20[b]	1942±22[b]	1731±25[b]	199±8[a]	12±1[b]	4.8±0.2[a]
	HC	Before	1140±110[a]	7.73±0.04[a]	2215±41[a]	2128±46[a]	2005±46[a]	86±7[a]	37±4[a]	2.1±0.2[a]
		End	780±43[b]	7.88±0.02[b]	2228±14[a]	2084±11[b]	1941±12[b]	117±6[b]	25±1[b]	2.8±0.1[b]

HNHP, 101 μmol L^{-1} dissolved inorganic nitrogen (DIN) and 10.5 μmol L^{-1} dissolved inorganic phosphate (DIP); LN, 8.8 μmol L^{-1} DIN; LP, 0.4 μmol L^{-1} DIP. Different letters indicate statistical difference between ~~two pCO_2 treatments~~ the beginning and end of the incubations within low or high pCO_2 level (Tukey Post hoc, $p < 0.01$). The values are expressed as mean ~~values~~ ± SD calculated from all light intensities ~~measurements before and in the final days of incubations~~.

Table 2. Results of three-way ANOVAs of the impacts of dissolved inorganic ~~nitrate (DIN) or phosphate (DIP)~~ nutrient concentrations~~s~~, pCO_2, light intensity and their interaction on growth rate, F_v/F_m, F_v'/F_m', POC and PIC production rates~~quotas~~, and PIC:POC ratio~~, F_v/F_m, F_v'/F_m' and ETR_{max}~~.

	~~Factor~~	~~*F* value~~	~~*p* value~~	~~Factor~~	~~*F* value~~	~~*p* value~~
~~Growth rate (d^{-1})~~	~~N~~	~~215.9~~	~~<0.001~~	~~P~~	~~1015.5~~	~~<0.001~~
	~~C~~	~~547.8~~	~~<0.001~~	~~C~~	~~213.3~~	~~<0.001~~
	~~L~~	~~1330.4~~	~~<0.001~~	~~L~~	~~1863.8~~	~~<0.001~~
	~~N×C~~	~~9.1~~	~~=0.004~~	~~P×C~~	~~147.6~~	~~<0.001~~
	~~N×L~~	~~11.8~~	~~<0.001~~	~~P×L~~	~~274.4~~	~~<0.001~~
	~~C×L~~	~~18.3~~	~~<0.001~~	~~C×L~~	~~11.1~~	~~<0.001~~
	~~N×C×L~~	~~4.1~~	~~=0.006~~	~~P×C×L~~	~~19.7~~	~~<0.001~~
~~POC quota ($pg\ C\ cell^{-1}$)~~	~~N~~	~~27.1~~	~~<0.001~~	~~P~~	~~13.7~~	~~<0.001~~
	~~C~~	~~0.6~~	~~=0.435~~	~~C~~	~~0.1~~	~~=0.731~~
	~~L~~	~~34.7~~	~~<0.001~~	~~L~~	~~103.2~~	~~<0.001~~
	~~N×C~~	~~13.2~~	~~<0.001~~	~~P×C~~	~~14.5~~	~~<0.001~~
	~~N×L~~	~~17.9~~	~~<0.001~~	~~P×L~~	~~0.4~~	~~=0.780~~
	~~C×L~~	~~1.0~~	~~=0.432~~	~~C×L~~	~~21.6~~	~~<0.001~~
	~~N×C×L~~	~~1.9~~	~~=0.125~~	~~P×C×L~~	~~7.3~~	~~<0.001~~
~~PIC quota ($pg\ C\ cell^{-1}$)~~	~~N~~	~~544.0~~	~~<0.001~~	~~P~~	~~619.1~~	~~<0.001~~
	~~C~~	~~70.5~~	~~<0.001~~	~~C~~	~~105.8~~	~~<0.001~~
	~~L~~	~~71.2~~	~~<0.001~~	~~L~~	~~55.3~~	~~<0.001~~
	~~N×C~~	~~2.8~~	~~=0.098~~	~~P×C~~	~~6.3~~	~~=0.015~~
	~~N×L~~	~~7.0~~	~~<0.001~~	~~P×L~~	~~9.7~~	~~<0.001~~
	~~C×L~~	~~11.4~~	~~<0.001~~	~~C×L~~	~~2.2~~	~~=0.078~~
	~~N×C×L~~	~~0.6~~	~~=0.639~~	~~P×C×L~~	~~7.0~~	~~<0.001~~
~~PIC:POC ratio~~	~~N~~	~~934.6~~	~~<0.001~~	~~P~~	~~395.0~~	~~<0.001~~
	~~C~~	~~81.8~~	~~<0.001~~	~~C~~	~~9.1~~	~~=0.004~~
	~~L~~	~~30.9~~	~~<0.001~~	~~L~~	~~47.6~~	~~<0.001~~
	~~N×C~~	~~6.6~~	~~=0.013~~	~~P×C~~	~~13.4~~	~~<0.001~~
	~~N×L~~	~~9.8~~	~~<0.001~~	~~P×L~~	~~14.4~~	~~<0.001~~
	~~C×L~~	~~6.8~~	~~<0.001~~	~~C×L~~	~~1.5~~	~~=0.202~~
	~~N×C×L~~	~~0.7~~	~~=0.567~~	~~P×C×L~~	~~4.7~~	~~=0.002~~
~~F_v/F_m~~	~~N~~	~~335.8~~	~~<0.001~~	~~P~~	~~171.2~~	~~<0.001~~
	~~C~~	~~1.5~~	~~=0.229~~	~~C~~	~~189.6~~	~~<0.001~~
	~~L~~	~~246.7~~	~~<0.001~~	~~L~~	~~153.9~~	~~<0.001~~
	~~N×C~~	~~16.1~~	~~<0.001~~	~~P×C~~	~~34.8~~	~~<0.001~~
	~~N×L~~	~~4.8~~	~~=0.002~~	~~P×L~~	~~13.8~~	~~<0.001~~
	~~C×L~~	~~12.6~~	~~<0.001~~	~~C×L~~	~~10.7~~	~~<0.001~~
	~~N×C×L~~	~~4.6~~	~~=0.003~~	~~P×C×L~~	~~2.6~~	~~=0.048~~

