To editor and reviewers,

First of all, we greatly appreciate the generosity of editor for allowing us the total of two extensions for our major revision. We also thank reviewers for very detailed comments and insights. Following your suggestions, we carried out substantial new analyses. We now believe that our results and conclusions are more robust and the quality of this manuscript has increased dramatically. Thank you once again for coordinated efforts under this very difficult time period.

Kind regards,

Tatsuro Tanioka (Corresponding author)

Response to reviewer 1

We thank the reviewer for insightful comments and suggestions for our manuscript. Please find attached all your comments and our responses (comments are in *italic*, our responses are in blue). Line numbers are those numbers used in the redlined manuscript (**p24 – p60 of this document**).

General Comments:

1. *The manuscript by Tanioka & Matsumoto is a well written and informative examination of the driving environmental factors of marine phytoplankton major element stoichiometry. The meta-analysis and use of the 's-factor' provides interesting new insights into the variability of different elemental ratios in the context of changing resource availability.*

   Thank you for these encouraging comments.

2. *Thought the article is well written and likely the subject of considerable interest, there are a number of serious issues that need to be addressed before it can be recommended for publication. These include: problems with the taxonomic affiliation of some 'diatoms' in the data analysis; a lack of discussion of the limitations, confounding factors and more basic details of the database; and the use of functional groups, which directly influences the conclusions.*

   We understand that four main issues are: 1) not correctly categorizing taxonomic affiliation of some 'diatoms' in the data, 2) not discussing limitation of the analysis, 3) database not providing enough information, and 4) not considering other plankton functional groups. We have addressed these issues extensively in the revised version.

3. *Looking through the figures it was clear that a number of non-diatoms were included in the meta-analysis for the diatom group. These include: the dinoflagellate Alexandrium minutum (diatom N:C and N:P, Fig. 2), the green algae Chlorella sp (diatom N:C and irradiance, Fig. 3), and the prymnesiophyte Phaeocystis antarctica (diatom P:C and temperature, Fig. 4). These taxa will need to be removed from the diatom grouping, leading to the need to re-run some of the statistical analysis.*

   We appreciate the reviewer for pointing our mistakes. We have corrected these misclassifications in the new database and have re-run all the statistical analyses.

4. *On discovering these mis-classifications, this reviewer began looking further into the taxonomy and ecology of the other species included in the functional groupings. This highlighted that in contrast to the diatom grouping, the eukaryotes included members of a huge range of taxonomic groups, with diverse ecologies (e.g. motility, biomineralisation), distributions (marine, estuarine) and likely physiologies. The cyanobacteria are another example of this issue, where single-celled oceanic and coastal species are simply grouped together with colonial species which are prominent nitrogen-fixing taxa. Simple traits within all the functional groups assessed, such as cell size or motility, cover a large range, despite their implications on nutrient uptake, cell metabolism and light harvesting (and hence likely elemental content). Using these groupings, with the assumption that such diverse taxa should confirm to a joint response to environmental variability, and then concluding that diatoms showed a more consistent response than the other functional groupings, is highly questionable. A more refined approach to the non-diatoms is needed, either in terms of sub-groupings to an appropriate taxonomic or functional level, or*

rephrasing the conclusions so that the lack of taxonomic diversity in the diatoms is recognized as allowing this group to show a consistent response.

Our original justifications were based on two reasons. First, we wanted to give a relatively balanced number of studies across each of the three categorical moderators (diatoms, non-diatom eukaryotes, and cyanobacteria). Second and critically, we wanted our results to be easily transferable to global ocean biogeochemical models with 3-4 phytoplankton functional groups. We therefore deliberately chose this broad classification.

That being said, we have followed the reviewer suggestion to analyze the data with a finer classification for the non-diatoms. In the revised version, we used more specific moderator for PFTs: 1) diatoms, 2) coccolithophores, 3) dinoflagellates, 4) other eukaryotes, 5) prokaryotes, and 6) diazotrophs. In addition, we carried out between moderator heterogeneity tests on 1) Eukaryotes vs Prokaryotes, and 2) Cold-water species vs Temperate species.

5. *Any data analysis is only as good as the quality of data it includes. Within the manuscript there is no examination, exploration or discussion of potential issues with the input data. Some analysis of the nutrient ranges (how replete or deplete where the experimental conditions?), irradiance gradients (where low light cultures light-limited? where high light cultures photo-inhibited?), or basic details of the growth conditions (temperature, salinity, light-dark cycle, light level) needs including. Were all cultures acclimated to experimental conditions for (e.g.) 10 generations? Did studies use natural seawater or artificial seawater? Where cultures grown under optimum temperature or salinity conditions? Are any of the species included in the eukaryote grouping euryhaline and were they grown under low (or high) salinity conditions? Such key details would have needed to be included and justified in the original studies, so why not in a meta-analysis of all the data? Could some of the strong responses that were distinct from other species be due to the growth conditions or other confounding factors (e.g. sub-optimal salinity, temperature, light-limitation)?*

   In the original dataset, we had already included the basic details of the growth conditions mentioned here (temperature, light-dark cycle, and light level). We have added in the revised version details on salinity, culture medium (natural or artificial seawater), acclimation (# of generations),growth mode (batch, semi-continuous, and chemostat), and growth phase (lag, exponential, decline, stationary).

Specific comments:

6. *Ln 6: 'The elemental stoichiometry of marine phytoplankton plays a critical role in the global carbon cycle through carbon export'. Surely elemental stoichiometry plays other critical roles in ocean biogeochemistry, such as differential nutrient cycling and subsequent nutrient limitation, or dictating the quantity and quality of organic matter formed through primary and secondary production?*

   Thank you for your suggestion. We mentioned in Line 7 the importance of elemental stoichiometry in nutrient cycling, remineralization, and secondary production.

