

Dear reviewers and editors,

The authors would like to thank the reviewers for the helpful comments provided in order to improve
5 the manuscript.

The manuscript has been carefully checked and revised based on the reviewer's comments. The responses to the detailed comments are listed below.

Response to RC1:

10 *“MAJOR COMMENTS:*

*Results: I am concerned about the “cellular content response (POC, PON, POP) to environmental drivers”. Organic matter quotas are strongly determined by the cell cycle. POC/cell, for example, will be much lower directly after cell division than right before. Thus, you can only compare cell quotas among treatments, when you are sure that all treatments were in the same cell cycle stage. The Authors
15 do not indicate if samples were taken at the same time. This information would be a step forward because it could then at least be assumed that cell division was synchronized during night. However, even if sampling times were identical, it remains questionable if this assumption is valid for every treatment. Growth rates are not reported here but I assume that they are below 0.69, at least in some treatments (e.g. the low temperature treatment). If the cells divide less than once per day and only
20 divide during night, it means that some cells of the population are packed with POC while others are depleted. Since you only sample once at an unknown cell cycle stage, it may become difficult (if not impossible) to disentangle the cell cycle-specific response from the actual treatment response. I therefore think that the results on cell quotas presented here (but also elsewhere in the literature) could potentially be misleading.*

25 *My suggestion would be to show production rates ($\mu \times$ cell quota) instead of cell quotas. These also have theoretical issues but should more robust. ”*

The authors agree that the cellular elemental quotas depend on the cell cycles. The samples in our study are taken during the same time window so that most of the cells were in the same stage during sampling.

The information was also added in the results section as “Samples were collected for cell counts, Chl-*a* biomass, and elemental components, including particulate organic carbon (POC), particulate inorganic carbon (PIC), particulate organic nitrogen (PON), and particulate organic phosphorus (POP), starting 2 hours after the beginning of the light incubation phase and finishing within 2 hours for all the experimental treatments”. In addition, the cells were examined under the microscope; there were no significantly enlarged cells in division observed, even at the lowest temperature. Therefore, the results of the elemental compositions presented in our study are comparable among different treatments.

ADDITIONAL COMMENTS:

10 *“Page 2 Line 1: the “each” could probably be removed.”*

The word “each” has been removed.

“Page 2 Line 1: perhaps remove “cellular” because PIC is extracellular.”

The word “cellular” has been removed.

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“Page 2 Line 2: “implications for coccolithophore biogeochemistry”. This is a rather vague formulation. What is coccolithophore biogeochemistry? Do you mean the influence coccolithophores have on biogeochemical cycles? I think a bit more precision would improve the final sentence? Do you mean their influence on the nutrient cycle? Carbon export?”

20 The text has been revised to “...with wide-reaching implications for coccolithophore related marine biogeochemical cycles...”.

“Section 2.1 provides a thorough description of the culturing methodology. One crucial information should be added, however. Were all samples taken at the same time (within an appropriate time window, e.g. ~2 hours)? This is important because cell quotas change over the day and these can only be compared when all treatments were in the same cell cycle state when sampled (see also MAJOR COMMENT).”

All the samples from each manipulation experiments were collected in a similar and appropriate time window. This description has been added in the results section as stated above.

“Page 6 Line 5: Agreed but a reference for this statement would probably be useful.”

5 A reference has been added.

“Page 12 Line 10: “with lowest nitrate and phosphate concentrations of 3.6 and 0.4 μM , respectively”. In this case, your results may not really be comparable to Paasche’s and others. Your nutrient concentrations were not leading to zero growth whereas those of Paasche et al were.”

10 The authors agree that the lowest nutrient concentrations were not as low (leading to zero) as those in the cited references. However, here we made the comparisons only to point out the difference of the results we observed in our study and those under nutrient depleted conditions. It is stated in the manuscript that “the present study used a semi-continuous incubation method with higher and relatively steady nutrient concentrations (with lowest nitrate and phosphate concentrations of 3.6 and 0.4 μM ,
15 respectively) and the cells were grown and sampled at a healthy exponential growth phase”. And thus “further studies at extremely low nutrient concentrations ($<0.1 \mu\text{M}$) in a steady-state growth phase are still needed to understand the potential connection between carbon production and extreme nutrient limitation”.

20 *“Page 13 Line 26: check spelling of ‘cell’.”*

The original typo has been corrected.

“Page 13 Line 26: It is unclear in this sentence whether you measured cell size or you refer to earlier results. Please clarify.”

25 The new supplemental figure (Fig. S1) has been added in order to provide the cell size information from the temperature experiments.

“Page 15 Line 1: This final speculation in the temperature section is a bit too extreme. It became clearer during the last couple of years that extrapolations from the (monoclonal) bottle to the global ocean should be avoided since way too many factors (e.g. ecology) are neglected.”

The last sentence has been revised to “Similarly, Toseland et al. (2013) suggested that future warming might accentuate nitrate limitation in the oceans” to avoid over extrapolations from our bottle incubation experiments.

“Page 15 Line 9: ‘In general, cell growth of E. huxleyi is less limited by low CO₂ concentrations than in other phytoplankton groups (Clark and Flynn, 2000; Paasche et al., 1996; Riebesell et al., 2000a).’ This statement implies that E. huxleyi would have a particularly efficient CCM but is this supported by the evidence provided in the cited references? I suggest to check the MIMS-based papers by for example Björn Rost’s group because these provide K_{1/2} values for carbon uptake and they have investigated quite a number of different species that can be compared with E. huxleyi.”

The reference of Rost et al. (2003) that examined the K_{1/2} values for carbon uptake of several phytoplankton species is now cited in the revised manuscript.

“Page 15 Line 27: I do not understand where the ‘both’ is referring to.”

The word “both” refers to the two parameters 1. cellular PIC:POC ratio in the present manuscript and 2. the ratio of calcification rate vs. photosynthesis rate in Feng et al. (2017) being commonly used in research papers to indicate the relative change of PIC vs. POC production in coccolithophores, and thus they have implications for the marine rain ratio.

“Page 15 Line 27: ‘ecological implications’? Do you mean ‘biogeochemical implications’?”

The word “ecological” has been revised to “biogeochemical”.

“Page 16 Line 2: Confusion: The ¹⁴C measurement is not referring to your study, or is it? ¹⁴C measurements have not been described in the methods or did I miss something?”

The paper Feng et al. (2017) is now referred to in the text.

“Page 16 Line 5: Reference missing in the reference list. (Please check the entire list since there some others missing as well).”

The missing references are thoroughly checked and added in the reference list.

5

“Page 16 Line 25: Semicolon”

The semicolon has been changed to comma.

10 “Page 17 Line 16: ‘...future research on a full environmental matrix is still necessary.’ It would be valuable to add that the goal of such a matrix should not be to simply combine different factors and then use the outcome to extrapolate it to the future. The goal of culture studies should be to understand the underlying mechanisms of synergistic effects. For example: ‘How does the light intensity modify the temperature response and why?’”

The goal of these research on full environmental matrix has been added in the revised manuscript as:
15 “These experiments will not only help to further explore the potential interactions (i.e. synergistic or agnostic effects) between environmental drivers, but also provide a better understanding of the underlying mechanisms of these interactive effects”.

20 “Page 18 Lines 14-17: I am not so sure about the final conclusion and the concomitant suggestion. If we design experiments to mimic anticipated physico-chemical conditions of the future as close as possible than the results can in most cases only be used to project findings from a culture experiment to the global ocean in a one to one manner. This, however, is questionable since many factors in that can significantly modify the outcomes are neglected in the experiment. Perhaps it may be more sustainable to suggest that experimentalists should design experiments in such a way that underlying mechanisms
25 for synergistic effects can be understood.”

The authors agree that there are many factors in the oceanic environments neglected in our experiment. However, it is a general limitation of laboratory manipulation experiments. Our manipulation experiments focused on the single driver effects, which provide some helpful diagnostic information for

further explaining the interactive effects of multiple drivers. As such, the final conclusions have been further extended as: “For future multi-factorial manipulation experimental designs, our results suggest that the magnitudes of change in each environmental driver need to be determined/decided cautiously and should have environmental relevance in order to make more accurate predictions, and the understanding of interactive effects of multiple environmental drivers and the underlying mechanisms should be further explored.”.

“Figure 7: Perhaps rather call it conceptual figure. Furthermore, were abbreviations “Q” defined in the text?”

The figure legend has been changed to “conceptual figure”, and the abbreviation of “Q” has also been defined as cellular quota.

Response to RC2:

Response to the general comments:

“Overall the manuscript is easy to follow, though there are a number of typos and inconsistencies that need to be addressed in the text and tables. The gradients across which the authors assess elemental composition are extensive, though regrettably there is no exploration of synergistic relationships between the variables (which the authors acknowledge). The authors state in the text that they were able to rank the importance of the different environmental variables, but the table containing that information (Table 3) was not included in the manuscript pdf, making it difficult to comment on that topic.”

The typos and the inconsistencies have been carefully checked and fixed. The potential synergistic relationships between the variables have now been further explored. The missing Table 3 is also added in the revised version.

Response to the specific comments:

“Not sure if this is a journal formatting issue but there should be either spaces or indentation to separate paragraphs. This is consistent throughout the manuscript.”

Spaces have been added to separate paragraphs.

“Inconsistent use of serial commas”

This problem has been fixed. The serial commas are now consistently used.

5

“The authors are inconsistent in using the modifier “cellular” when referring to the various forms of particulate organic matter. If, as I suspect, they are only referring to cellular forms of such matter, then the continual use of the “cellular” term is unnecessary.”

In this manuscript, the elemental composition mainly refers to the cellular elemental contents and ratios,
10 and the inconsistent use of the modifier “cellular” is now fixed.

“Pg7 line7: Delete ‘then’.”

The word “then” has been deleted.

15 *“Figures 1-5: If you are fitting curves through data points, would it not be better to plot all of your data points using a scatterplot as opposed to using bar plots? This would give the reader a much better sense of the variability within the data.”*

The bar plots instead of scatter plots are used in order to make comparisons between different treatments for each of the manipulation experiments. And the fitting curves are used to describe the
20 variability of the trends within the data.

“Section 3.1: You don’t mention anything about the effects of nutrients on POC.”

There were no significant effects of nutrient concentrations on the POC contents based on our experimental results.

25

“Why are the values for goodness of fit in the supplement and not in the manuscript?”

There are 7 figures and 3 tables in the manuscript. Therefore, the table containing the fitting equations and the values for goodness of fit are in the supplement in order to keep the manuscript to a reasonable length.

- 5 *“Pg9, line 15: The ‘dramatic’ decline was predominately seen between 4 and 7°C and leveled out thereafter. Maybe change the wording to more correctly state this response.”*

The wording has now been changed to “The C:Chl-*a* ratio dramatically decreased with warming, especially between 4°C and 7°C (Fig. 6d)”.

- 10 *“Table 2: The meaning of bold values should be stated in the table caption, not in the manuscript text. Table 2: There are numerous values that are stated as being significantly different in the text but are not bold in the table.”*

The meaning of bold values has been stated in the table caption in the revised manuscript. The other significantly different values in the table have also been formatted in bold font.

15

“Table2: Why are these data presented as a table instead of plots as were used for the previous metrics?”

The differences of the elemental ratios between different treatments are less significant compared to the cellular elemental contents. Therefore, the ratios are presented in one table and not in 3 separate figures,
20 to keep a reasonable total number of figures of the manuscript.