~~F_v'/F_m'~~	~~N~~	~~10.1~~	~~=0.002~~	~~P~~	~~675.4~~	~~<0.001~~
	~~C~~	~~33.6~~	~~<0.001~~	~~C~~	~~134.0~~	~~<0.001~~
	~~L~~	~~670.5~~	~~<0.001~~	~~L~~	~~1007.7~~	~~<0.001~~
	~~N×C~~	~~11.7~~	~~=0.001~~	~~P×C~~	~~195.5~~	~~<0.001~~
	~~N×L~~	~~3.4~~	~~=0.014~~	~~P×L~~	~~22.8~~	~~<0.001~~
	~~C×L~~	~~14.6~~	~~<0.001~~	~~C×L~~	~~8.2~~	~~<0.001~~
	~~N×C×L~~	~~12.6~~	~~<0.001~~	~~P×C×L~~	~~3.5~~	~~=0.012~~
~~ETR_{max}~~ ~~(mol e^- g^{-1} Chl *a* h^{-1})~~	~~N~~	~~811.2~~	~~<0.001~~	~~P~~	~~335.2~~	~~<0.001~~
	~~C~~	~~67.9~~	~~<0.001~~	~~C~~	~~71.3~~	~~<0.001~~
	~~L~~	~~176.6~~	~~<0.001~~	~~L~~	~~625.4~~	~~<0.001~~
	~~N×C~~	~~11.2~~	~~=0.001~~	~~P×C~~	~~20.2~~	~~<0.001~~
	~~N×L~~	~~15.3~~	~~<0.001~~	~~P×L~~	~~151.0~~	~~<0.001~~
	~~C×L~~	~~4.8~~	~~=0.002~~	~~C×L~~	~~35.1~~	~~<0.001~~
	~~N×C×L~~	~~12.7~~	~~<0.001~~	~~P×C×L~~	~~9.4~~	~~<0.001~~

1202

	Factor	*F* value	*p* value
Growth rate (d^{-1})	Nut	264.7	<0.01
	C	875.6	<0.01
	L	2035.8	<0.01
	Nut×C	53.6	<0.01
	Nut×L	84.2	<0.01
	C×L	9.3	<0.01
	Nut×C×L	26.8	<0.01
F_v/F_m	Nut	68.6	<0.01
	C	184.7	<0.01
	L	225.8	<0.01
	Nut×C	10.3	<0.01
	Nut×L	8.1	<0.01
	C×L	15	<0.01
	Nut×C×L	5.2	<0.01
F_v'/F_m'	Nut	63.9	<0.01
	C	181.8	<0.01
	L	1161.8	<0.01
	Nut×C	51.9	<0.01
	Nut×L	15.3	<0.01
	C×L	9.9	<0.01
	Nut×C×L	8.1	<0.01
POC production rate (pg C $cell^{-1}$ d^{-1})	Nut	11.8	<0.01
	C	128.9	<0.01
	L	293.7	<0.01
	Nut×C	4.9	=0.01
	Nut×L	19.0	<0.01
	C×L	8.47	<0.01

	Nut×C×L	1.94	=0.06
PIC production rate (pg C cell^{-1} d^{-1})	Nut	624.4	<0.01
	C	142.0	<0.01
	L	147.2	<0.01
	Nut×C	1.9	=0.16
	Nut×L	17.3	<0.01
	C×L	8.1	<0.01
	Nut×C×L	4.6	<0.01
PIC:POC ratio	Nut	326.7	<0.01
	C	57.7	<0.01
	L	41.8	<0.01
	Nut×C	8.3	<0.01
	Nut×L	12.5	<0.01
	C×L	4.0	<0.01
	Nut×C×L	3.3	<0.01

~~N, dissolved inorganic nitrogen (DIN, μmol L^{-1}); P, dissolved inorganic phosphate (DIP, μmol L^{-1})~~ Nut, dissolved inorganic nutrient concentrations (μmol L^{-1}); C, pCO_2 (μatm); L, light intensity (μmol photons m^{-2} s^{-1}); POC and POC production rates ~~quota~~, particulate organic and inorganic carbon ~~content~~production rates; ~~PIC quota, particulate inorganic carbon content;~~ F_v/F_m, maximum photochemical quantum yield; F_v'/F_m', effective photochemical quantum yield~~; ETR_{max}, maximum electron transport rate~~.

Table 3. Experimental treatments, growth rate, F_v/F_m, F_v'/F_m', particulate organic (POC) and inorganic carbon (PIC) production rates, and PIC:POC ratio ~~carbon quotas, photosynthesis parameter~~ in dilute bath cultures.