7. *Ln 31-32: What about supply of nitrate from nitrification?.*

Since ammonium that is converted to nitrate via nitrification are produced via recycling of organic matter, nitrogen will not be newly added to the system by nitrification per se. Therefore, nitrification will not affect the balance of N:P over geologic timescale.

What about the loss terms? The balance of N:P will depend on the supply and loss terms over geological time scales

The loss terms, burial and denitrification, are important on geologic timescale. We mention this in Lines 48-49.

8. *Ln 157: Meta-analysis within 3 plankton functional types (diatoms, eukaryotes excluding diatoms, cyanobacteria) as a categorical moderator – not three functional types (i.e. eukaryotes not functional type and contain diverse taxa with distinct ecology and physiology). Also cyanobacteria grouping contains both nitrogen-fixing taxa and nonnitrogen fixing taxa, with highly differential impacts on the N:C and P:C ratios and the impact of N, P and Fe availability on their stoichiometry.*

   As mentioned in our reply to the general comment #4, we have redefined new categorial phytoplankton classes (Lines 233-235): 1) diatoms, 2) coccolithophores, 3) dinoflagellates, 4) other eukaryotes, 5) prokaryotes, and 6) diazotrophs.

9. *Ln 186: 'NO3 is one of the primary drivers of N:C'. What about the availability of other N sources?*

   In our meta-analysis, we conducted a moderator test on different types of N source (nitrate, ammonium, nitrate + ammonium, diazotrophy) and found no statistically significant differences amongst them for N:C (Table S1).

10. *Ln 186-187: So the s-factor for NO3 and N:C is 0.22 $\pm$0.04 for diatoms and 0.17 $\pm$0.04 for eukaryotes, are these statistically different enough to support the statement that 'diatoms are the most sensitive PFT'?*

    Thank you for clarifying. The difference between PFTs is not in fact statistically significant for N:C (Table S1, Figure S1d). However, eukaryotes are stoichiometrically more sensitive than prokaryotes ($P < 0.05$, Fig. 3a). We have rephrased our conclusion and abstract accordingly.

11. *Ln 243-244: How often does nutrient toxicity impact natural communities of phytoplankton? The phrasing of this statement should be modified to reflect just how high nutrient concentrations need to be to induce nutrient toxicity – i.e. nutrient concentrations are in excess of requirements during early spring prior to the spring bloom when phytoplankton biomass is low.*

    Although nutrient toxicity, especially that of iron (II), is quite common in some lagoon environments (Demirel et al., 2009; Swanner et al., 2015), it is not the case for other nutrients. We have therefore removed this sentence.

12. *Ln 250-253: What about fundamental taxonomic differences?*

Since this sentence was vague and not well supported, we have removed it in the revised manuscript.

13. *Ln 357-358: Is it the length of the light period per se or the total daily light dose that is important in terms of the effects of different light regimes? Does the data base not contain this information i.e. light-dark cycle and irradiance level)?*

    Thank you for this suggestion. We have conducted a between moderator heterogeneity test with continuous light versus periodic light and have shown that lighting regime does indeed have a significant effect on N:C (Fig. 3d, $P < 0.05$).

14. *Ln 362-364: Surely N availability has a stronger influence on N:C in light-replete low latitudes (i.e. the subtropical gyres)?*

    Our message here is that light availability affects N:C the most in high latitudes, where N is high but light is low. N availability does indeed have a larger influence on N:C than irradiance (Fig. 2). We have therefore rephrased Lines 824-827.

15. *Ln 377-378: Is 'temperature arguably the most important environmental factor affecting growth and survival' of phytoplankton?*

    Although this phrase is a direct quote from the well-known text of microbiology (Brock, Biology of Microorganisms) we agree that it is not supported by our meta-analysis. We therefore removed this sentence in the revised edition.

16. *Ln 419-422: The authors state that differences in the overall conclusions in their metaanalysis with previous ones (e.g. Yvon-Durocher et al., 2015) is due to the two analyses assessing different sets of studies (over different time-scales).*

    After conducting re-analysis with our new dataset, our result does indeed agree with the study by Yvon-Durocher et al, in which they found that increase in temperature leads to lower P:C (higher C:P) (Fig. 2). We have rephrased Lines 831-836 accordingly.

    If this is true as the only reason for the divergence of conclusions, can we expect a different conclusion from a future study done in another (e.g.) 20 years?

    This is possible, although it is obviously impossible to predict the outcome of a future meta-analysis. However, as we are beginning to get a better understanding of the physiological/mechanistic causes behind change in stochiometric ratios due to temperature, it is unlikely that future meta-analyses would yield radically different results.

17. *Ln 432-434: The use of 'that' early in the sentence skews the meaning and interpretation of the statement: 'This suggests <that> an increase in the carbon assimilation via photosynthesis and/or a reduction in the formation of nitrogen rich compounds such as porphyrin and phycobiliproteins that are essential for light harvesting..'.*

    Thank you for pointing this out. We modified the sentence (Lines 720-722) accordingly.

Reference:

Demirel, S., Ustun, B., Aslim, B. and Suludere, Z.: Toxicity and uptake of Iron ions by Synechocystis sp. E35 isolated from Kucukcekmece Lagoon, Istanbul, J. Hazard. Mater., 171(1–3), 710–716, doi:10.1016/j.jhazmat.2009.06.058, 2009.

Swanner, E. D., Mloszewska, A. M., Cirpka, O. A., Schoenberg, R., Konhauser, K. O. and Kappler, A.: Modulation of oxygen production in Archaean oceans by episodes of Fe(II) toxicity, Nat. Geosci., 8(2), 126–130, doi:10.1038/ngeo2327, 2015.