“Section 3.8: I could not find the Table 3 that is referenced in the text, making it difficult to review this section.”

The table has been added in the manuscript.

25

“Pg13, line26: typo; Pg13, line27: typo; Pg14, line2: typo”

These typos have all been corrected.

“Pg14, line5: Why are cell size data not presented (in text or supplement) in either this manuscript or Feng et al., 2017?”

The cell size data from the temperature manipulation experiments is presented in the supplements as Fig. S1.

5

“Pg14, line8: Don’t you mean greater than 11°C, since 10°C was not tested in this study and PIC values did not appear to differ amongst the 4, 7, and 11°C treatments?”

The previous 10°C is now revised to 11°C.

10 Pg14, line10: Again referring to data (cell volume) that is not presented.

Now the supplemental data in Fig. S1 is referred to.

Pg14, line11: 10°C was not a treatment level in this study.

The previous 10°C is now revised to 11°C.

15

“Pg14, line15: The best-fit line does not follow this description. Given the poor fit based on the low R2 value, why is this fitting included?”

The PIC:POC ratio at 4°C was significantly lower than the other treatments, indicating lower cellular PIC:POC production under extreme low temperature; therefore, this fitting is included.

20

“Pg14, line16: 24°C was not a temperature used in this study or Feng et al. (2017)”

The authors agree that 24°C was not a temperature used in the experiment; however, here 24°C was the optimal temperature for photosynthetic rate from the fitting in Fig. 3d of Feng et al. (2017).

25 “Pg14, line23: A 74% increase is not really ‘almost double’.”

The original wording of “...almost double...” has been revised to “the cellular N:P ratio of *E. huxleyi* at 20°C increased by 74%...”.

“Pg16, line2: This study did not use any isotopic labeling. I assume that this is referring to Feng et al. (2017).”

Yes, this is referring to Feng et al. (2017). And the reference has been added in the text.

- 5 “Pg17, line23: You could also cite Blanco-Ameijeiras et al. (2016) in PLoS ONE since they tested 13 strains under the same environmental conditions, avoiding interlaboratory experimental variability that is an issue when comparing results from different experiments.”

The reference of Blanco-Ameijeiras et al. (2016) has been cited.

- 10 We have also attached the mark-up manuscript version showing the changes we have made on the previous version.

We look forward to hearing back from you again. Thank you very much.

- 15 Sincerely,

Yuanyuan Feng and the coauthors

Environmental controls on the elemental composition of a Southern Hemisphere strain of the coccolithophore *Emiliana huxleyi*

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Abstract. A series of semi-continuous incubation experiments were conducted with the coccolithophore *Emiliana huxleyi* strain NIWA1108 (Southern Ocean isolate) to examine the effects of five environmental drivers (nitrate concentration, phosphate concentration, irradiance, temperature, and $p\text{CO}_2$) on both the physiological rates and elemental composition [of the coccolithophore](#). Here, we report the alteration ~~of~~ the elemental composition of *E. huxleyi* in response to the changes in these environmental drivers. A series of dose response curves for the cellular elemental composition of *E. huxleyi* were fitted for each of the five drivers across an environmentally-representative gradient. The importance of each driver in regulating the elemental composition of *E. huxleyi* was ranked using a semi-quantitative approach. The percentage variations [in](#) elemental composition arising from the change in each driver between present day and model-projected conditions for the year 2100 were calculated. Temperature was the most important driver controlling both cellular particulate organic and inorganic carbon content, whereas nutrient concentrations were the most important regulator of cellular particulate nitrogen and phosphorus of *E. huxleyi*. In contrast, elevated $p\text{CO}_2$ had the greatest influence on cellular particulate inorganic carbon to organic carbon ratio, resulting in a decrease in the ratio. Our results indicate that the different environmental drivers ~~each~~ play specific roles in regulating the ~~cellular~~

elemental composition of *E. huxleyi* with wide-reaching implications for coccolithophore--related marine biogeochemical cycles, as a consequence of the regulation of *E. huxleyi* physiological processes.

1 Introduction

5 Climate change is causing a wide range of alterations to the marine environment including ocean acidification (OA), rising sea surface temperature (SST), and intensified stratification due to increased density gradients between surface and subsurface waters, with associated shifts in mean irradiance levels and nutrient availability in the upper water column (Boyd and Doney, 2002; Rost and Riebesell, 2004; Stocker, 2013). All these global changes in environmental variables will affect the physiology and ecology of phytoplankton, both individually and interactively, in a complex way (Boyd and Hutchins, 10 2012; Boyd et al., 2010; Boyd et al., 2016; Feng et al., 2017).

Phytoplankton elemental composition is an important cellular property that reflects the metabolic rates of phytoplankton (Raven and Geider, 1988). Elemental composition is strongly influenced by environmental conditions and by phytoplankton adaptations to these conditions (Sterner and Elser, 2002) 15 which in turn influences marine foodweb structure, particulate carbon export to the deep ocean, and ultimately marine biogeochemistry (Finkel et al., 2010 and references therein). The widely-recognized average molar elemental ratio of C:N:P is 106:16:1 for marine phytoplankton assemblages - the Redfield ratio (Redfield et al., 1963). However, individual phytoplankton species may have elemental ratios deviating, on short timescales (days to months) from Redfield depending on the 20 environmental conditions they encounter. Such deviations subsequently influence the accumulation of these elements in the upper food web and also marine biogeochemistry (Finkel et al., 2010; Ho et al., 2003; Sardans et al., 2012).

Different environmental drivers may play a range of roles in regulating the stoichiometry of marine phytoplankton. Nutrient availability (Hecky et al., 1993; Perry, 1976) has been proven to affect 25 phytoplankton stoichiometry directly. Irradiance provides the energy source for nutrient assimilation in the cells (Goldman, 1986). In addition, temperature changes, which mainly alter metabolic rates, can also influence the diffusive uptake of nutrients into cells (Raven and Geider, 1988; Roleda et al., 2013).

Increased levels of dissolved CO₂ during cell growth may result in higher cellular C:N and C:P ratios, due to increased CO₂ availability as a substrate for photosynthesis (Beardall et al., 2009; Feng et al., 2008; Fu et al., 2007; Fu et al., 2008). However, the dependency of C:N or C:P ratios on CO₂ availability can be species-specific (Burkhardt and Riebesell, 1997; Burkhardt et al., 1999). The effect of rising pCO₂ on the N:P ratio is still unclear, due to large variations observed in previous environmental manipulation studies (Sardans et al., 2012). For example, the N:P ratio of *Synechococcus* increased with elevated CO₂ concentration, but remained unchanged for *Prochlorococcus* (Fu et al., 2007) and *Emiliania huxleyi* (Feng et al., 2008).

Marine coccolithophores are responsible for almost half of the global marine calcium carbonate production, and are important in the marine carbon cycle through both the organic carbon pump and the inorganic carbon counter pump (Rost and Riebesell, 2004). *Emiliania huxleyi* is the most widely distributed coccolithophore species (Balch et al., 1991; Holligan et al., 1993; Holligan et al., 1983), and has been selected as a model phytoplankton species in the context of the marine carbon cycle (Westbroek et al., 1993). A wide range of environmental drivers, such as CO₂ concentration, nutrient concentration, irradiance, and temperature influence the growth, photosynthesis, and calcification of *E. huxleyi* both individually and interactively (Feng et al., 2017; Raven and Crawford, 2012; Zondervan, 2007). Changes in these physiological processes may in turn alter the elemental stoichiometry and composition of coccolithophores. Knowledge of how different environmental drivers will affect the elemental composition of *E. huxleyi* is important for a more complete understanding of the physiological responses of this species to the changing environment and the consequent effects on biogeochemical cycles. In addition, the magnitude of change of each environmental driver will be different with the future climate change, depending on location and scenario; hence a systematic study across a gradient of each driver is required.

This study advances previous findings by relating the change in elemental composition of *E. huxleyi* cells, in response to environmental forcing, to the physiological rate responses presented in the study of Feng et al. (2017). The major objective of the present study is to investigate and rank the importance of the environmental drivers, including the nitrate and phosphate concentrations, irradiance, temperature, and pCO₂, on setting the elemental composition of a Southern Ocean strain of *E. huxleyi*. The combined

results of this study and Feng et al. (2017) provide new insights into how ~~changing~~-environmental-
drivers changes will impact the marine biogeochemical cycles related to *E. huxleyi*.

2 Materials and methods

2.1 Experimental setup

5 ___ The marine coccolithophore *Emiliana huxleyi* (morphotype A, strain NIWA1108) was isolated from
the Chatham Rise, east of New Zealand by Dr. H. Chang in 2009, as detailed in Feng et al. (2017). The
stock culture was maintained in the laboratory at 14°C and an irradiance of $\sim 140 \mu\text{mol m}^{-2} \text{s}^{-1}$, under a
light/dark cycle of 12 h/12 h. The medium used for maintaining the stock culture was seawater obtained
from Otago Harbour, New Zealand (nutrient concentrations: phosphate 0.3-0.6 μM , nitrate 3-6 μM),
10 filtered using 0.2 μm pore size filtration cartridge (WhatmanTM) and supplemented with nutrient stock
solutions to give final concentrations of nitrate 96 μM and phosphate 6 μM , without silicate addition.
Trace metal and vitamin stock solutions were added according to the f/20 recipe for both stock culture
and the manipulation experiments (10 times dilution of f/2 level; Guillard and Ryther, 1962).

___ For the manipulation experiments, *E. huxleyi* cells, in exponential growth phase, were transferred
15 into acid-cleaned 500-mL polycarbonate bottles with screw caps and subjected to a series of semi-
continuous incubation experiments under different nutrient, irradiance, temperature, and $p\text{CO}_2$
conditions (Feng et al., 2017). Only one environmental driver was manipulated at a time for each
incubation experiment, with the other environmental drivers remaining the same as the stock culture
conditions. The manipulation of each of the different drivers was carefully selected to cover a broad
20 range of conditions observed in the natural environment and those commonly employed for laboratory
incubations (Feng et al., 2017). Initial cell abundances were $\sim 10^4 \text{ cell mL}^{-1}$ and *in vivo* chlorophyll *a*
(Chl-*a*) fluorescence readings were monitored daily as indicators of Chl-*a* biomass and cell growth.
After 2-3 days of acclimation under the experimental conditions daily dilution was started by adding
freshly made seawater medium into each incubation bottle to adjust the biomass to the level of the
25 previous day. All the incubation experiments were carried out in walk-in growth chambers (Model 650,
Contherm, New Zealand), with metal halide lamps (full spectrum) as the light source, under a light/dark

cycle of 12 h/12 h. Irradiance levels inside the incubation bottles were measured using a quantum PAR sensor (2100 series, Biospherical Instruments Inc., USA). The temperature in each incubation experiment was monitored continuously using a HOBO Pendant[®] temperature/light data logger (Onset, Australia), with variation no more than $\pm 0.5^{\circ}\text{C}$.