~~Initial N/P~~	~~pCO_2~~	~~L~~	~~Growth rate~~	~~POC quota~~	~~PIC quota~~	~~PIC: POC~~	~~F_v/F_m~~	~~F_v'/F_m'~~	~~ETR_{max}~~
~~101/ 10.5~~	~~435~~	~~80~~	~~1.11(0.02)~~	~~8.8(0.5)~~	~~1.6(0.4)~~	~~0.19(0.05)~~	~~0.59(0.01)~~	~~0.58(0.03)~~	~~1.25(0.07)~~
		~~120~~	~~1.21(0.03)~~	~~9.1(0.3)~~	~~2.3(0.7)~~	~~0.25(0.08)~~	~~0.55(0.00)~~	~~0.54(0.01)~~	~~1.52(0.12)~~
		~~200~~	~~1.37(0.02)~~	~~8.5(0.6)~~	~~2.8(0.7)~~	~~0.33(0.08)~~	~~0.55(0.01)~~	~~0.48(0.01)~~	~~1.65(0.02)~~
		~~320~~	~~1.29(0.03)~~	~~9.7(1.0)~~	~~5.0(1.3)~~	~~0.52(0.16)~~	~~0.47(0.03)~~	~~0.37(0.03)~~	~~1.58(0.09)~~
		~~480~~	~~1.17(0.03)~~	~~12.3(0.7)~~	~~3.5(0.4)~~	~~0.28(0.04)~~	~~0.45(0.06)~~	~~0.31(0.02)~~	~~1.63(0.06)~~
	~~970~~	~~80~~	~~1.06(0.01)~~	~~7.7(0.4)~~	~~0.9(0.1)~~	~~0.12(0.02)~~	~~0.58(0.01)~~	~~0.57(0.02)~~	~~1.16(0.01)~~
		~~120~~	~~1.19(0.03)~~	~~8.9(0.2)~~	~~2.2(0.4)~~	~~0.25(0.04)~~	~~0.54(0.01)~~	~~0.52(0.01)~~	~~1.69(0.16)~~
		~~200~~	~~1.32(0.01)~~	~~8.2(0.7)~~	~~2.3(0.4)~~	~~0.28(0.06)~~	~~0.53(0.01)~~	~~0.47(0.01)~~	~~1.61(0.01)~~
		~~320~~	~~1.21(0.02)~~	~~9.9(0.8)~~	~~2.9(0.7)~~	~~0.30(0.09)~~	~~0.49(0.03)~~	~~0.37(0.02)~~	~~1.60(0.09)~~
		~~480~~	~~1.16(0.01)~~	~~11.7(1.2)~~	~~1.7(0.4)~~	~~0.14(0.02)~~	~~0.33(0.03)~~	~~0.28(0.02)~~	~~1.24(0.1)~~
~~8.8/ 10.5~~	~~410~~	~~80~~	~~1.08(0.01)~~	~~7.3(0.4)~~	~~2.9(0.6)~~	~~0.39(0.09)~~	~~0.59(0.01)~~	~~0.58(0.01)~~	~~1.44(0.04)~~
		~~120~~	~~1.21(0.01)~~	~~8.4(0.4)~~	~~4.7(0.9)~~	~~0.57(0.12)~~	~~0.57(0.00)~~	~~0.55(0.01)~~	~~2.03(0.11)~~
		~~200~~	~~1.31(0.01)~~	~~8.1(0.3)~~	~~5.9(0.8)~~	~~0.74(0.08)~~	~~0.59(0.01)~~	~~0.53(0.01)~~	~~2.50(0.15)~~
		~~320~~	~~1.29(0.01)~~	~~9.9(0.4)~~	~~8.7(0.7)~~	~~0.87(0.07)~~	~~0.45(0.04)~~	~~0.37(0.04)~~	~~2.10(0.07)~~
		~~480~~	~~1.12(0.02)~~	~~7.9(0.8)~~	~~6.8(0.8)~~	~~0.87(0.17)~~	~~0.41(0.03)~~	~~0.35(0.04)~~	~~1.69(0.14)~~
	~~936~~	~~80~~	~~1.00(0.01)~~	~~7.8(0.3)~~	~~2.4(0.7)~~	~~0.31(0.11)~~	~~0.59(0.01)~~	~~0.57(0.01)~~	~~1.66(0.04)~~
		~~120~~	~~1.11(0.01)~~	~~8.9(0.5)~~	~~4.3(0.3)~~	~~0.48(0.04)~~	~~0.55(0.01)~~	~~0.54(0.02)~~	~~1.86(0.06)~~
		~~200~~	~~1.25(0.01)~~	~~8.3(0.5)~~	~~5.6(0.8)~~	~~0.68(0.09)~~	~~0.54(0.01)~~	~~0.44(0.01)~~	~~2.35(0.16)~~
		~~320~~	~~1.21(0.01)~~	~~9.7(0.2)~~	~~5.4(0.4)~~	~~0.56(0.05)~~	~~0.50(0.01)~~	~~0.41(0.03)~~	~~2.00(0.08)~~
		~~480~~	~~1.06(0.06)~~	~~7.2(1.1)~~	~~4.2(0.6)~~	~~0.54(0.06)~~	~~0.37(0.02)~~	~~0.33(0.04)~~	~~1.76(0.15)~~
~~101/ 0.4~~	~~372~~	~~80~~	~~1.00(0.02)~~	~~8.7(0.3)~~	~~3.2(0.5)~~	~~0.36(0.06)~~	~~0.59(0.01)~~	~~0.55(0.01)~~	~~1.01(0.05)~~
		~~120~~	~~1.24(0.01)~~	~~8.3(0.2)~~	~~4.2(0.4)~~	~~0.51(0.05)~~	~~0.59(0.01)~~	~~0.55(0.01)~~	~~1.58(0.04)~~
		~~200~~	~~1.39(0.01)~~	~~8.1(0.3)~~	~~5.3(0.5)~~	~~0.66(0.09)~~	~~0.56(0.01)~~	~~054(0.02)~~	~~2.10(0.06)~~
		~~320~~	~~1.31(0.02)~~	~~9.6(0.5)~~	~~4.1(0.6)~~	~~0.43(0.08)~~	~~0.47(0.02)~~	~~0.38(0.01)~~	~~1.85(0.06)~~
		~~480~~	~~1.18(0.05)~~	~~10.8(0.6)~~	~~2.7(0.5)~~	~~0.25(0.03)~~	~~0.38(0.08)~~	~~0.29(0.04)~~	~~1.61(0.18)~~
	~~852~~	~~80~~	~~0.97(0.02)~~	~~6.9(0.5)~~	~~2.6(0.4)~~	~~0.38(0.04)~~	~~0.58(0.01)~~	~~0.54(0.02)~~	~~0.91(0.03)~~
		~~120~~	~~1.08(0.01)~~	~~9.0(0.1)~~	~~3.7(0.7)~~	~~0.41(0.07)~~	~~0.55(0.01)~~	~~0.49(0.01)~~	~~1.29(0.02)~~
		~~200~~	~~1.27(0.01)~~	~~8.1(0.1)~~	~~4.0(0.3)~~	~~0.49(0.04)~~	~~0.55(0.01)~~	~~0.51(0.02)~~	~~2.16(0.07)~~
		~~320~~	~~1.22(0.01)~~	~~8.6(0.1)~~	~~3.1(0.4)~~	~~0.36(0.05)~~	~~0.47(0.03)~~	~~0.37(0.03)~~	~~2.18(0.09)~~
		~~480~~	~~0.90(0.01)~~	~~12.8(0.6)~~	~~3.5(0.6)~~	~~0.28(0.06)~~	~~0.25(0.03)~~	~~0.17(0.01)~~	~~1.21(0.09)~~