Response to reviewer 2

We thank the reviewer for insightful comments and suggestions for our manuscript. Please find attached all your comments and our responses (comments are in *italic*, our responses are in blue). Line numbers are those numbers used in the redlined manuscript (**p24 – p60 of this document**).

General Comments:

1. *This study addresses the very important topic of stoichiometric variability in marine phytoplankton. Understanding the magnitude and drivers of this variability as well as its taxonomic variation are essential for developing new and more accurate global biogeochemical models. The authors take a novel approach to this problem by performing a meta-analysis through which they calculate a sensitivity factor for major stoichiometries (N:C, P:C, and N:P) in response to a suite of environmental drivers. The goal of such a quantitative approach - to estimate the group-specific response of these stoichiometries to expected changes in ocean conditions - is laudable.*

   Thank you for these encouraging comments.

2. *However, there are several major flaws in how this approach is applied and how studies are selected and screened for this meta-analysis that would need to be addressed for this to be published in Biogeosciences. Additionally, these major flaws in approach receive little or no discussion throughout the manuscript.*

   The major issues we addressed in the revision are: 1) study selection criteria (comment #19), 2) S-factor calculation (comment #20), 3) and the overall discussion of the methodological limitations. Please refer to the responses to specific comments for more detail. We note that what are referred to as major flaws (i.e., application of the power law metric to studies with 3 environmental levels) are deliberate and justifiable choices we made given our motivation to develop possibly nonlinear stoichiometric formulations for use in global biogeochemical models. As discussed below, there are tradeoffs in selecting studies with 2 or 3 levels. But in our revision, we heeded the suggestion of the reviewer and considered the additional selection criteria.

3. *The authors present their approach to estimating a response to an environmental condition as more nuanced and informative than simply calculating a response between two end points or experimental treatments. While those simplistic, past approaches have numerous limitations, they were generally acceptable for meta-analyses due to two major challenges: 1) the high variability in experiment conditions of individual studies; and 2) the fact that some environmental drivers may produce linear or at least monotonic responses within a range of natural variability (e.g. the response to nutrient availability), while other drivers produce responses that are distinctly antitonic (e.g. temperature and irradiance). Essentially the authors have suggested a more complex metric for such meta-analyses without addressing these two major challenges. As a result, ambient nutrient concentrations are treated as a measure of a study species' nutrient status that is comparable across different experiment types (semi-continuous batch vs. chemostat), which is inappropriate for several reasons (addressed below in my specific comments). The flaws of this approach are not discussed in the manuscript and the approach is used to make the study's strongest conclusion, that diatom P:C and N:C are*

particularly sensitive to N and P availability. It should be added that this result is based on meta-analysis of only four studies, one of which was on a dinoflagellate and incorrectly categorized.

Thank you for this suggestion. As suggested, we conducted new meta-analysis using two end points which should resolve the two major challenges mentioned here: 1) the high variability in experiment conditions of individual studies; and 2) the fact that some environmental drivers may produce linear or at least monotonic responses. We conducted two point meta-analysis using two different measures of effect size (natural-log response ratios and stoichiometric sensitivity factor) for the same dataset and have shown that both of these measures yield the same results (Figure 2a and b).

4. *This approach also results in deeming a given stoichiometry as sensitive to a driver like irradiance or temperature if that stoichiometry has a monotonic response to these drivers. Considering that the responses of phytoplankton to light and temperature are distinctly non-linear and antitonic (usually displaying a clear central optimum), this approach seems very flawed.*

   As mentioned in the previous point (#3), we conducted two point meta-analysis which should have satisfactorily resolved the issues mentioned here.

5. *Considering its novelty and potential value, the approach used by the authors should not be discarded, but refinement and far more discussion of its limitations would be necessary to present it in a manuscript.*

   We kept our s-factor (fractional change in X:C over fractional change in independent variable) as our effect size but made refinement to data selection by choosing two end points instead of > 3 in the previous version. We also conducted a meta-analysis using more traditional measure of effect size (log response ratio) and obtained consistent results.

6. *The computational needs of the sensitivity factor that the authors use (requiring experiments where the response to at least 3 levels of an environmental driver were measured) also seems to have resulted in a meta-analysis of a somewhat limited number of studies. While this criteria is strict, there is no study selection criteria mentioned that address the many other confounding factors that could differ among studies and little or no discussion of such factors.*

   There is a tradeoff between using two points (more studies but linear response) and three points (fewer studies but possibly nonlinear response). We focused on the latter in the previous version but now acknowledge the merits of using just two points. As suggested, we added a new selection criterion to include studies with 2 levels and we were able to increase the number of studies for meta-analysis from 64 studies to 104 studies.

7. *Along with this lack of evaluation of the original studies used in the meta-analysis, there is also little comparison of the results of this work to the findings of several other narrative reviews and quantitative meta-analyses of phytoplankton stoichiometry, most of which considered a larger number of original studies. These past studies are generally just mentioned for comparison of approaches, but not their results are not critically evaluated in light of the authors' contributions to this topic.*

Thank you for this suggestion. We have more critically compared and discussed our results with those from previous synthesis studies especially with these more recent studies: Moreno and Martiny (2018); Villar-Argaiz et al. (2018); Yvon-Durocher et al. (2015).

8. *As mentioned above, there also seems to be several studies that were incorrectly categorized, with non-diatom species appearing to be grouped with diatoms in the group-specific meta-analyses.*

   Thank you for picking out our errors. We updated our database and re-conducted meta-analysis with correct classification.