5 | ___ The experimental conditions for each environmental driver used for the manipulation experiments are presented in Table 1, and described in Feng et al. (2017). All the treatments were conducted in triplicate. For each culture, the final sampling was performed after the daily monitored growth rate remained relatively constant (daily variations $<10\%$) for more than seven generations (Feng et al., 2008). This yielded total acclimation of the cultures to the experimental conditions for ~ 20 days. Samples were
10 | collected for cell counts, Chl-*a* biomass, and elemental components, including particulate organic carbon (POC), particulate inorganic carbon (PIC), particulate organic nitrogen (PON), and particulate organic phosphorus (POP). startinged 2 hours after the beginning of the light incubation phase and finishinged within 2 hours for all the experimental treatments.-

2.2 Sample analyses

15 2.2.1 Cell counts and Chl-*a*

___ One mL subsamples for cell counts were taken from each incubation bottle, preserved by adding 6 μL modified Lugol's solution, prepared by dissolving 10 g KI and 5 g iodine crystals in 20 mL Milli-Q water, then adding 50 mL of 5% anhydrous sodium acetate solution, and stored in dark at 4°C . Cell abundance was determined with a nanoplankton counting chamber (PhycoTech, USA) using a Zeiss
20 | microscope (Axiostar plus, Germany). *In vitro* Chl-*a* concentration was analysed using a Turner 10-AU fluorometer (Turner design, USA) with 90% acetone extracted samples, as detailed in Welschmeyer (1994).

2.2.2 Elemental composition

___ Subsamples from each incubation bottle for PON, total particulate carbon (TPC), and POC
25 | measurement were filtered onto pre-combusted GF/F glass fibre filters (WhatmanTM), and analysed using an elemental combustion system (Elementar vario EL III, Germany). Filters for POC analysis,

were fumed with saturated HCl for 3 hours in order to remove all the inorganic carbon (Zondervan et al., 2002). The PIC content was calculated by subtracting POC from TPC values. Subsamples for particulate organic phosphate (POP) measurements were filtered onto pre-combusted GF/F filters (Whatman™), and analysed following the molybdate colorimetric method of Solórzano and Sharp (1980). The particulate inorganic nitrogen (PIN) and particulate inorganic phosphorus (PIP) contents were both neglected ~~for the calculation~~, due to their relatively low values for laboratory cultures (Feng et al., 2008).

2.2.3 Data analyses

The effects of different environmental drivers on the elemental composition of *E. huxleyi* and their stoichiometric ratios were identified with one-way analysis of variance (ANOVA) using the commercial statistical software package SigmaStat (Version 3.5; Jandel Scientific, San Rafael, CA, US). Differences between treatments were considered significant when $p < 0.05$. Post-hoc comparisons using the Student-Neuman-Keuls (SNK) test were conducted to determine any differences between particular treatments.

For the environmental drivers that had significant effects on the elemental composition and ratios of *E. huxleyi* within the examined range, the response curves to the drivers were fitted using the models listed in Table S1. All of the curve-fitting was performed using least square fit with Prism software (version 5.0; GraphPad Prism Software, US) with all the triplicate data for each of the experimental treatments.

The same approach as used in Feng et al. (2017) was performed to rank the relative importance of each environmental driver, that was found to have significant effects using the one-way ANOVA analyses, on the elemental composition of *E. huxleyi*. Firstly, the two values for the elemental composition at the average present-day conditions and the projected conditions for the year 2100 were derived from each fitted dose-response curve for the environmental driver that had significant effects. The environmental conditions were projected using Coupled Model Intercomparison Project phase 5 (CMIP5) models (Boyd and Law, 2011; Law et al., 2016; Rickard et al., 2016), which suggested 33% decreases in both nitrate and phosphate concentrations, 2°C warming, a 25% increase in irradiance, and an increase in $p\text{CO}_2$ from 39 to 75 Pa in the Chatham Rise area for the year 2100 compared to present

day conditions (Feng et al., 2017). The magnitude and direction of percentage change in the elemental composition under projected future conditions relative to the present day condition for each environmental driver was then calculated. The ranking was finally determined by comparing the absolute values of the calculated percentage changes of the physiological metrics caused by each driver.

5 The driver that caused the largest percentage change was selected as the most important controlling driver.

3 Results

3.1 Changes in cellular POC content in response to environmental drivers

___ Cellular POC content was significantly affected by alteration of irradiance, temperature, and $p\text{CO}_2$ (Fig. 1). Increasing irradiance from 14 to 80 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ increased the cellular POC content by around two-fold from 8.20 ± 2.39 to $14.07 \pm 1.17 \text{ pg cell}^{-1}$ ($p < 0.05$). POC content ~~then~~ decreased at the two highest irradiance levels (350 and 650 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, Fig. 1c). A trend of decreased *E. huxleyi* cellular POC content with elevated temperature was evident from the temperature manipulation experiment (Fig. 1d). The cellular POC content ($28.85 \pm 6.98 \text{ pg cell}^{-1}$) was significantly higher than all the other treatments ($p < 0.05$) at the lowest temperature of 4°C and significantly reduced by ~ 70% at both 20°C and 25°C ($p < 0.05$). Raising $p\text{CO}_2$ from 8 to 15 Pa significantly increased the cellular POC content from 9.63 ± 1.67 to $12.93 \pm 1.84 \text{ pg cell}^{-1}$ (Fig. 1e), with cellular POC content being relatively uniform from 15 to 109 Pa.

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3.2 Alteration of cellular PIC content in response to environmental drivers

___ Temperature was the only driver that significantly altered the cellular PIC content (Fig. 2). There was a general trend of decreased cellular PIC content of *E. huxleyi* with warming from 11°C to 20°C (Fig. 2d). The cellular PIC content was significantly lower at 20°C and 25°C compared to the other ~~four~~ temperature treatments ($p < 0.05$). More than a 50% decrease in cellular PIC content was observed at the two highest temperature conditions, relative to the 7°C treatment. However, there were no significant differences in cellular PIC content between the other temperature treatments. The fitted

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Q_c value (the plateau for one phase decay) was 6.94 ± 0.93 pg cell⁻¹, close to the average value at the two highest temperatures (Table S1).

3.3 Changes in the cellular PIC:POC ratio in response to environmental drivers

As for cellular POC, the cellular ratio of PIC:POC was mainly affected by changes in irradiance, temperature, and $p\text{CO}_2$ (Fig. 3). The highest cellular PIC:POC ratio of 1.20 ± 0.09 was observed at the lowest irradiance ($19 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$; $p < 0.05$, compared to all other irradiance treatments). The ratio then decreased with increasing irradiance to 0.72 ± 0.10 at $190 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$ and slightly increased again at the two highest irradiances ($p < 0.05$ between 190 and $650 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$, Fig. 3c). In the temperature manipulation experiment, the PIC:POC ratio was significantly lower ($p < 0.05$) at the lowest temperature (4°C) than any other treatment, with a value of 0.45 ± 0.03 pg cell⁻¹. The PIC:POC value then leveled off between the range of 7°C to 25°C , with the average value more than double that at 4°C (Fig. 3d). With the variation of $p\text{CO}_2$ levels, the cellular PIC:POC ratio decreased by more than 40% from 1.46 ± 0.02 pg cell⁻¹ at 8 Pa to 0.90 ± 0.15 at 39 Pa and stayed similar between the range of 39 and 109 Pa ($p < 0.05$) (Fig. 3e), mainly due to the increased cellular POC quota with rising $p\text{CO}_2$.

3.4 Alteration of cellular PON content in response to environmental drivers

The cellular PON content increased with increasing nitrate concentration. The content at the two lowest nitrate concentrations of 3.7 and $6.0 \mu\text{M}$ was less than half of the average value (2.06 ± 0.36 pg cell⁻¹) of the three highest nitrate treatments (Fig. 4a). Warming from 4°C to 25°C decreased the cellular PON content ($p < 0.05$). The value of 4.07 ± 0.00 pg cell⁻¹ at 4°C was double that at 15°C (1.93 ± 0.10 pg cell⁻¹); and three-fold greater than the PON content of 1.31 ± 0.24 pg cell⁻¹ at 25°C (Fig. 4d).

3.5 Changes in cellular POP content in response to environmental drivers

The cellular POP content of *E. huxleyi* was significantly altered by nitrate, phosphate, temperature, and $p\text{CO}_2$. POP content was slightly less at the three low nitrate concentrations (3.7, 6.0 , and $12 \mu\text{M}$), compared to those at 96 and $200 \mu\text{M}$ ($p < 0.05$; Fig. 5a). Cellular POP content significantly increased with rising phosphate concentration (Fig. 5b), with the highest POP content observed at $20 \mu\text{M}$

phosphate. As observed for cellular POC and cellular PON contents, warming greatly decreased the cellular POP content (Fig. 5d), with a reduction of 65% from $1.08 \pm 0.14 \text{ pg cell}^{-1}$ at 4°C to $0.38 \pm 0.04 \text{ pg cell}^{-1}$ at 11°C , but then only a further decrease of $\sim 0.1 \text{ pg cell}^{-1}$ from 15°C to 25°C . Significant differences in POP content were detected between the two lowest temperature treatments compared to all others. Conversely, with rising $p\text{CO}_2$ level there was a trend of increased cellular POP content (Fig. 5e), which almost doubled from $0.20 \pm 0.04 \text{ pg cell}^{-1}$ at 8 Pa to $0.38 \pm 0.02 \text{ pg cell}^{-1}$ at 109 Pa ($p < 0.05$).

3.6 Alteration of cellular C to Chl-*a* ratio in response to environmental drivers

Alteration of all the five environmental drivers greatly affected the cellular ratio of POC to Chl-*a* content (C:Chl-*a*, g:g) ($p < 0.05$) (Fig. 6). C:Chl-*a* decreased exponentially with increased nitrate concentration up to $50 \text{ }\mu\text{M}$, but stabilised between 50 and $200 \text{ }\mu\text{M}$ (Fig. 6a). The highest ratio of 422.36 ± 74.28 was observed at the lowest nitrate concentration of $3.7 \text{ }\mu\text{M}$, significantly higher than all other treatments ($p < 0.05$). The ratio then decreased by 87% at $200 \text{ }\mu\text{M}$. An increase in phosphate concentration, however, only slightly decreased the C:Chl-*a* ratio (Fig. 6b). Compared to the ratios at the two lowest concentrations, a significant decrease ($p < 0.05$) at $6.0 \text{ }\mu\text{M}$ and $20 \text{ }\mu\text{M}$ was observed (by $\sim 20\%$ each). Increased irradiance increased the C:Chl-*a* ratio linearly, with more than a doubling at $650 \text{ }\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ compared to the ratio of 47.45 ± 12.58 at $14 \text{ }\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ (Fig. 6c). The C:Chl-*a* ratio dramatically decreased with warming, especially between 4°C and 7°C (Fig. 6d)~~The C:Chl-*a* ratio dramatically decreased with warming, with a decline of 64% from 4°C to 25°C (Fig. 6d).~~ The ratio of 131.26 ± 42.96 observed at 4°C was significantly higher than all the other temperatures ($p < 0.05$). Significantly lower C:Chl-*a* ratios were observed at the two lowest $p\text{CO}_2$ levels of 8 and 15 Pa compared with the other treatments ($p < 0.05$, Fig. 6e), with the ratio increasing by 42% from low to high $p\text{CO}_2$.

3.7 Shifts in cellular elemental molar ratios in response to environmental drivers

The cellular PON to POP (N:P) ratio was significantly lower ($p < 0.05$) at the two lowest nitrate treatments compared to the others (Table 2)~~(bold values in Table 2)~~. In contrast, the cellular POC to PON (C:N) ratio was significantly higher ($p < 0.05$) at the two lowest nitrate concentrations. There was

no significant difference in C:N ratios across the other four nitrate treatments ($p > 0.05$). Changes in nitrate concentration did not significantly affect the cellular POC to POP (C:P) ratio.