Initial N/P	pCO_2	L	Growth rate	F_v/F_m	$F_v^{'}/F_m^{'}$	POC/cell/d	PIC/cell/d	PIC:POC
101/10.5	439	80	1.11(0.02)	0.59(0.01)	0.58(0.03)	9.70(0.45)	1.81(0.43)	0.19(0.05)
		120	1.21(0.03)	0.55(0.00)	0.54(0.01)	11.03(0.28)	2.80(0.88)	0.25(0.08)
		200	1.37(0.02)	0.55(0.01)	0.48(0.01)	11.67(0.71)	3.82(0.97)	0.33(0.08)
		320	1.29(0.03)	0.47(0.03)	0.37(0.03)	12.59(1.35)	6.44(1.67)	0.52(0.16)
		480	1.17(0.03)	0.45(0.06)	0.31(0.02)	14.54(0.89)	4.06(0.47)	0.28(0.04)
	973	80	1.06(0.01)	0.58(0.01)	0.57(0.02)	8.25(0.30)	0.99(0.14)	0.12(0.02)
		120	1.19(0.03)	0.54(0.01)	0.52(0.01)	10.50(0.19)	2.65(0.39)	0.25(0.04)
		200	1.32(0.01)	0.53(0.01)	0.47(0.01)	10.74(1.06)	3.02(0.61)	0.28(0.06)
		320	1.21(0.02)	0.49(0.03)	0.37(0.02)	12.04(0.91)	3.55(0.92)	0.30(0.09)
		480	1.16(0.01)	0.33(0.03)	0.28(0.02)	13.50(1.32)	2.02(0.50)	0.14(0.02)
8.8/10.5	409	80	1.08(0.01)	0.59(0.01)	0.58(0.01)	7.93(0.39)	3.08(0.61)	0.39(0.09)
		120	1.21(0.01)	0.57(0.00)	0.55(0.01)	10.26(0.40)	5.78(1.10)	0.57(0.12)
		200	1.31(0.01)	0.59(0.01)	0.53(0.01)	10.60(0.30)	7.81(1.00)	0.74(0.08)
		320	1.29(0.01)	0.45(0.04)	0.37(0.04)	12.76(0.47)	11.17(1.10)	0.87(0.07)
		480	1.12(0.02)	0.41(0.03)	0.35(0.04)	8.84(0.91)	7.60(0.85)	0.87(0.17)
	936	80	1.00(0.01)	0.59(0.01)	0.57(0.01)	7.85(0.37)	2.39(0.74)	0.31(0.11)
		120	1.11(0.01)	0.55(0.01)	0.54(0.02)	9.89(0.53)	4.76(0.35)	0.48(0.04)
		200	1.25(0.01)	0.54(0.01)	0.44(0.01)	10.37(0.60)	7.02(0.94)	0.68(0.09)
		320	1.21(0.01)	0.50(0.01)	0.41(0.03)	11.73(0.20)	6.53(0.53)	0.56(0.05)
		480	1.06(0.06)	0.37(0.02)	0.33(0.04)	8.44(0.57)	5.63(2.17)	0.54(0.06)
101/0.4	371	80	1.00(0.02)	0.59(0.01)	0.55(0.01)	8.74(0.33)	3.15(0.46)	0.36(0.06)
		120	1.24(0.01)	0.59(0.01)	0.55(0.01)	10.23(0.23)	5.22(0.45)	0.51(0.05)
		200	1.39(0.01)	0.56(0.01)	054(0.02)	11.22(0.41)	7.35(0.97)	0.66(0.09)
		320	1.31(0.02)	0.47(0.02)	0.38(0.01)	12.67(0.78)	5.42(0.71)	0.43(0.08)
		480	1.18(0.05)	0.38(0.08)	0.29(0.04)	12.84(0.84)	3.26(0.58)	0.25(0.03)
	852	80	0.97(0.02)	0.58(0.01)	0.54(0.02)	6.66(0.42)	2.51(0.33)	0.38(0.04)
		120	1.08(0.01)	0.55(0.01)	0.49(0.01)	9.72(0.22)	3.96(0.74)	0.41(0.07)
		200	1.27(0.01)	0.55(0.01)	0.51(0.02)	10.33(0.19)	5.09(0.34)	0.49(0.04)
		320	1.22(0.01)	0.47(0.03)	0.37(0.03)	10.57(0.19)	3.76(0.49)	0.36(0.05)
		480	0.90(0.01)	0.25(0.03)	0.17(0.01)	11.57(0.49)	3.19(0.56)	0.28(0.06)

Initial N/P, the ratio of dissolved inorganic nitrogen to phosphate at the beginning of experiment~~; L, light intensity (μmol photons m^{-2} s^{-1}). See Table 2 for detailed~~

~~information.~~ More detailed information is given as in Table 2. Data in the brackets are the standard deviations for four replicates.

Table 4. Results of two-way ANOVAs of the effects of dissolved inorganic nutrient concentration and pCO_2 on fitted a and maximum value (V_{max}) of growth, POC and PIC production rates. More detailed information is given as in Table 2.