Specific comments:

Abstract:

9. *Line 18-20: It seems overly simplistic to imply that the temperature response of cyanobacteria is responsible for global P:C patterns without acknowledging the effect of macronutrient availability, which you have also shown to have a strong effect on P:C and N:C. The global patterns in C:N:P (lower P:C and N:C in subtropics, higher in subpolar and upwelling regions) has also been attributed to macronutrient availability and phytoplankton biogeography with the relative impact of all three drivers being a rich and contentious area of research. Linking your findings to this on-going area of study should either be excluded from the abstract or addressed in a more complete fashion by noting that the macronutrient sensitivity of diatom C:N:P and the temperature sensitivity of cyanobacteria C:N:P you observe are both helpful in explaining the persistent global patterns in C:N:P.*

   Our intent was that temperature is possibly an important factor along with other factors such as macronutrients in explaining the subtropical cyanobacteria C:N:P. As pointed out by the reviewer, we neglected to note the other factors. We modified the sentence (Lines 21-23) to read: "Along with other oceanographic conditions of the subtropical gyres (e.g., low macronutrient availability), elevated temperature may explain why P:C is consistently low in subtropical oceans."

Introduction:

10. *Line 43-45: This sentence should be supported by citations. It is not clear which of the citations in the previous sentence (if any) are the sources for this information.*

    Information comes from the review paper by Moreno and Martiny (2018). We now cite this paper in the revised manuscript (Line 63).

11. *Line 53-55: This statement is vague and detailed specific support for this should be given.*

    The main message of this sentence is that environmentally induced trait change is variable because it is driven by both plasticity (acclimation) and adaptation, which differs amongst species (Collins et al., 2020; Ward et al., 2019). We rephrased this sentence (Lines 89-92) to make the meaning clearer.

    It's worth clarifying why previous studies have not yielded a broader understanding of how phytoplankton C:N:P varies across taxa and environmental conditions (and thus justifying your meta-analysis).

As the field of marine ecological stoichiometry itself is new (i.e., the transition from traditional Redfieldian view), fundamentally, there is not yet many studies that give **broad and quantitative** views on how marine environmental factors affect plankton C:N:P. Our main motivation for this work therefore was to build a database that could be used to calibrate power-law based flexible C:N:P model of phytoplankton (Tanioka and Matsumoto, 2017) that can easily be incorporated into marine biogeochemical models. We also aim to build on previous phytoplankton cellular models (e.g., Pahlow and Oschlies, 2009) that are usually calibrated with very few selected studies from 20-30 years ago (e.g., Laws and Bannister, 1980).

Also, the inherent genetic differences among taxa don't simply correspond to differences in environmental responses, they correspond to inherent differences in steady-state C:N:P under ideal conditions among major phytoplankton groups (Quigg et al. 2003; Garcia et al. 2018) that likely reflect basic differences in cellular structure and size (Finkel et al. 2016a; Finkel et al. 2016b). See references below.

- *Quigg, A., Finkel, Z. V., Irwin, A. J., Rosenthal, Y., Ho, T. Y., Reinfelder, J. R., ... & Falkowski, P. G. (2003). The evolutionary inheritance of elemental stoichiometry in marine phytoplankton. Nature, 425(6955), 291.*
- *Garcia, N. S., Sexton, J., Riggins, T., Brown, J., Lomas, M. W., & Martiny, A. C. (2018). High variability in cellular stoichiometry of carbon, nitrogen, and phosphorus within classes of marine eukaryotic phytoplankton under sufficient nutrient conditions. Frontiers in microbiology, 9, 543.*
- *Finkel, Z. V., Follows, M. J., Liefer, J. D., Brown, C. M., Benner, I., & Irwin, A.J. (2016a). Phylogenetic diversity in the macromolecular composition of microalgae. PLoS One, 11(5), e0155977.*
- *Finkel, Z. V., Follows, M. J., & Irwin, A. J. (2016b). Size-scaling of macromolecules and chemical energy content in the eukaryotic microalgae. Journal of Plankton Research, 38(5), 1151-1162.*

Thank you for this insight. We touched this point in the discussion section comparing the fundamental differences in responses between eukaryotic versus prokaryotic phytoplankton groups (Lines 699-703). We note however that our study is more concerned with **changes in C:N:P** under a transient condition rather than C:N:P value at a steady-state.

12. *Line 55-58: In addition to the point made in the previous comment, there are many reasons why it is hard to draw consensus from the various studies of phytoplankton C:N:P, but an inconsistency of statistical analyses seems like one of the least compelling of these reasons. What about the differences in how experimental treatments are applied, particularly for macronutrient limitation (e.g. steady-state vs batch cultures and differences in the duration of nutrient stress)? What about confounding experimental conditions (e.g. bacterial contamination, low CO2 availability/high pH in dense batch cultures)? Or more simply, the fact that many studies only measure one or two of the three major elements and few measure the biochemical components that determine elemental quotas. These are all factors that make understanding how phytoplankton C:N:P varies across taxa and conditions difficult when using existing literature and seem much more important than the selection of statistical analyses. Not mentioning these factors in the introduction and, more importantly, in the methods section when considering selection criteria is a major omission in this paper.*

Thank you for this suggestion. Although it is inherently impossible to consider all of the factors mentioned above, we conducted analyses on the effects of some of these important moderators (Section 2.3.5). These include growth mode (batch, semi-continuous, chemostat), N type, growth phase at harvest, and the light regime (Fig. 3, Table S1).