5 The N:P ratio of *E. huxleyi* increased at low phosphate concentrations (0.4 and 2 μM), with highest value in the 0.4 μM phosphate treatment ($p < 0.05$). There was a significant increase in the C:P ratio ($p < 0.05$) at the two lowest phosphate concentrations compared to the others. The highest C:P ratio, recorded at the lowest phosphate concentration (0.4 μM), was almost double the value at 2 μM ; and more than three times the average ratio of the other treatments (Table 2). In contrast, there were no significant differences in the calculated C:N ratio across the phosphate treatments ($p > 0.05$).

10 Decreased C:N ratios were observed for low irradiances; the value at 14 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ being significantly lower than the three highest irradiances ($p < 0.05$). Similarly, a decreased C:P ratio was found at low irradiance, with a significantly lower value at 14 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ compared to the three highest irradiances. Warming significantly increased the N:P ratio from 4°C to 20°C (Table 2).

3.8 Ranking the importance of environmental drivers in altering *Emiliania huxleyi* elemental composition

15 Ranking the response of the Southern Ocean *E. huxleyi* isolated to projected future changes in oceanic properties revealed differential responses between drivers and processes (Table 3, Fig. 7). Cellular POC and cellular PIC:POC ratio were both significantly influenced by CO₂ and temperature, with temperature affecting cellular POC content the most, while CO₂ was the most important factor regulating PIC:POC. However, only one driver (temperature) significantly regulated cellular PIC, with a
20 4°C warming causing a 14.2 % decrease. The cellular PON content was significantly affected by future nitrate concentration and temperature, with nitrate ranking the most important. ~~However, only one driver (temperature) significantly regulated cellular PIC, with a 4°C warming causing a 14.2 % decrease.~~
Four (phosphate, temperature, CO₂, and nitrate) out the five environmental drivers, under end of the century conditions, significantly affected cellular POP content, with future phosphate concentration
25 playing the most important role. The rankings associated with statistically non-significant differences among the treatment intervals, as marked in Table 3 and Fig. 7, need to be considered with caution (see Feng et al. (2017)).

4 Discussion

___ This is the first detailed study of the individual effect of five environmental drivers (nitrate concentration, phosphate concentration, irradiance, temperature, and $p\text{CO}_2$) on the cellular elemental composition of the coccolithophore *E. huxleyi*. Moreover, it is the first to rank the importance of the predicted changes in these environmental drivers on *E. huxleyi* elemental stoichiometry for the year 2100 relative to the present-day conditions. Relating changes in elemental composition is an important addition to the responses of growth, photosynthesis, and calcification rates (Feng et al., 2017), providing insights into the biogeochemical consequences of the physiological effects induced by change in the five essential environmental drivers.

4.1 Effects of nutrient concentration on the elemental stoichiometry of *Emiliania huxleyi*

___ The PON and POP cell quotas of *E. huxleyi* in the present study were mainly controlled by nitrate and phosphate concentrations, respectively, as phytoplankton relies on seawater nutrient availability as the external elemental source (Hecky et al., 1993; Price, 2005; Sakshaug and Holmhansen, 1977). Nitrate concentration plays an important role in regulating the growth, photosynthetic, and calcification rates of *E. huxleyi* (Feng et al., 2017); however, the three lowest nitrate concentrations only resulted in slightly decreased cellular POP contents and had no significant effect on cellular POC or PIC content. This indicates that the regulation of the nitrate concentration on the POC and PIC productivity in our study was mainly a consequence of decreased growth rate of the cells under nitrate limitation, as shown by Feng et al. (2017). This finding is in contrast to Paasche (1998) who observed higher *E. huxleyi* cellular PIC:POC ratios under nitrate limitation as a result of decreased cellular POC and increased coccolith abundance per cell in *E. huxleyi* strain BOF 92 isolated from the North Atlantic. Higher PIC:POC ratios under nitrate limitation was alternatively attributed to increased calcite mass per lith of *E. huxleyi* strain CCMP 378 isolated from the Gulf of Maine (Fritz, 1999). In addition, phosphate concentration did not significantly affect *E. huxleyi* cellular carbon content nor the PIC:POC ratio of cells in the present study. However, Paasche (1998) observed greatly increased PIC content of *E. huxleyi* (strain BOF 92) under phosphate limiting conditions, and Riegman et al. (2000) observed that a greater increase PIC quotas under phosphate limitation than nitrate limitation for *E. huxleyi* (strain L).

These discrepancies between studies in the nitrate or phosphate effects on cellular PIC:POC ratio are mainly due to the different nutrient concentrations in the culturing media. Paasche (1998) observed an increase in *E. huxleyi* PIC cell quota under the stationary phase of batch incubation, i.e. when cell division ceased as nitrate dropped to $\leq 0.2 \mu\text{M}$ and phosphate dropped to $\leq 0.03 \mu\text{M}$. This supports the findings of both Riegman et al. (2000) and Fritz (1999) who conducted continuous incubations with high cell densities of *E. huxleyi*. These studies observed an increased cellular PIC content when phosphate concentration fell below 0.4 nM (Riegman et al., 2000) or nitrate concentration was below the detection limit (Fritz, 1999). However, the present study used a semi-continuous incubation method with higher and relatively steady nutrient concentrations (with lowest nitrate and phosphate concentrations of 3.6 and 0.4 μM , respectively) and the cells were grown and sampled at a healthy exponential growth phase. Similarly, Müller et al. (2008) only found higher *E. huxleyi* (strain CCMP371) cellular calcite content during the stationary but not the exponential growth phase under both nitrate and phosphate limitation, due to the different cell cycle phases during which the calcification and cell division occurred. The authors explained that calcification continued during the G1 phase of cell assimilation when cell division was restricted under nutrient limitation, and thus the cellular PIC content was increased (Müller et al., 2008). Further studies at extremely low nutrient concentrations ($< 0.1 \mu\text{M}$) in a steady-state growth phase are still needed to understand the potential connection between carbon production and extreme nutrient limitation, given reports of areal expansion of oligotrophic waters in the world oceans with global climate change (Polovina et al., 2008).

20 **4.2 Irradiance effects on the elemental stoichiometry of *Emiliana huxleyi***

In the present study, irradiance was the main environmental factor affecting cellular POC content which in turn altered the PIC:POC ratio. The increased PIC:POC cellular ratio at low irradiance indicates that calcification is less dependent on irradiance than organic carbon fixation, as discussed in Feng et al. (2017). Although both processes require light as an energy source, calcification requires less energy (Anning et al. 1996) than photosynthesis (Paasche, 1965; Balch et al., 1992). Therefore, the calcification rate is generally saturated at lower irradiance levels than photosynthesis (Paasche, 1964; Zondervan, 2007). Feng et al. (2017) reported greatly reduced photosynthetic rates under the two lowest

irradiance levels, while observing that this trend was less significant for the calcification rate. Hence, limiting irradiance will lead to less POC content in the cells compared to the cellular PIC quota, and thus a higher cellular PIC:POC ratio would be expected at low irradiance when growth and photosynthesis are light-limited (Raven and Crawford, 2012), as confirmed by the response of calcification:photosynthesis in Feng et al. (2017).

Increasing irradiance also elevated the C:Chl-*a* ratio linearly in the present study, due to the increase in POC quota and a decrease in Chl-*a* quota, as also reported for diatoms and dinoflagellates (Geider, 1987). The reduced cellular pigment quota under high irradiance helps to reduce the energy required for light harvesting in phytoplankton cells, which is a strategy to balance the energy demands for growth and POC production with photon harvesting (Kiefer, 1993). In addition, the present study revealed that the C:N and C:P ratios of *E. huxleyi* both increased at high light levels, as a consequence of increased cellular POC content driven by increased irradiance but no significant change in cellular PON or POP quota, further suggesting that organic carbon content is more light dependent than the accumulation of cellular N or P (Geider et al., 1998).

4.3 Temperature effects on the elemental stoichiometry of *Emiliana huxleyi*

Temperature is important in regulating dissolved chemical diffusion and transport, non-enzymatic and enzymatic reactions, and the metabolic rates of phytoplankton (Raven and Geider, 1988). In our accompanying study, the growth, photosynthetic, and calcification rates all increased with rising temperature until the optimal temperature was reached (Feng et al., 2017). Conversely, the cellular POC, PON, and POP content all reduced significantly as temperature increased in the present study. It has been proposed that reduced cell size is a universal strategy in response to increasing temperature for both terrestrial and aquatic organisms (Gardner et al., 2011), following a hypothesis suggested by Atkinson et al. (2003). A study on the coccolithophores *E. huxleyi* (strain EH2) and *Gephyrocapsa oceanica* (strain GO1) observed decreased cell size and thinner coccospheres upon raising temperature from 10°C to 25°C, which was attributed to the relatively suppressed cell division at low temperature (Sorrosa et al., 2005). This decrease in cell volume (Fig. S1) could be the main cause of reduced cellular elemental components in the present study. Previous studies also reported that warming

resulted in reduced cell volume of *E. huxleyi* (strain AC481, De Bodt et al., 2010; strain L, Van Rijssel and Gieskes, 2002), and decreased cellular POC and PIC quotas of coccolithophore *Coccolithus pelagicus* when the temperature was raised from 10°C to 15°C (Gerecht et al., 2014). Similarly, warming significantly decreased the cellular elemental contents to their lowest levels measured in the present study over the range from 4°C to 25°C, with a decrease in cell size at higher temperatures (Fig. S1 data not shown), as growth rate increased (Feng et al, 2017).

However, contrary to the observed changes in POC, PON, and POP cell quota, the cellular PIC content of *E. huxleyi* only decreased when temperature was higher than 11°C in the present study, due to the strongly reduced calcification and malformation at low temperatures of 4°C and 7°C (Feng et al., 2017). The reduced cell division rate (i.e. enlarged cell volume, Fig. S1) offset the reduced calcification rate at lower temperatures, and so there was no significant difference in PIC cell quota at temperatures below 11°C. Consequently, the cellular PIC:POC ratio was lower at 4°C and 7°C, consistent with the trend observed for the calcification: photosynthesis ratio (Feng et al., 2017), indicating suppression of PIC formation relative to POC production at low temperature (Watabe and Wilbur, 1966). The PIC:POC ratio then decreased with warming from 11°C to 15°C and remained relatively steady afterwards, mainly due to the lower optimal temperature for calcification (20°C) compared to photosynthesis (24°C) as suggested in Feng et al. (2017).

Furthermore, warming from 4°C to 20°C significantly increased the *E. huxleyi* cellular N:P ratio in the present study, in agreement with the recent model study on a natural phytoplankton community (Toseland et al., 2013). Toseland et al. (2013) found that with increasing temperature the rate of cellular protein synthesis in phytoplankton was higher, but with a lower number of phosphorus-rich ribosomes, thereby increasing the cellular N:P ratio. In the present study, the cellular N:P ratio of *E. huxleyi* at 20°C increased by 74% from that was almost double that at 4°C, in spite of both cellular PON and cellular POP decreasing with warming. Although this study presents results for a single strain of *E. huxleyi*, if the temperature dependency of cellular resource allocation is a universal trend for all the *E. huxleyi* genotypes, we can speculate that the diverse *E. huxleyi* strains growing in different temperature regions might have different requirements for nitrogen vs. phosphorus, and that the growth of *E. huxleyi* strains in the temperate to tropical regions might be more readily limited by nitrate than sub-polar strains.