		Factor	F value	p value
a	Growth rate	Nut	18.08	<0.001
		CO_2	0.186	0.6711
		Nut×CO_2	0.398	0.6776
	POC production rate	Nut	7.21	0.005
		CO_2	7.78	0.0121
		Nut×CO_2	2.50	0.11
	PIC production rate	Nut	21.73	<0.001
		CO_2	2.32	0.145
		Nut×CO_2	2.56	0.105
V_{max}	Growth rate	Nut	24.9	<0.001
		CO_2	572.7	<0.001
		Nut×CO_2	14.8	<0.001
	POC production rate	Nut	7.301	0.0048
		CO_2	15.95	0.0009
		Nut×CO_2	1.91	0.177
	PIC production rate	Nut	56.06	<0.001
		CO_2	86.84	<0.001
		Nut×CO_2	0.168	0.85

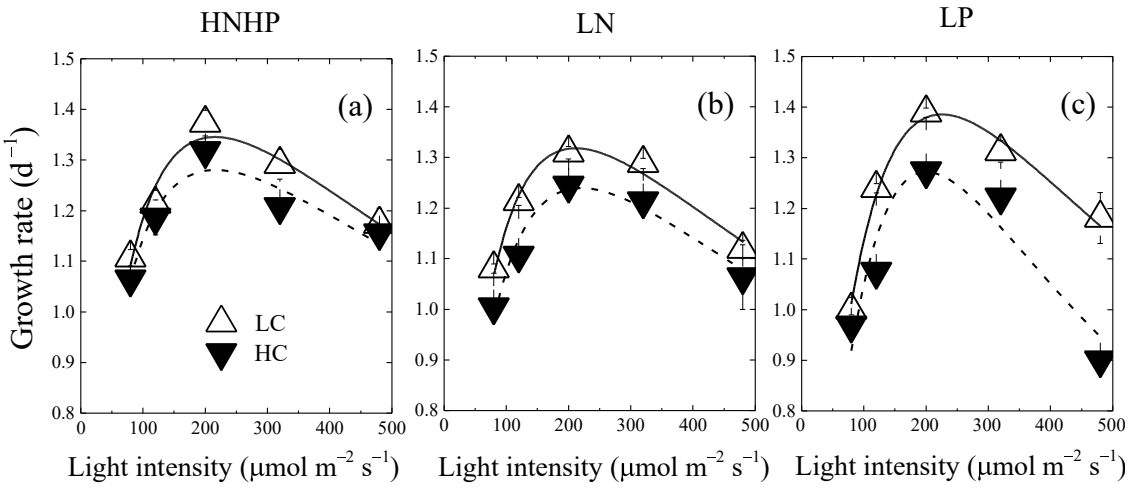

HNHP
LN
LP
(a)
(b)
(c)
Growth rate (d^{-1})
LC
HC
Light intensity (μmol m^{-2} s^{-1})
Light intensity (μmol m^{-2} s^{-1})
Light intensity (μmol m^{-2} s^{-1})

Figure 1

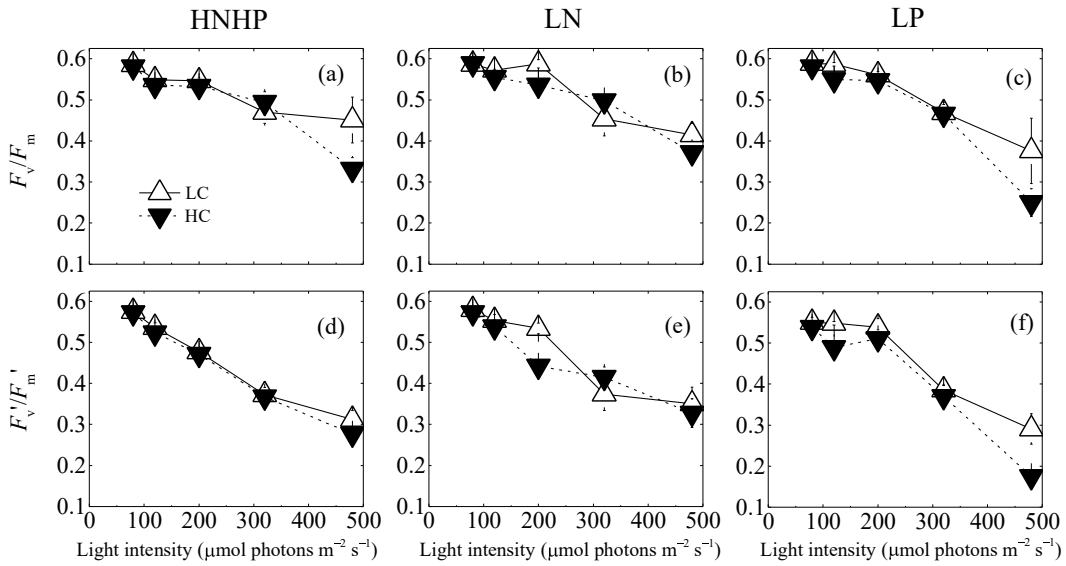

HNHP
LN
LP
(a)
(b)
(c)
(d)
(e)
(f)
LC
HC
F_v/F_m
F_v'/F_m'
0.1
0.2
0.3
0.4
0.5
0.6
0
100
200
300
400
500
Light intensity (μmol photons m^{-2} s^{-1})