13. *Line 59-67: This paragraph seems mostly unnecessary. The value of a quantitative meta-analysis is self-evident for the audience and can be stated by a simple statement of the goal of this work later in the introduction. Shortening this also leaves more room for more helpful introductory information regarding the causes of phytoplankton C:N:P variability or the factors that make this meta-analysis challenging (see previous two comments).*

    We agree that this paragraph was a little lengthy. In the revised manuscript, we shortened this paragraph and focused more on the causes of phytoplankton C:N:P variability based on the results from previous synthesis studies (Lines 93-105).

14. *Line 69-72: While previous meta-analyses that focus on only one environmental driver are indeed limited, these studies must still have some value or informative conclusions. This introduction contains no mention of the actual findings or major conclusions of these previous studies. Addressing the findings and relative value of previous, similar work should be a fundamental part of any introduction. Again, addressing this omission seems more helpful than the paragraph explaining why meta-analyses are valuable.*

    Thank you for this suggestion. As mentioned in the response to the previous comment (#13), we addressed the findings and relative values of previous meta-analysis studies in more depth.

15. *Line 76: The sentence contained here is incomplete and seems like a typo.*

    We move this sentence to Methods section (Lines 165-169) and turned it into a complete sentence.

Methods:

16. *Line 93: For readers who might not be familiar with search operators, you should define "TS" as in its first usage as a field tag for "topic" (or some other appropriate definition).*

    Thank you for this suggestion. We defined "TS" as a field tag for topic and included in the caption of Table 1.

17. *Line 94-100: As with the previous comment, it would be good to explain the meaning of "*" as a wildcard search operator.*

    We explained " * " as a wild card search in the caption of Table 1.

18. *Line 94-100: These descriptions of search terms are not accessible when listed in a paragraph. This information should be placed in a table.*

    Thank you for this suggestion. We placed these search terms in Table 1 for a better accessibility.

19. *Study Selection Criteria: The way in which studies were selected for the meta-analysis and the lack of analysis or discussion of the confounding factors that various studies present are where some of my strongest critiques lie. I've presented these critiques as a list below:*

a) *Limitation of 3 experimental levels: The value of setting the study selection criteria to 3 experimental levels for each environmental factor of interest seems overstated. The terms X and Y (the fractional response and fractional change in conditions) could be calculated with just two experimental levels for each experimental unit. Granted this does not allow the error associated with a linear regression of 3 X and Y values to be used or for a non-linear response to be detected, but I would question the value of such an error term or description of a non-linear response that was based on a linear regression of only three points. Give the limits of this additional explanatory power, this criterion seems unnecessarily limiting (see next points).*

It is true that our effect size of meta-analysis (i.e., s-factor; the fractional response over fractional change in conditions) can in theory be calculated with just two points. There is a tradeoff between using two points (more studies but linear response) and three points. Given that one of our main motivations was to incorporate a possibly nonlinear stoichiometric response in a global ocean model, the three point metric was originally selected. However, we acknowledged the merits of using just two points as suggested. In the revised manuscript, we provide results of meta-analysis using 2 levels instead of 3.

b) *Excluding valuable studies: Having only two levels of an experimental factor is not the major failing of most studies of phytoplankton elemental composition. There are many studies that I would deem of high quality that would have made excellent additions to this metaanalysis that only use two experimental levels for a given type of nutrient stress (e.g. Bertilsson et al. 2003; Fu et al. 2007 J. Phycol.). Considering this, the criteria of 3 levels unnecessarily diminishes the data density of the meta-analysis. Again, perhaps a better explanation of the selection criteria and meta-analysis calculations is needed if I am mistaken. It seems like a meta-analysis that utilizes a greater number of individual experimental units by including experiment with only two levels would have much greater breadth and power.*

As mentioned, in the previous point #19a), we carried out new analysis using two levels. This has greatly increased the number of studies from 64 to 104. Supporting Information Appendix S1 lists all the studies included in this paper.

c) *Not addressing major confounding factors: The more important failing in studies of phytoplankton C:N:P is the lack of consistent experimental conditions or poorly described conditions. Many studies do not offer verification that some desired growth state was successfully applied, particularly in the case of N or P stress. For example, many studies do not describe the growth rate at a given experimental level of a limiting nutrient. How an author defines N or P starvation or to what extent these conditions were applied (e.g. were they applied until growth ceased, or just until growth slowed) can greatly affect the observed response. Additionally, many nutrient starvation experiments are done in dense batch cultures where the additional stressors of light limitation, high pH, and low carbon availability arise as cultures increase in density and coincide with the onset of nutrient starvation. I mention this not to say that the authors should have determined such confounding factors in every study (in many cases, experimental conditions are not described well enough to do this), but rather to point out that such factors are not addressed at all in the selection criteria. In other words, a poorly executed*

study that did not fully apply nutrient starvation (even across 3 levels) would be included, but a well-described and well-executed experiment across only 2 levels (e.g. nutrient replete vs. nutrient starved) would be excluded. Again, this gets to the point that basing s-factors on a linear regression of 3 or more experimental levels has applied a major constraint on the meta-analysis and the value of this constraint is unclear, yet other major confounding factors are not addressed in the selection criteria.

Since all the experiments are "unique" in the sense that they do not have all the same controlled variables, it is pragmatically impossible to take into the account of all the factors (e.g., pH, carbon availability etc.). That being said, we updated our database to include these extra factors such as salinity, growth mode, phase at extraction, daily light/dark cycle (Lines 233-238). We then conducted between moderator heterogeneity analyses (section 2.3.5 of the main manuscript). The new data collection strategy has significantly increased our data points and made our results more robust.