~~Moreover, this trend of temperature dependent N:P ratio also indicates that future warming might accentuate nitrate limitation in the oceans (Toseland et al., 2013). Similarly, Toseland et al. (2013) suggested that future warming might accentuate nitrate limitation in the oceans.~~

4.4 Effects of CO₂ on the elemental stoichiometry of *Emiliana huxleyi*

5 | ___ The photosynthesis of *E. huxleyi* was saturated at a higher $p\text{CO}_2$ than that for growth rate (Feng et al., 2017). In the present study, CO₂ plays the most important role in regulating the cellular PIC:POC ratio. The PIC:POC ratio was significantly higher at the lowest $p\text{CO}_2$ level, as a consequence of the lower cellular POC and higher cellular PIC at 8 Pa. In general, cell growth of *E. huxleyi* is less limited by low CO₂ concentrations than in other phytoplankton groups (Clark and Flynn, 2000; Paasche et al., 1996; 10 | Riebesell et al., 2000a; [Rost et al., 2003](#)). Moreover, recent studies suggest that *E. huxleyi* operates an active carbon concentrating mechanism (CCM) to utilize HCO₃⁻ through the enzyme carbonic anhydrase (CA; Reinfelder, 2011), and may have high affinity for CO₂ in photosynthesis (Stojkovic et al., 2013). However, the efficiency of CCMs in *E. huxleyi* (strain B92/11) is considered to be low as a consequence of the leakage of CO₂ from the cell (Rost et al., 2006), and so coccolithophore 15 | photosynthesis is more dependent than cell growth on CO₂ concentration (Rost and Riebesell, 2004). This discrepancy between growth and organic carbon fixation can lead to a decrease in cellular POC at low $p\text{CO}_2$. This difference in CO₂ requirements between the two processes may also have resulted in the lower cellular POP content at 8 Pa compared to other $p\text{CO}_2$ treatments.

20 | ___ The increasing trend observed for cellular POC and POP was not apparent for cellular PIC quota, as calcification rates significantly decreased with increasing $p\text{CO}_2$ level >40 Pa (Feng et al., 2017). Hence the cellular PIC:POC ratio was significantly higher at the two lowest $p\text{CO}_2$ levels, consistent with previous findings for CO₂ manipulations at saturating irradiances on *E. huxleyi* (strain PML B92/11A; Zondervan et al., 2002; Zondervan et al., 2001). No further significant change in cellular carbon content or PIC:POC ratio occurred at higher $p\text{CO}_2$, in contrast to the linear decrease in the 25 | calcification:photosynthesis ratio with rising $p\text{CO}_2$ (Feng et al., 2017). This difference is noteworthy ~~as for~~ both cellular PIC:POC and calcification:photosynthesis ratios are commonly used to examine the relative change of PIC and POC production in coccolithophores (Raven and Crawford, 2012). These

changes ~~both~~ have ~~ecological~~ biogeochemical implications for the marine rain ratio in the carbon cycle (Klaas and Archer, 2002; Rost and Riebesell, 2004), which is the export ratio of calcite to organic carbon into the deep ocean. The ^{14}C -labelling technique used in this study (see Feng et al., 2017) to measure carbon fixation (photosynthesis and calcification) rates was conducted during the light period, thus the measured rate is an indicator of net carbon fixation, that does not account for the energy-consuming respiratory process or CO_2 leakage out of the cells (Bach et al., 2015, 2013; Rost et al., 2006). Conversely, the cellular carbon content indicates the gross accumulated carbon in the cells over longer period of growth (Engel et al., 2010; Fabry and Balch, 2010). The most compelling reason for the relatively higher PIC:POC ratio (~ 1.5) than calcification: photosynthesis ratio (~ 1.0) in the lowest $p\text{CO}_2$ treatment (8 Pa, 79 ppm) in our study may then be attributed to diffusive CO_2 loss limiting inorganic carbon active uptake from the substrate (Bach et al., 2013), resulting in less POC fixation into the cells relative to the PIC fixation by the calcification process.

 The C:Chl-*a* ratio of *E. huxleyi* was lowest at $p\text{CO}_2$ of 8 Pa across all the $p\text{CO}_2$ treatments in the present study, mainly due to the decreased cellular POC at low $p\text{CO}_2$, rather than any change in cellular Chl-*a* content. However, increasing $p\text{CO}_2$ did not have significant effects on the C:N, N:P or C:P ratios in the present study. This is in accordance with a recent study on *E. huxleyi* (strain PML B92/11A), which also exhibited constant C:N:P ratios across a $p\text{CO}_2$ range of 18 to 75 Pa for cultures at steady growth phase under phosphate-limited continuous incubation (Engel et al., 2014).

4.5 Biogeochemical implications and future directions

 The comparisons between present day conditions and those projected for year 2100 for the Chatham Rise area are summarized in the conceptual figure-model (~~in~~-Fig. 7). These results indicate that the 2°C warming will decrease both POC and PIC cellular quotas of *E. huxleyi*, but may slightly increase the PIC:POC ratio. Rising $p\text{CO}_2$ alone will result in decreased cellular PIC:POC ratio. Although the 33% decrease in nitrate concentration is the major factor controlling the growth, photosynthetic, and calcification rates (Feng et al., 2017), change in nitrate concentration did not significantly affect the elemental stoichiometry except for the cellular PON contents of *E. huxleyi*. In addition, increasing temperature may increase the cellular N:P ratio, while rising $p\text{CO}_2$ will decrease the N:P and C:P ratios.

These results provide a more detailed perspective that can improve our knowledge on how the model coccolithophore species, *E. huxleyi*, may respond to future environmental changes. For example, our results suggest that rising $p\text{CO}_2$ in the future oceanic environment will decrease the *E. huxleyi* cellular PIC:POC ratio by 5.4%; however, the projected warming and increase in irradiance level may offset this decreased PIC:POC by 2.4% and 0.3%, respectively. The changes in PIC:POC have implications for the marine “rain ratio” and so alter the marine carbon cycle (Rost and Riebesell, 2004). Similarly, the cellular N:P ratio will be decreased by rising $p\text{CO}_2$, although this trend may be canceled out by warming. The altered C:N:P stoichiometry will in turn affect the nutrient cycle at higher trophic levels (Jones and Flynn, 2005) and marine biogeochemical cycles (Beardall and Raven, 2004).

10 | ___ It is noteworthy that the research presented here only examined the physiological response norms of *E. huxleyi* to a single environmental driver when other drivers were all kept at the stock culture growth condition (i.e. a set of single dimensional space experiments). However, these responses (such as the shape of the curves and the optimal conditions) may be different when the other background conditions are changed. For example, Sett et al. (2014) observed the dose-response curves of calcification of *E.*
15 | *huxleyi* PML B9/11 to CO_2 concentration was regulated by temperature. Therefore, in order to comprehensively understand how *E. huxleyi* physiology will respond to multiple environmental drivers and fill this knowledge gap, future research on a full environmental matrix is still necessary. These experiments will not only help to further explore the potential interactions (i.e. synergistic or agnostic effects) between environmental drivers, but also provide a better understanding of the underlying mechanisms of these interactive effects. In addition, the present study is only based on a single strain of southern hemisphere *E. huxleyi*. Due to the wide distribution of this species in the natural marine environment, *E. huxleyi* presents high variability in terms of genetic, morphological, and physiological characteristics (Cook et al., 2011; Read et al., 2013; Young et al., 2014). Therefore, the physiology of different *E. huxleyi* strains isolated from different geographic locations might respond differently to
20 | changing environmental drivers. For example, within the context of OA research, extensive previous studies suggest a strain-specificity of *E. huxleyi* in response to changes in seawater carbonate chemistry (Langer et al., 2009; Raven and Crawford, 2012; Blanco-Ameijerías et al., 2016). It has also been observed that different *E. huxleyi* ecotypes/morphotypes responded differently to OA (Müller et al.,
25 |

2015), which is likely a consequence of their genetic variation (Cook et al., 2011). The present study and Feng et al. (2017) demonstrate the important roles of different environmental drivers in controlling the physiology of *E. huxleyi* strain NIWA1108, and so further work is required to determine if the findings apply to other strains.

5 In summary, this study, in combination with Feng et al. (2017), have a number of implications for
research into the response of *E. huxleyi* to ocean acidification and global climate change. In addition to
seawater carbonate chemistry (Riebesell et al., 2010), it is necessary to report the experimental
conditions of all the environmental drivers carefully. The predictions presented will provide useful
information for biogeochemical models, such as that of Bopp et al. (2001), of how the elemental
10 stoichiometry of *E. huxleyi* will respond to the alteration of these environmental conditions individually,
in order to predict the future changes in the marine biogeochemical cycles. In addition, multiple
environmental drivers tend to change simultaneously in the future global climate change scenario (Boyd
and Hutchins, 2012), and so future studies should also investigate the interactions between these
multiple drivers on phytoplankton physiology. The predicted future changes in marine physical
15 properties (such as sea surface temperature (SST) and mixed layer depth) will vary from one oceanic
region to another (Boyd and Doney, 2002). The dose response curves from our study suggest that the
range of alteration in environmental drivers may control the outcome of the effects of environmental
perturbation on *E. huxleyi* physiology and biogeochemistry. For future multi-factorial manipulation
experimental designs, our results suggest that the magnitudes of change in each environmental driver
20 need to be determined/decided cautiously and should have environmental relevance in order to make
more accurate predictions, and the understanding of interactive effects of multiple environmental
drivers and the underlying mechanisms should be further explored. Therefore, for future multi-factorial
manipulation experiments, our results suggest that the magnitudes of change in each environmental
driver need to be determined/decided cautiously and should have environmental relevance in order to
25 make more accurate predictions.