Figure 2

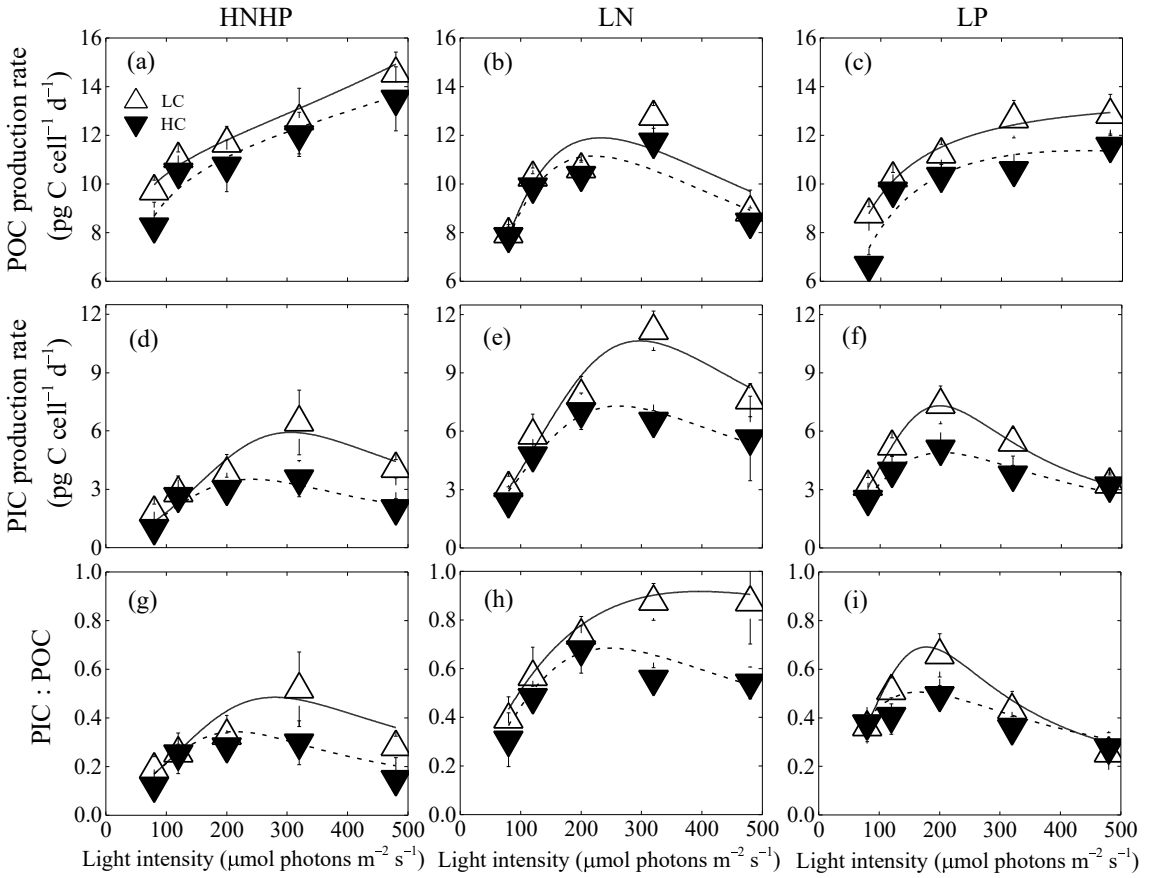
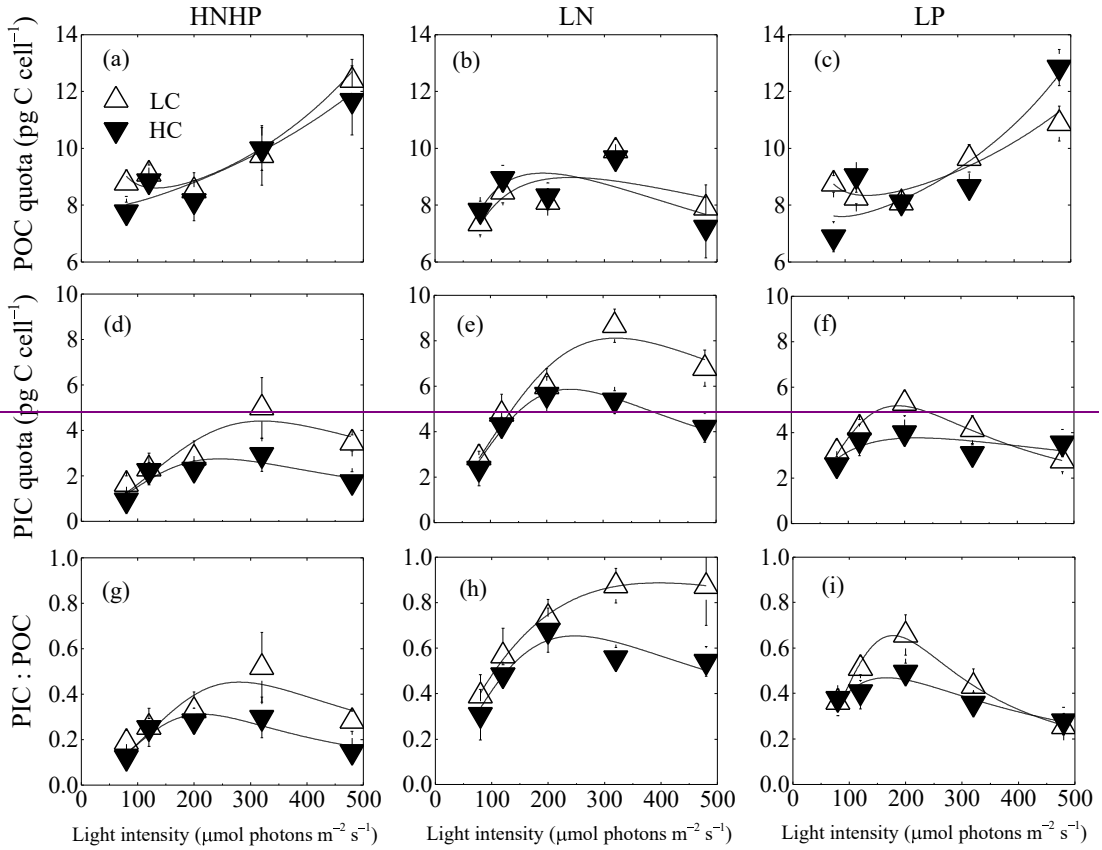
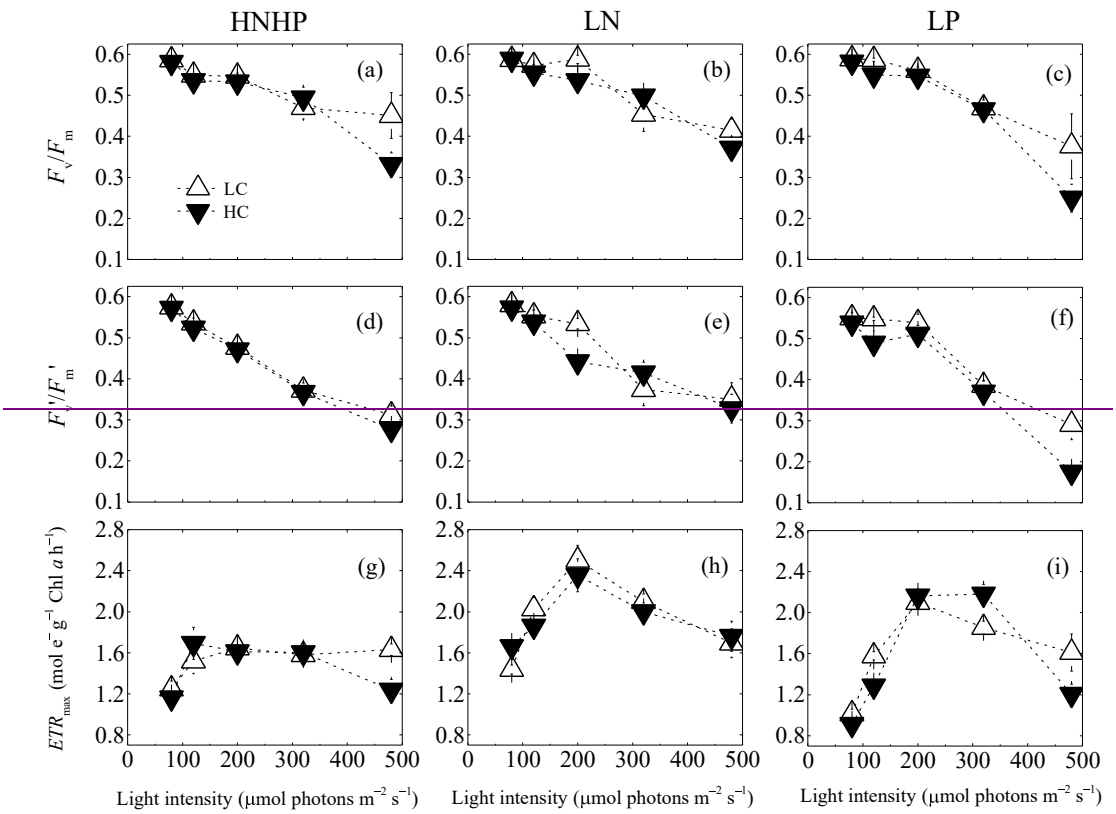