20. *S-factor Calculation for Meta-Analysis: My other major critiques pertain to how s-factor was calculated, particularly for macronutrient stress experiments. Again, I've presented these critiques as a list below:*

a) *How was standard error propagated when calculating s-factors? Does the error reflect both the error associated with each P:C or N:C measurement and the error associated with the regression of X and Y for each experimental unit? How the error associated with the original measurements was accounted for and propagated must be described (if this was done).*

In the revised manuscript using two experimental levels, we describe the method for propagating uncertainties in individual P:C or N:C measurements to standard error for s-factor for each individual experimental unit (equations (3) and (4)). We also describe how to compute variance-weighted mean s-factor (equations (5) and (6)).

b) *With respect to the error associated with the weighted mean s-factors, I realize that the metafor R package is used for this calculation, but some general description of how this package calculates error should be provided. In other words, you should be explicit about what the error bars shown in the figures actually mean.*

Mean s-factor is weighted with respect to the variance of individual experimental unit (equation (5)). We described this in detail at section 2.3.4 how this was calculated with R package *metaphor*. We also provide R codes in our Zenodo data repository (http://doi.org/10.5281/zenodo.3723121).

c) *It is not at all clear how the fractional change in nitrate or phosphate stress was calculated. Was this simply based on the ambient nitrate or phosphate concentration reported for each experimental level? If so, how can the level of N stress be determined if ammonium or nitrate are not accounted for?*

In this revised version, we selected two end-members (nutrient limited and nutrient replete) based on the definition given in the original studies. For batch and semi-continuous batch experiments, we compared fractional change in initial concentrations between nutrient replete and limited conditions when calculating stoichiometry sensitivity factor (Lines 199-202). For

continuous (chemostat) nutrient experiments, we used difference in the inflow concentrations of the nutrient replete and limited cultures to determine stoichiometry sensitivity factor (Lines 202-204). When multiple levels of concentrations are used, we selected two end-member points, one with the lowest growth rate and the other highest growth rate (Lines 204-206).

d) *Batch, semi-continuous batch, and continuous chemostat experiments were used in the meta-analysis of macronutrient response. I do not understand how a simple measurement of ambient inorganic nutrient concentration can be used to determine experimental levels of N or P stress across these different experiment types. Even between a semi-continuous batch experiment where authors claim cultures are in balanced growth and a chemostat experiment, the measured nutrient concentrations or nutrient concentrations in fresh or inflow media mean different things with respect to extent of nutrient stress. In other words, moving from a nitrate concentration of 1.0 to 0.2 would mean very different things depending on whether they are in semi-continous or continuous mode, the concentration of other forms of dissolved inorganic nitrogen (ammonium, nitrite) or what the concentration of other potentially limiting nutrients are. The extent of nutrient stress cannot be compared between these different growth modes based on dissolved nutrient concentrations alone. Some would argue the extent of nutrient stress cannot be compared across these growth modes at all, and thus they can't be pooled into one type of meta-analysis. Again, a strict criterion of 3 experimental levels has been applied in this meta-analysis to serve a computational need, but other major confounding factors have been ignored. Additionally, these 3 experimental levels have been used to calculate a fractional change in conditions that does not have a consistent meaning across experiment types. The only way to deal with these problems while still using the current meta-analysis approach (sfactors, based on 3 experimental levels) would be to separate experimental units based on their growth mode and apply a more rigorous means of determining experimental levels of nutrient stress (i.e. growth rate) in the semi-continuous and continuous growth experiments.*

Firstly, as we increased the number of studies by including 2 level experiments, we now have enough studies to separate out "P-limited → P-replete studies" (for calculating s-factor with respect to change in PO4) from "N-limited → N-replete studies" (for calculating s-factor with respect to change in NO3) based on the definition given in the original study and/or based on the change in growth rate. We were also able to test the effects for the different forms of N but we did not find any significant differences (Table S1).

As for difference in the growth modes, it would be best ideally to use chemostat experiment for assessing the macronutrient availability on C:N:P because this growth mode can achieve constant growth rate. However, there are two issues: 1) there are significantly fewer chemostat studies compared to batch/semi-continuous, 2) different chemostat studies use different dilution rates. Therefore, we had decided to put all the three form of experiments (batch, semi-continuous, chemostat) together in our analysis. In the revised manuscript with more data available, we have had enough experimental units to conduct heterogeneity test between three growth modes. The difference in growth mode does indeed lead to statistically significant heterogeneity in stoichiometric responses (Fig. 3b).

e) *Similar problems with the s-factor calculation of using a linear fractional change in growth conditions also apply to temperature and irradiance. Such a formulation ignores the growth optimum of a particular species or strain and thus treats an extremely non-linear response as something that can be compared across studies and taxa with a simple linear relationship.*

Consider a scenario where an experiment measured N:C at four temperatures in a species with growth optimum of 22C and had the following result: 15C = 0.14, 20C=0.154, 25C=0.156, and 30C=0.14. An s-factor calculated as a linear regression of X and Y from this experiment would be very small in magnitude and imply that this species is insensitive to temperature changes, when in fact these are actually large changes in N:C with respect to global conditions and what is generally observed in temperature responses. This experiment also shows that N:C declines at supraoptimal temperatures, the most relevant result with respect to climate change scenarios, but something that would be missed by the s-factor. In other words, the s-factor is a poor metric for a biological variable that does not have a monotonic response to some condition as is the case with light and temperature responses.

A prior meta-analysis study by Yvon-Durocher et al. (2015) found a linear relationship between temperature and C:P, hence the assumption of monotonic relationship is somewhat justifiable. However, we do agree that we should take growth rate into an account. In the revised manuscript we chose two end member temperature or irradiance values, one with the lowest growth rate and the other with the highest growth rate. When growth rate was not explicitly mentioned we selected the lowest and highest treatment values with the assumption that the phytoplankton is temperature or light limited within the range of values considered (Lines 210-213).