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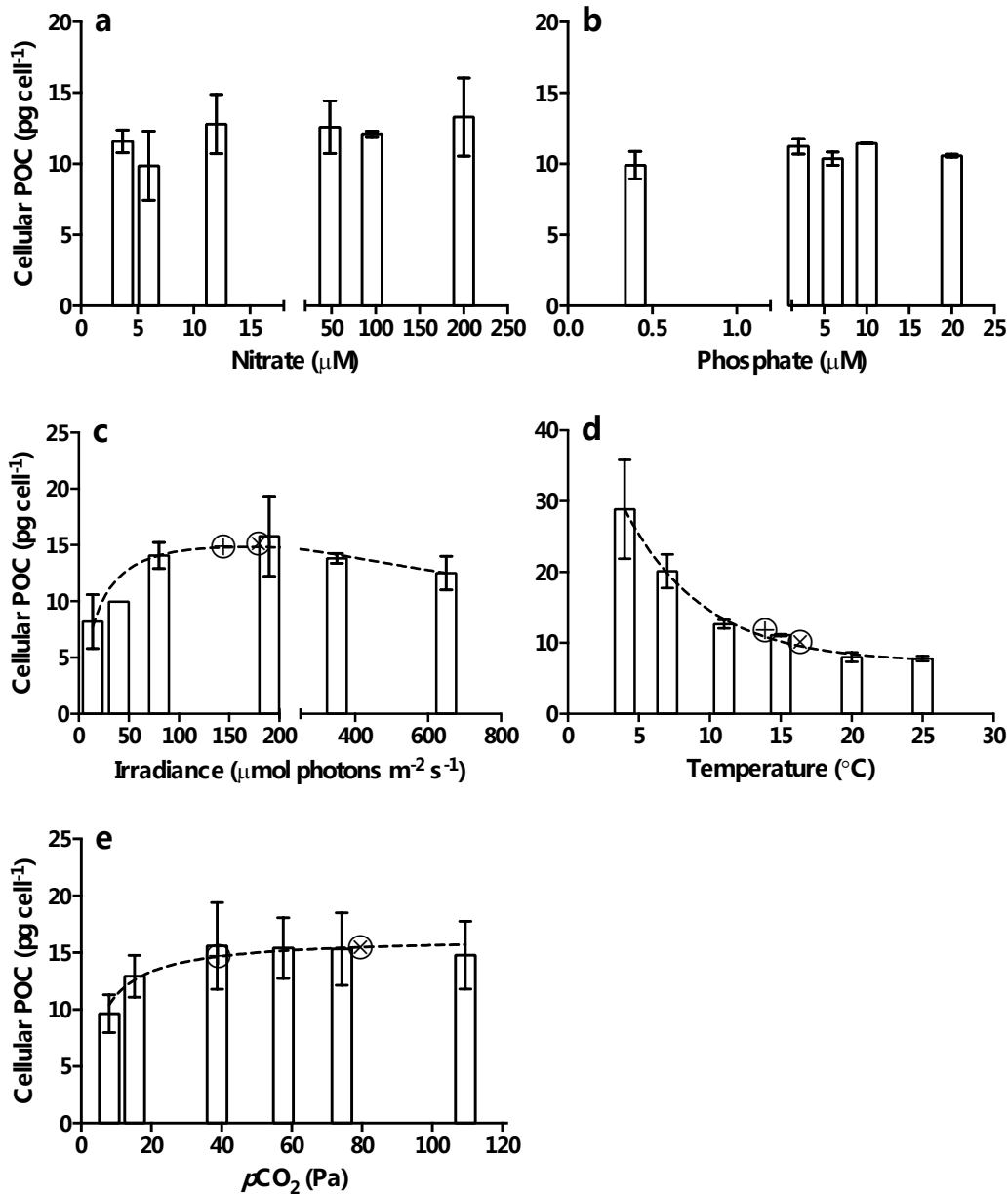


Figure 1. Changes in *Emiliania huxleyi* cellular particulate organic carbon (POC) content in response to different environmental drivers: a) cellular POC vs. nitrate concentration; b) cellular POC vs. phosphate concentration; c) cellular POC vs. irradiance; d) cellular POC vs. temperature; and e) cellular POC vs. $p\text{CO}_2$. Error bars represent standard deviations (n=3).

- 5 For Figures 1-5: The dashed lines represent the fitted dose-response curves. “⊕” represent the fitted values for the present day conditions in the Chatham Rise area, and “⊗” represent the fitted values for the predicted future conditions (2100) in the Chatham Rise area. Error bars represent standard deviation (n=3).

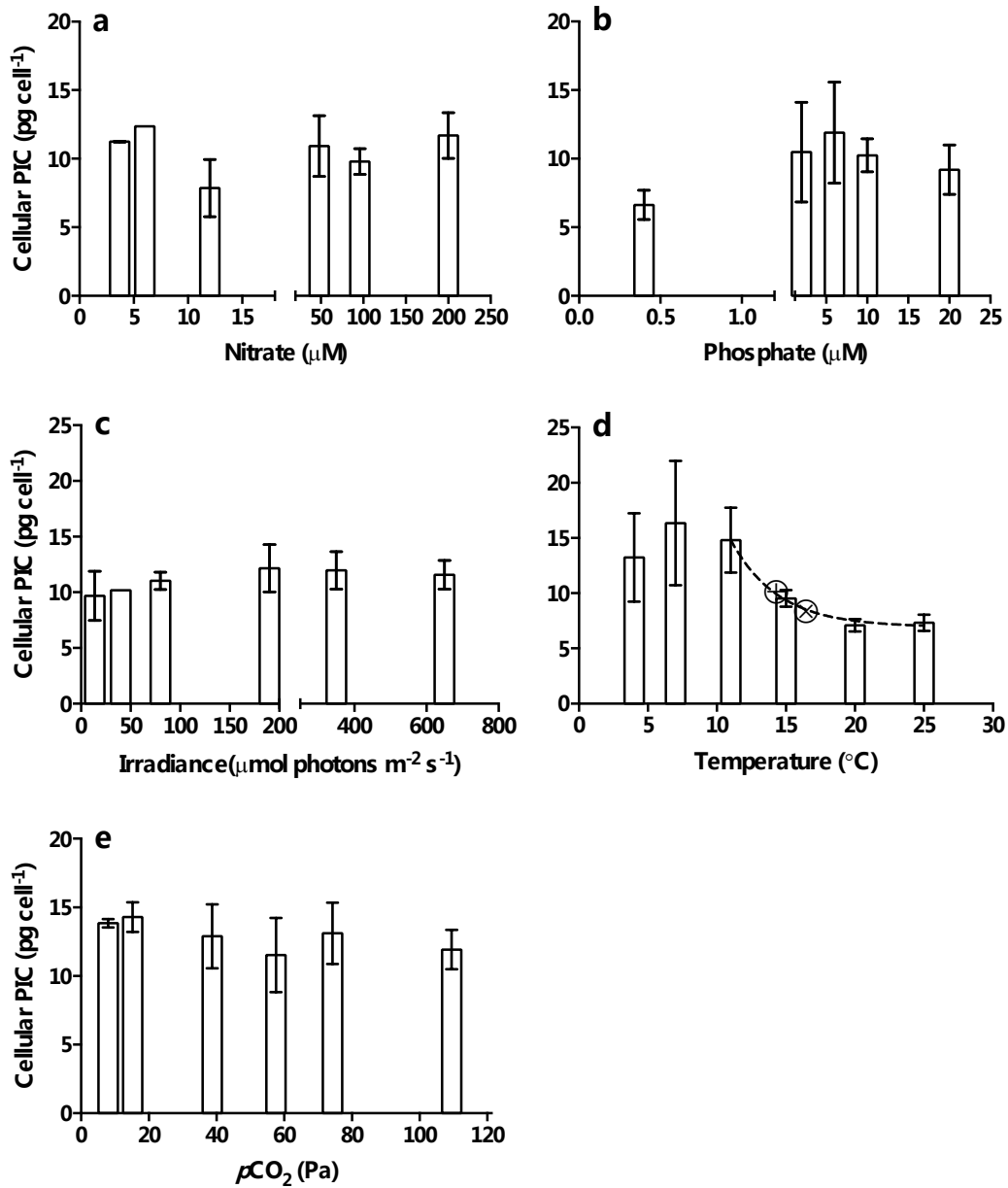


Figure 2. Changes in *Emiliana huxleyi* cellular particulate inorganic carbon (PIC) content in response to different environmental drivers: a) cellular PIC vs. nitrate concentration; b) cellular PIC vs. phosphate concentration; c) cellular PIC vs. irradiance; d) cellular PIC vs. temperature; and e) cellular PIC vs. $p\text{CO}_2$.

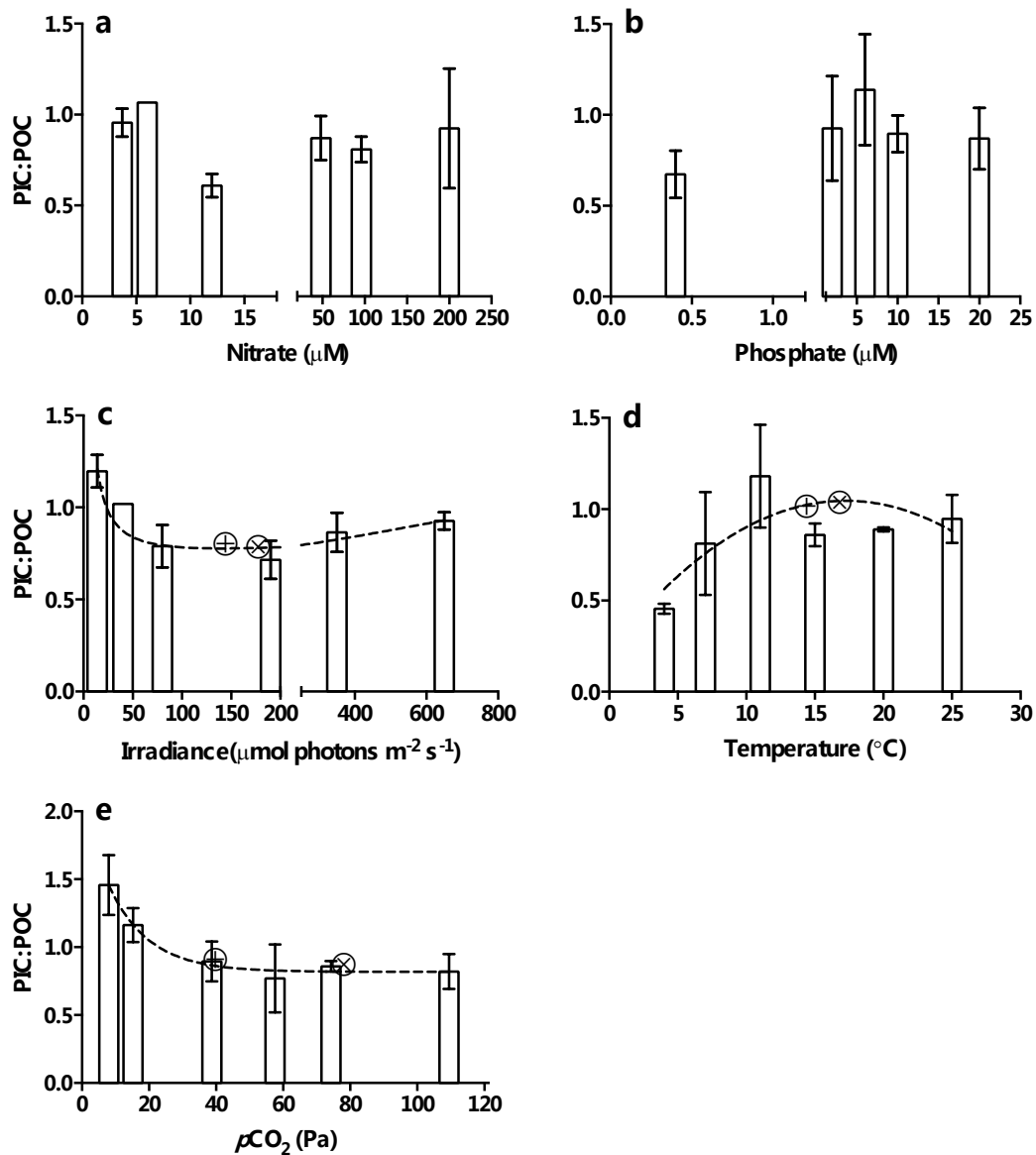


Figure 3. Changes in the ratio of *Emiliana huxleyi* cellular particulate inorganic carbon content to particulate organic carbon content (PIC:POC) in response to different environmental drivers: a) PIC:POC ratio vs. nitrate concentration; b) PIC:POC ratio vs. phosphate concentration; c) PIC:POC ratio vs. irradiance; d) PIC:POC ratio vs. temperature; and e) PIC:POC ratio vs. $p\text{CO}_2$.