HNHP
LN
LP
(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
(i)
LC
HC
POC production rate (pg C cell^{-1} d^{-1})
PIC production rate (pg C cell^{-1} d^{-1})
PIC : POC
Light intensity (µmol photons m^{-2} s^{-1})
Light intensity (µmol photons m^{-2} s^{-1})
Light intensity (µmol photons m^{-2} s^{-1})

Figure 3


HNHP
LN
LP
(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
(i)
LC
HC
POC quota (pg C cell^{-1})
PIC quota (pg C cell^{-1})
PIC : POC
Light intensity (µmol photons m^{-2} s^{-1})
Light intensity (µmol photons m^{-2} s^{-1})
Light intensity (µmol photons m^{-2} s^{-1})

~~Figure 2~~


HNHP
LN
LP
(a)
(b)
(c)
(d)
(e)
(f)
(g)
(h)
(i)
LC
HC
F_v/F_m
F_v'/F_m'
ETR_{max} (mol e^- g^{-1} Chl a h^{-1})
Light intensity (μmol photons m^{-2} s^{-1})

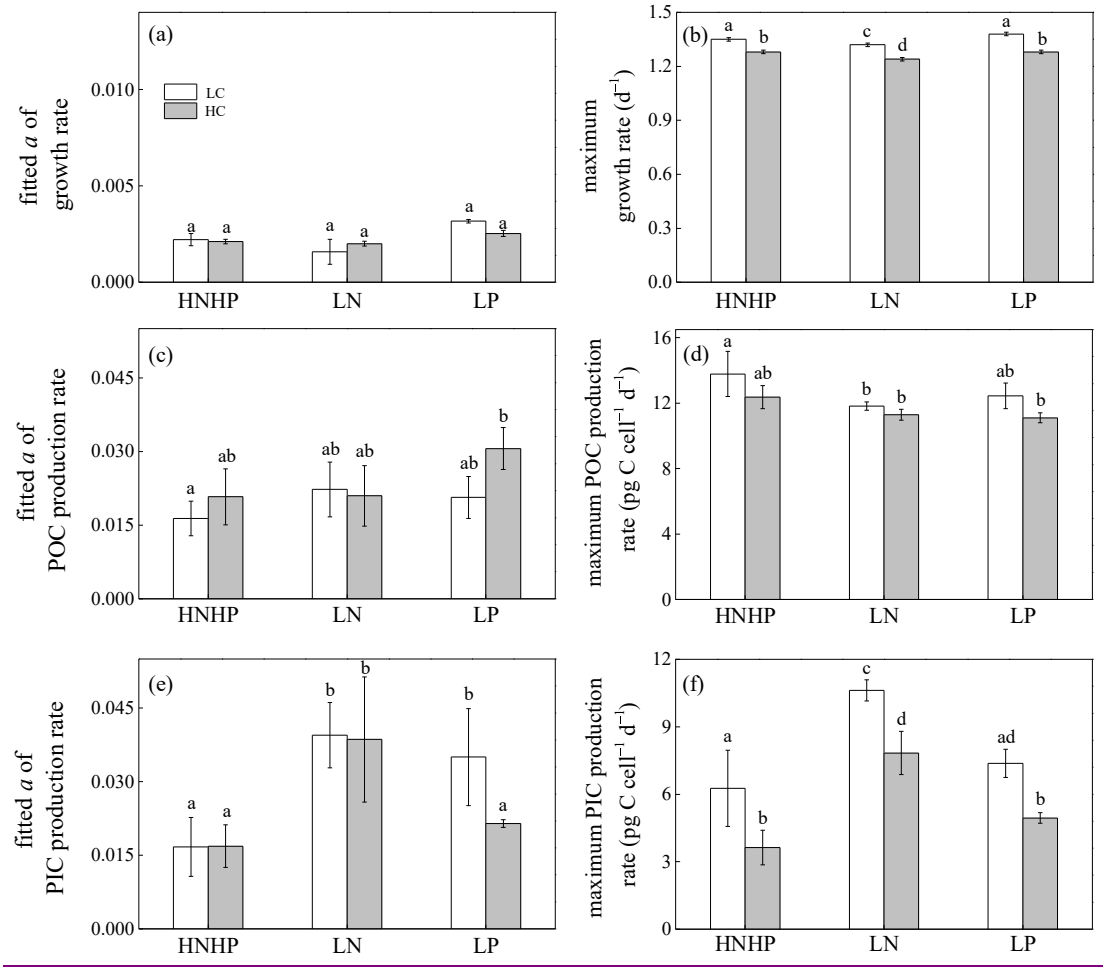

(a)
fitted a of growth rate
LC
HC
0.010
0.005
0.000
HNHP
LN
LP
(b)
maximum growth rate (d⁻¹)
1.5
1.2
0.9
0.6
0.3
0.0
(c)
fitted a of POC production rate
0.045
0.030
0.015
(d)
maximum POC production rate (pg C cell⁻¹ d⁻¹)
16
12
8
4
0
(e)
fitted a of PIC production rate
(f)
maximum PIC production rate (pg C cell⁻¹ d⁻¹)
12
9
6
3