Also, depending on the light or temperature levels selected in a given experiment with respect to the study species growth optimum, a fractional change in these conditions means very different things and are not directly comparable.

We agree that results can be more accurately comparable if optimal temperature was used throughout. As mentioned in the previous paragraph, we followed this criterion as much as possible to ensure a fair comparison of studies.

21. *Line 147-149: the symbol used to denote dissolved iron should be a mathematical prime symbol(′), not an apostrophe or single quotation mark.*

    Thank you for pointing this out. We corrected the usage of this symbol.

22. *Line 150-151: "only selected experiments where NO3 concentrations were kept constant." This is either a writing error or a misunderstanding of the experiments selected. The non-limiting macronutrient was not kept constant in many of the experiments selected and this is rarely achieved even in chemostat experiments (see the nutrient concentrations described in Leonardos and Geider 2004 for example). Again, the selection criteria and calculation of fractional change for macronutrient stress experiments is either poorly described, problematic, or both.*

    For chemostat experiments (e.g., Leonardos and Geider, 2004) NO_3 concentration is referring to the "inflow" NO3 concentration and this was kept constant at 100 μmol/L for experiment conducted by Leonardos and Geider (2004). In addition, this paper explicitly states that "We[Leonardos and Geider] infer N limitation at N: P $\leq$ 15 and P limitation at N: P $\geq$ 30" (page 2107 of Leonaros and Geider, 2004). We therefore chose two end-member P concentrations under P-limitation based on their definition. We note that we carefully went through each paper

in this revision and obtained information on nutrient limitation given by the authors of original studies. If the nutrient limitation was not explicitly stated, we assumed P limitation if addition of P increased growth rate, from the lowest to the maximum given that all the other drivers are kept at a constant value.

In calculating s-factors from macronutrient concentrations, we compared inflow concentrations between control (nutrient limited) and treatment (nutrient replete) for chemostat experiments. For a batch or semi-continuous batch experiment. we compared initial nutrient concentrations between the control and the treatment. We made this point clearer in the revised manuscript (Lines 199-209).

Results:

23. *Figures 2 – 5: The structure of the figures seems likely to confuse readers. Tables are often arranged such that inclusive categories are listed above subcategories. When first looking at figure 1, I see "Diatoms" in bold and then genus names for various eukaryotes below it and was disoriented for a moment. The figures may be more intuitive if you listed an inclusive group (e.g. "Diatoms") and then listed taxa within that group immediately below it with an indentation.*

    Thank you for this suggestion. We have made changes to the way we present results. We hope that new figures (Figure 2-3) are more concise and easier to understand.

    Also, why are the figures arranged as nutrient limitation (Fig. 2), Light (Fig. 3), Temperature (Fig. 4), nutrient-limitation (Fig. 5). I understand if this was done because there is very limited data for Iron limitation, but a more logical arrange of the figures would be better for comparison.

    For a better comparison, we will place iron after P and N experiments.

24. *There also appears to be a few taxonomic assignment errors in the meta-analysis based on the figures. Alexandrium minutum (a dinoflagellate) is listed among the diatoms in the Figure 2, Chlorella sp. (a chlorophyte) is listed among the diatoms in Figure 3, and Phaeocystis (a haptophyte) is listed among the diatoms in Figure 4. Does this error extend to the meta-analysis or was this an error in figure preparation?*

    Thank you for pointing this point. These were indeed misclassifications and analyses were redone with the correct classification.

25. *There also seems to be errors or inconsistencies in how studies were characterized with respect to N or P limitation. For example, why is Leonardos and Geider 2004 only listed among "Phosphate" experiments. This is a chemostat study that spanned both N-limited, balanced growth and P-limited balanced growth and thus could also be included with the "Nitrate" and "Nitrate/Phosphate" meta-analyses. The fact that these chemostats were controlled by manipulating inflow phosphate is irrelevant and does not make them simply "phosphate" experiments. Neither nitrate or phosphate values were constant across experimental levels in this experiment, what matters is that these were chemostats where inflow N:P was manipulated. I did not closely examine every study in the meta-analysis, but I am concerned that other such inconsistencies are present.*

The reason Leonardo and Geider (2004) study was included in "Phosphate" experiments and not in "Nitrogen" was because inflow phosphate concentration was manipulated while inflow nitrate concentration was kept constant. As mentioned in the reply to #22, authors of this original study explicitly state their experiment was P-limited under certain NO3:PO4 supply ratio. In addition, we got rid of the "Nitrate/Phosphate" category as it was redundant and not clear.

Discussion:

26. *Line 230: the word "the" before "chemical" should be removed*

    Changed as suggested (Line 571).

27. *Line 241: "making of … reductase". Do you mean "reductant" (i.e. NADPH) rather than reductase (an enzyme)?*

    Thank you for pointing out our mistake. We changed this to reductants (Line 582).

28. *Line 243-246: These are specific statements that should be supported with references.*

    We decided to remove this sentence as it was not directly relevant.

29. *Line 237-238 and other parts of paragraph: There seems to be a misunderstanding of the term "balanced growth". A natural population or culture can be both nutrient-limited and in steady-state, balanced growth if the limiting nutrient is supplied at a consistent rate. Despite the various factors that limit phytoplankton growth and the natural conditions that represent clearly unbalanced growth (spring blooms), a balanced growth model of natural populations (the "steady-state ocean") is still very relevant for the vast subtropical oceans where consistent and actively growing populations occur amidst apparent chronic nutrient limitation.*

    Thank you for pointing this out. We modified this paragraph and removed the phrase "balanced growth" in lines 571-573 as suggested.