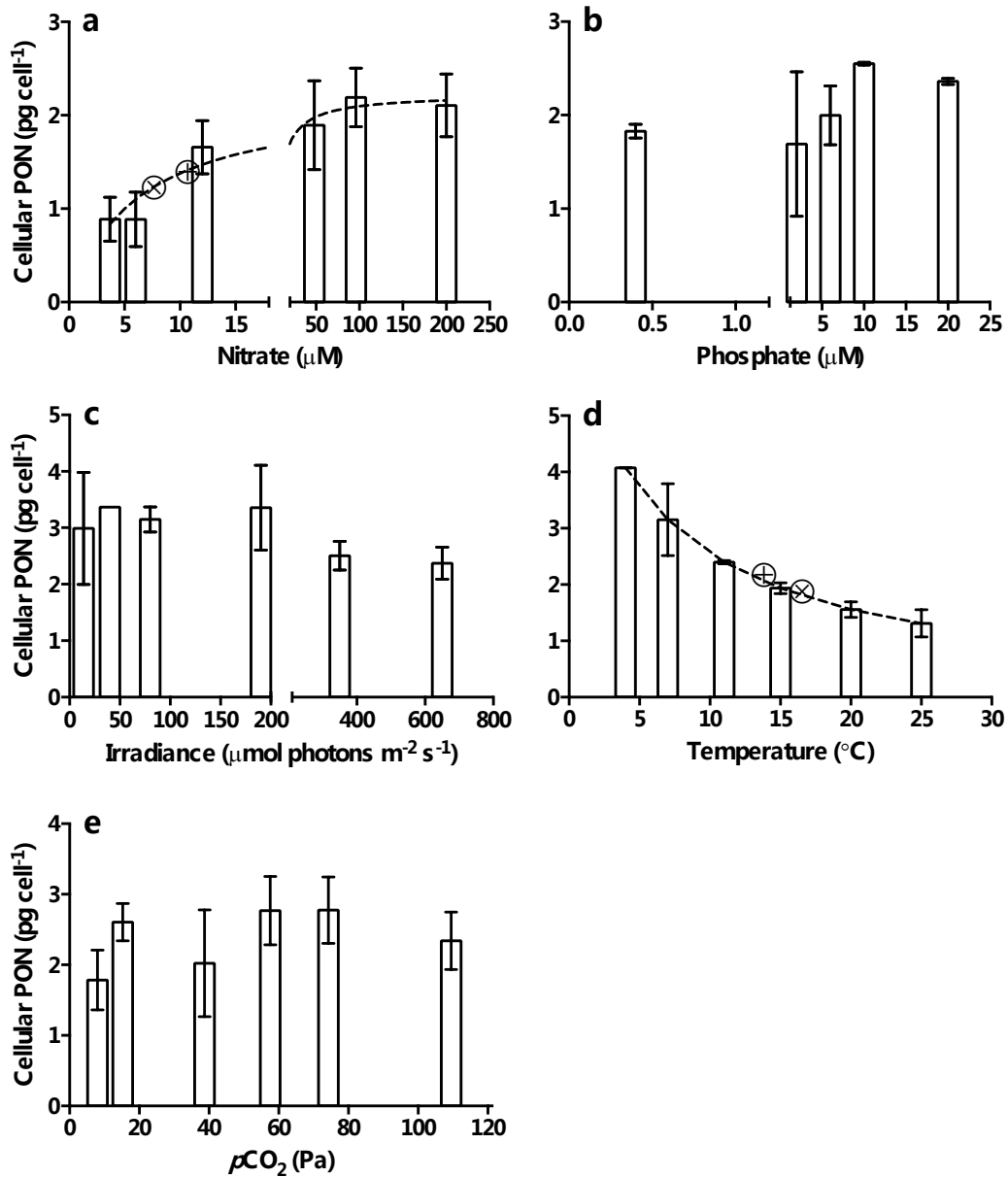


Figure 4. Changes in *Emiliana huxleyi* cellular particulate organic nitrogen (PON) content in response to different environmental drivers: a) cellular PON vs. nitrate concentration; b) cellular PON vs. phosphate concentration; c) cellular PON vs. irradiance; d) cellular PON vs. temperature; and e) cellular PON vs. $p\text{CO}_2$.

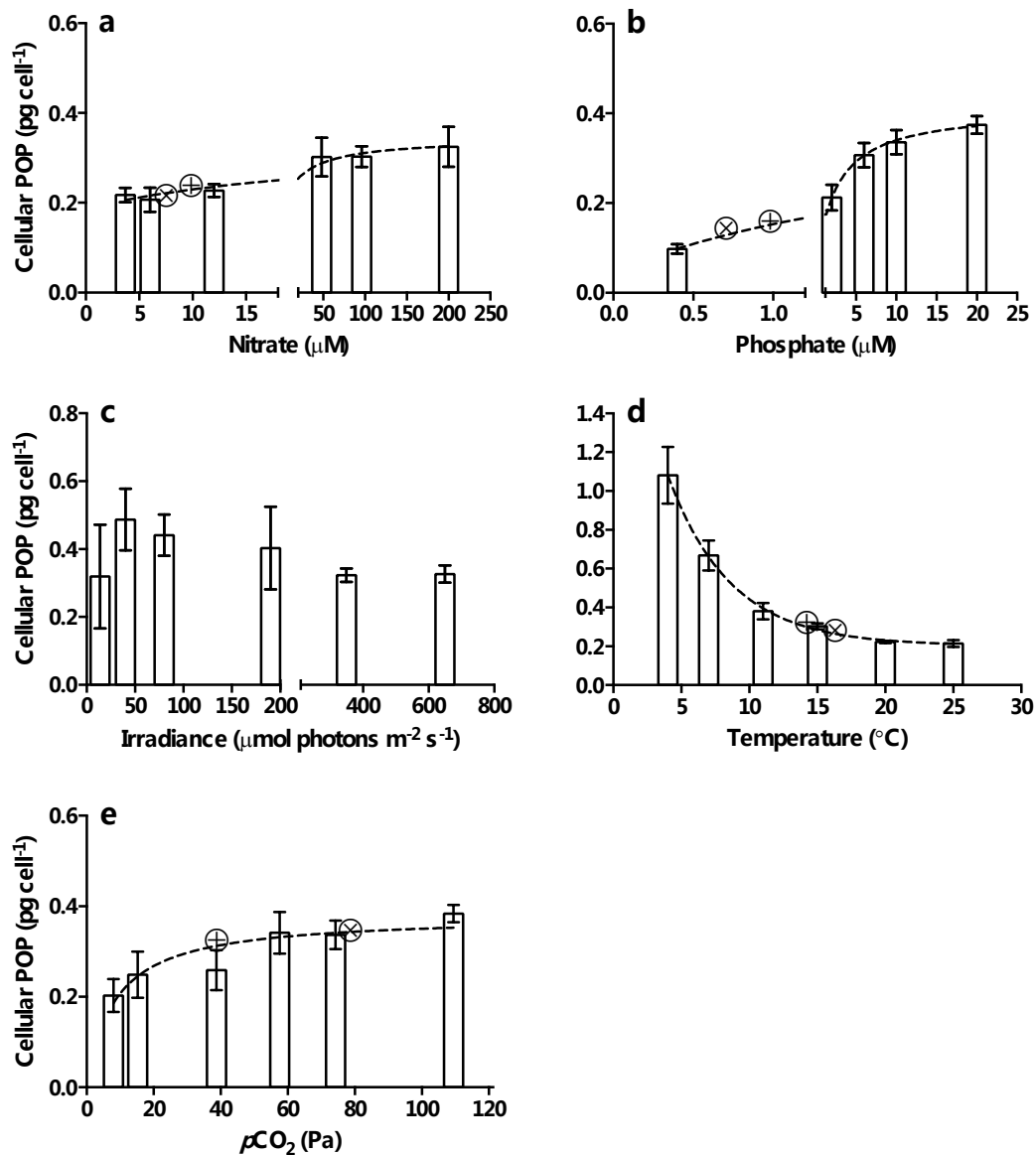


Figure 5. Changes in *Emiliania huxleyi* cellular particulate organic phosphorus (POP) content in response to different environmental drivers: a) cellular POP vs. nitrate concentration; b) cellular POP vs. phosphate concentration; c) cellular POP vs. irradiance; d) cellular POP vs. temperature; and e) cellular POP vs. $p\text{CO}_2$.

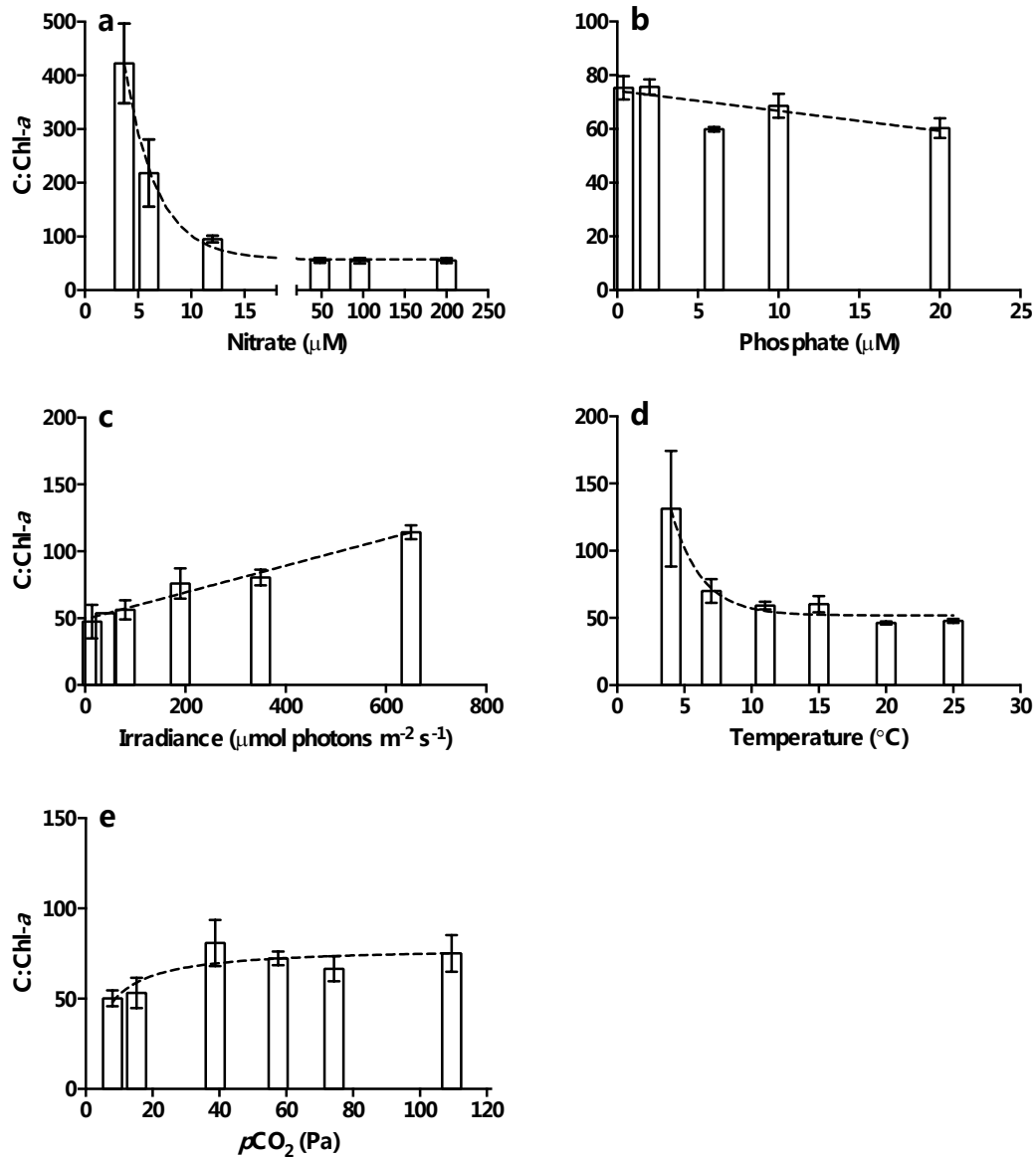


Figure 6. Changes in the ratio of *Emiliana huxleyi* cellular particulate organic carbon content to chlorophyll *a* content (C:Chl-*a*) in response to different environmental drivers: a) C:Chl-*a* ratio vs. nitrate concentration; b) C:Chl-*a* ratio vs. phosphate concentration; c) C:Chl-*a* ratio vs. irradiance; d) C:Chl-*a* ratio vs. temperature; and e) C:Chl-*a* ratio vs. $p\text{CO}_2$. Error bars represent standard deviations (n=3).