Figure 4

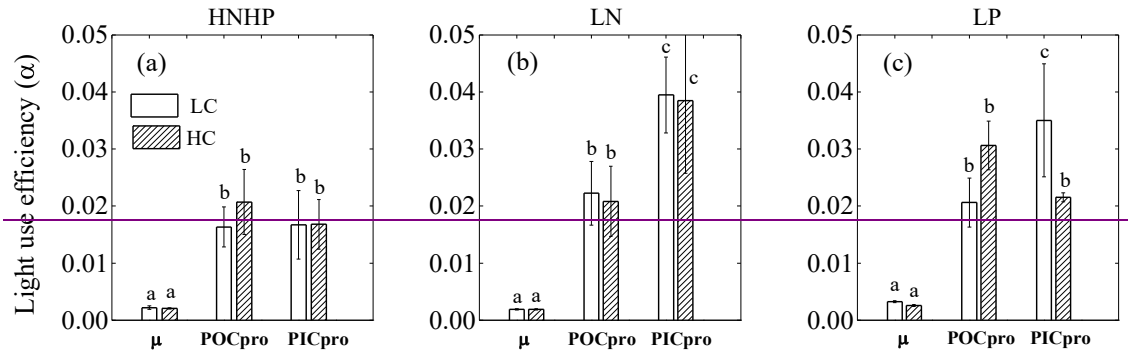
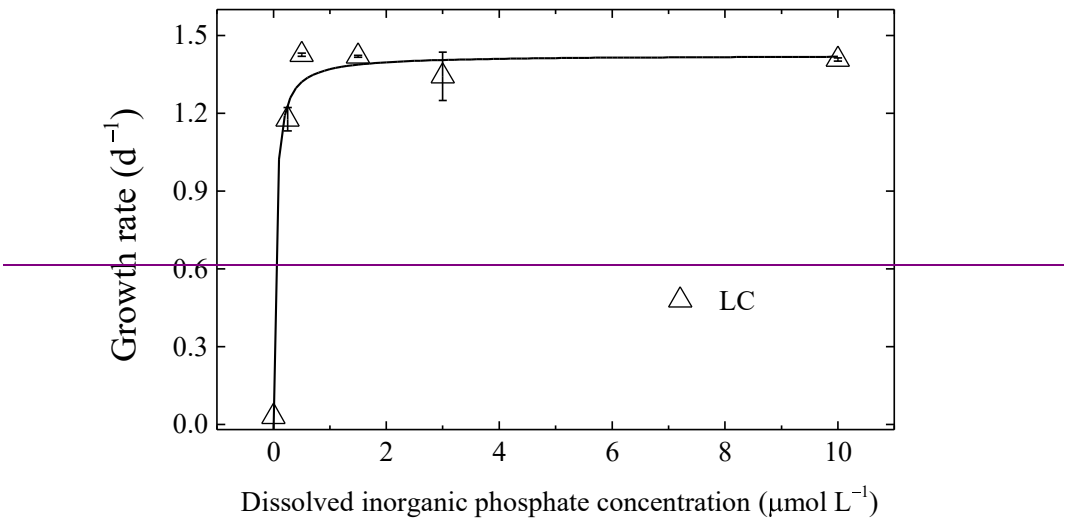

HNHP
LN
LP
(a)
(b)
(c)
LC
HC
Light use efficiency (α)
0.05
0.04
0.03
0.02
0.01
0.00
a a
b b b b
a a
b b c c
a a
b b c b
μ
POCpro
PICpro

Figure 4


Growth rate (d^{-1})
1.5
1.2
0.9
0.6
0.3
0.0
0
2
4
6
8
10
LC
Dissolved inorganic phosphate concentration (μmol L^{-1})

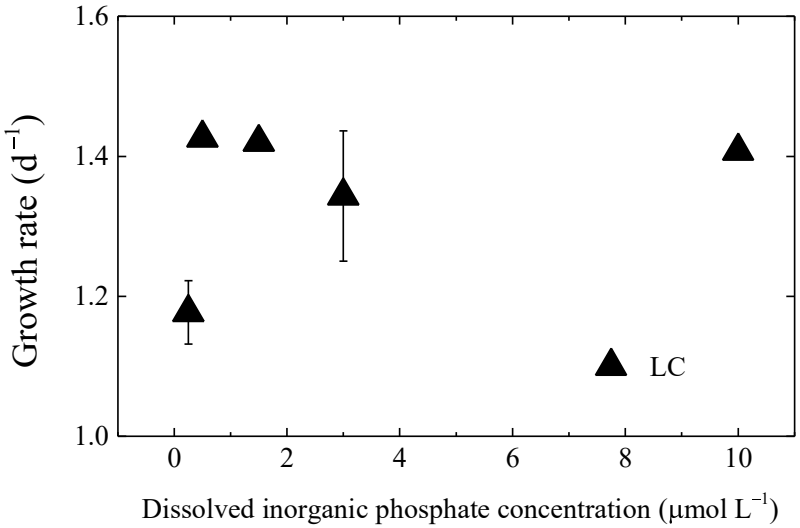

1.6
1.4
1.2
1.0
Growth rate (d^{-1})
0
2
4
6
8
10
Dissolved inorganic phosphate concentration (μmol L^{-1})
LC

Figure 5