5

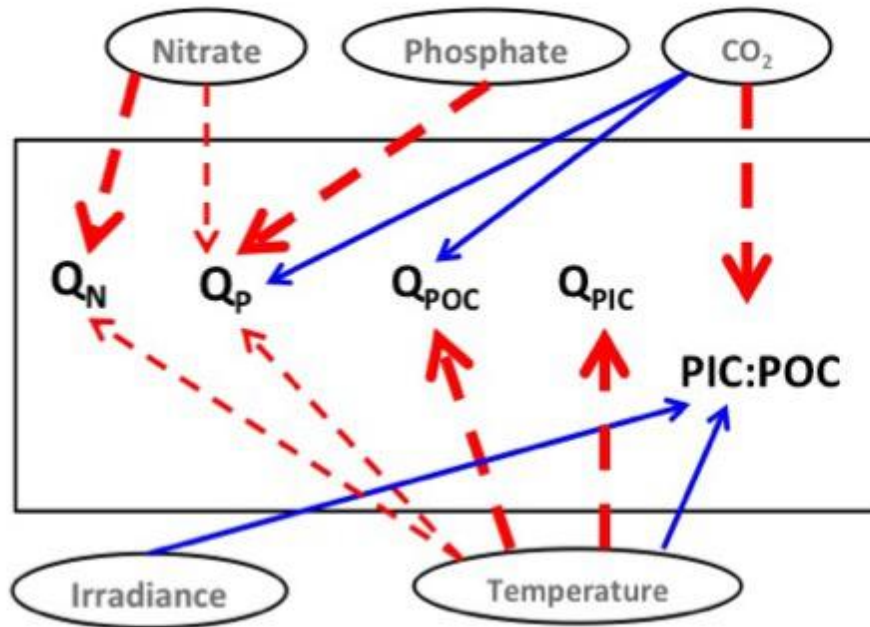


Figure 7. Conceptual **figure model** of the specific effects of each the five environmental drivers, under the projected future conditions (year 2100), on the elemental composition of *Emiliana huxleyi*. **Q represents the cellular quota of each element of *Emiliana huxleyi*.**

- 5 ***The box denotes the *E. huxleyi* cell. Solid blue arrows indicate positive effects of the future environmental changes; and dash red arrows indicate negative effects of the future environmental changes. Arrows in bold indicate the environmental drivers that play the most important role regulating the connected physiological metrics under the predicted environmental conditions for the year 2100.

Table 1. Treatment conditions for each environmental driver used in the manipulation experiments.

| Environmental driver | Treatments |
|-------------------------------------------------------------|---------------------------|
| Nitrate (μM) | 3.7; 6; 12; 48; 96; 200 |
| Phosphate (μM) | 0.4; 2; 6; 10; 20 |
| Irradiance ($\mu\text{mol photons m}^{-2} \text{s}^{-1}$) | 14; 40; 80; 190; 350; 650 |
| Temperature ($^{\circ}\text{C}$) | 4; 7; 11; 15; 20; 25 |
| $p\text{CO}_2$ (Pa) | 8; 15; 39; 58; 74; 109 |

Table 2. Elemental molar ratios of N:P, C:N and C:P of *Emiliania huxleyi* from the five single-factorial manipulation experiments. The errors are standard deviations around the mean (n=3). The values in bold are significantly different compared to other treatments.

| Environmental driver | Treatment | N:P (mol:mol) | C:N (mol:mol) | C:P (mol:mol) |
|-----------------------------------------------------------------|-----------|-------------------|-------------------|---------------------|
| Nitrate (μM) | 3.7 | 9.09±2.48 | 15.90±4.09 | 137.69±8.27 |
| | 6 | 9.07±1.55 | 13.16±1.13 | 118.45±10.06 |
| | 12 | 15.90±1.38 | 9.00±0.07 | 143.08±11.28 |
| | 48 | 14.46±1.39 | 7.01±0.64 | 107.76±11.93 |
| | 96 | 16.16±3.45 | 6.56±1.07 | 103.56±6.54 |
| | 200 | 14.31±0.40 | 7.32±0.47 | 104.82±8.34 |
| Phosphate (μM) | 0.4 | 41.57±4.14 | 6.32±0.42 | 261.67±17.30 |
| | 2 | 21.47±0.28 | 9.27±5.08 | 137.57±11.31 |
| | 6 | 14.17±3.59 | 6.16±1.24 | 85.00±4.51 |
| | 10 | 17.06±2.06 | 5.24±0.06 | 89.28±9.80 |
| | 20 | 13.99±0.89 | 5.22±0.08 | 73.04±3.48 |
| Irradiance ($\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) | 14 | 22.03±7.27 | 4.26±0.29 | 73.82±6.48 |
| | 40 | 14.27** | 4.38** | 62.50** |
| | 80 | 16.03±2.90 | 5.24±0.69 | 83.96±17.05 |
| | 190 | 18.71±1.74 | 5.99±0.23 | 112.39±14.60 |
| | 350 | 17.15±0.83 | 6.47±0.54 | 110.63±3.98 |
| | 650 | 16.11±1.77 | 5.70±0.31 | 91.49±6.66 |
| Temperature ($^{\circ}\text{C}$) | 4 | 8.92±1.29 | 8.67±2.64 | 71.34±27.92 |
| | 7 | 10.46±2.05 | 7.60±1.32 | 78.70±14.78 |
| | 11 | 13.58±1.91 | 6.21±0.32 | 86.56±11.92 |
| | 15 | 14.12±0.66 | 6.70±0.31 | 94.47±3.84 |
| | 20 | 15.53±1.06 | 5.98±0.15 | 92.88±6.33 |
| | 25 | 13.67±2.99 | 7.08±1.39 | 93.96±4.87 |
| $p\text{CO}_2$ (Pa) | 8 | 19.39±2.41 | 6.81±1.09 | 122.61±9.97 |
| | 15 | 24.01±6.80 | 5.80±0.71 | 137.50±33.51 |
| | 39 | 16.96±3.62 | 7.41±0.06 | 155.64±31.42 |
| | 58 | 17.89±0.80 | 6.55±1.16 | 116.67±16.15 |
| | 74 | 18.22±2.45 | 6.42±0.43 | 116.93±17.60 |
| | 109 | 13.56±2.78 | 7.41±1.20 | 99.25±17.70 |

** Sample loss during analysis resulted in only single values at this irradiance.

Table 3. Comparison of cellular particulate organic carbon (POC) contents, particulate inorganic carbon (PIC) contents, PIC:POC ratios, particulate organic nitrogen (PON) contents and particulate organic phosphorus (POP) contents of *Emiliania huxleyi* between projected (year 2100) and present day Chatham Rise conditions, with rankings of the importance of the environmental drivers which caused significant effects on each physiological parameter. The numbers of the ranking scheme represent the gradient of the most (1) to least (4) important effects. Effect of “+” represents an increase and “-“ represents a decrease in the elemental composition/ratio in the future, respectively.

| Physiological parameter | Environmental driver | Fitted values at different conditions of environmental drivers* | | Future vs. present day comparisons | | |
|-----------------------------------------------|----------------------|-----------------------------------------------------------------|--------|------------------------------------|---------------|-----------|
| | | Present day | Future | Change (%)** | Effects (+/-) | Ranking |
| Cellular POC content (pg cell ⁻¹) | Temperature | 10.798 | 9.713 | 10.0 | - | 1* |
| | CO ₂ | 14.632 | 15.436 | 5.5 | + | 2 |
| | Irradiance | 14.774 | 14.827 | 0.3 | + | 3 |
| | Nitrate | | | | | n.s. |
| | Phosphate | | | | | n.s. |
| Cellular PIC content (pg cell ⁻¹) | Temperature | 10.206 | 8.753 | 14.2 | - | 1 |
| | Nitrate | | | | | n.s. |
| | Phosphate | | | | | n.s. |
| | Irradiance | | | | | n.s. |
| | CO ₂ | | | | | n.s. |
| PIC:POC | CO ₂ | 0.868 | 0.821 | 5.4 | - | 1 |
| | Temperature | 1.017 | 1.042 | 2.4 | + | 2 |
| | Irradiance | 0.777 | 0.780 | 0.3 | + | 3 |
| | Nitrate | | | | | n.s. |
| | Phosphate | | | | | n.s. |
| Cellular PON content (pg cell ⁻¹) | Nitrate | 1.380 | 1.162 | 15.8 | - | 1 |
| | Temperature | 2.013 | 1.819 | 9.6 | - | 2 |
| | Phosphate | | | | | n.s. |
| | Irradiance | | | | | n.s. |
| | CO ₂ | | | | | n.s. |
| Cellular POP content (pg cell ⁻¹) | Phosphate | 0.106 | 0.078 | 25.9 | - | 1 |
| | Temperature | 0.304 | 0.269 | 11.6 | - | 2 |
| | CO ₂ | 0.312 | 0.342 | 9.6 | + | 3 |
| | Nitrate | 0.249 | 0.227 | 8.9 | - | 4 |
| | Irradiance | | | | | n.s. |

*The fitted values for “present day” and “future” were extracted from the fitted dose-response curves (Fig.1 - 5) at the stock culture growing conditions, average present day conditions in the Chatham Rise area, and the predicted future conditions (2100) of Chatham Rise, respectively.

** The percentage changes were calculated as the changes caused by each environmental driver under the future predicted condition relative to that under the present day condition.

*Numbers in bold indicate statistically significant difference between the range of present day and future conditions (nitrate treatments: 6.0 and 12.0 μM; phosphate treatments: 0.4 and 2 μM; irradiance treatments: 80 and 190 μmol photons m⁻² s⁻¹; temperature treatments: 11°C, 15°C and 20°C) based on the one-way ANOVA. “n.s.” indicates non-significant difference (one-way ANOVA) among all the treatments used for the fitting.