30. *Line 282: This should be corrected to "we observe a consistent trend" or "we observe consistent trends"*

    We change to "we observed a consistent trend" (Line 641).

31. *Line 296: I think "… the level…" should be changed to "…the same level…". If this is not just a typo, than this sentence should rewritten and clarified*

    This was a typo. We corrected to "… the same level…" (Line 650).

32. *Line 298: the phrase "number of…" or "abundance of…" should be placed before "…ribosomes"*

    The phrase "number of …" was added (Line 652).

33. *Line 300: revise to "… in a cell, resulting in … " or "… in a cell and result in…"*

We changed to "… in a cell, resulting in …" (Line 654).

34. *Line 309: The Garcia reference is not appropriate here. References that actually describe this mechanism should be cited:*

    - *Dortch, Q., Clayton, J. R., Thoresen, S. S., & Ahmed, S. I. (1984). Species differences in accumulation of nitrogen pools in phytoplankton. Marine Biology, 81(3), 237-250.*
    - *Lourenço, S. O., Barbarino, E., Lavín, P. L., Lanfer Marquez, U. M., & Aidar, E. (2004). Distribution of intracellular nitrogen in marine microalgae: calculation of new nitrogen-to-protein conversion factors. European Journal of Phycology, 39(1), 17-32.*
    - *Grover, J. P. (1991). Resource competition in a variable environment: phytoplankton growing according to the variable-internal-stores model. The American Naturalist, 138(4), 811-835.*
    - *Tozzi, S., Schofield, O., & Falkowski, P. (2004). Historical climate change and ocean turbulence as selective agents for two key phytoplankton functional groups. Marine Ecology Progress Series, 274, 123-132.*
    - *Talmy, D., Blackford, J., Hardman-Mountford, N. J., Polimene, L., Follows, M. J., & Geider, R. J. (2014). Flexible C: N ratio enhances metabolism of large phytoplankton when resource supply is intermittent.*

    Thank you for these references. We now cite these papers instead (Line 701-702).

35. *Line 320: The word "and" should be inserted after "significantly"*

    We removed this sentence as it is no longer an accurate description of our new results.

36. *Line 328: "Large stoichiometry sensitivity…" should be changed to "The larger stoichiometric sensitivity…" or "The larger sensitivity of P:C…"*

    We removed this sentence as it is not consistent with our new results.

37. *Line 339-340: "Excess carbon…" – this sentence is a non-sequitur and should be modified to connect with the topic of irradiance effects.*

    We rephrased this as "Excess carbon that is fixed under high irradiance condition is …" (Line 782).

38. *Line 349-350: This statement may not be true and should be supported by some reference. The light harvesting apparatus will still be expected to be down-regulated under N-replete conditions in order to avoid oxidative stress and photodamage and also to maximize growth rate and N allocation.*

    We removed these lines. It is true that down-regulation of light harvesting apparatus is expected under nutrient-replete condition as well (Geider et al., 1996; Laws and Bannister, 1980) and our original description here is not correct.

39. *Line 351-355: Amidst all these explanations of why irradiance has little effect on C:N:P, there is a fundamental explanation that has not been addressed. Although N-content may be expected to*

decline as irradiance increases due to a down regulation of the light harvesting apparatus, one could also expect an increase in N allocation to other cellular functions including nutrient uptake, biosynthesis, and repair of the light harvesting apparatus in order to match an increase in C-fixation. This shift in N allocation from light harvesting content to nutrient acquisition and biosynthesis is essential to an increase in growth rate with irradiance and could be expected at light levels that are below some photoinhibitory level. I don't know if this reallocation of N is sufficient to offset the expected decline in N content due down regulation of the light harvesting apparatus, but at least this is based on fundamental biological processes rather than critiques of experimental conditions that are not followed by any details or substantiation.

This is a very good suggestion. As suggested, we included these factors to illustrate why irradiance could have muted effect on C:N:P (Lines 811-817).

1) Increase in N allocation to nutrient uptake apparatus following the "chain model" concept (Ågren, 2004; Pahlow and Oschlies, 2009).
2) Increased N requirement for Rubisco at high irradiance offsetting reduction in N-content due to a down regulation of the light harvesting apparatus (Li et al., 2015).
3) Increased demand of proteins (e.g., D1 protein) for the repair of light harvesting apparatus at high irradiance (Demmig-Adams and Adams, 1992; Li et al., 2015; Talmy et al., 2013).

40. *Line 351-364: It seems odd that the variation in experimental conditions is invoked here to explain the limited the effect of irradiance on C:N:P, but this was not addressed with respect to macronutrient limitations. It seems logically inconsistent to note these methodological issues only when a clear effect is not found.*

We now address the variation due to the difference in experimental condition (batch vs semi-continuous batch vs continuous) (see Fig. 3b).

41. *Line 359-360 and 372-373: "We speculate…" – Aren't these concepts easy to verify or discuss further considering the small number of studies used in the meta-analyses rather than just speculate? Were the experiments used for the irradiance meta-analysis diel or continuous light. What proportion were continuous light?*

As the reviewer suggested, we analyzed if the length of light vs dark hours does lead to statistically significant difference in s-factors. Indeed, there is a statistically significant difference between N:C of periodic vs continuous light(Fig. 3d).

Were these experiments mostly done at optimal temperature?

Most studies just state the temperature at which the experiment was conducted and do not comment whether the temperature is optimal temperature or not. So unfortunately, we could not determine whether the experiments are conducted at optimal temperature or not simply by reading though methods section of the original studies.

Also, I thought your selection criteria examined studies where irradiance was manipulated, but nutrient status was not. How can nutrient status then be invoked as a possible confounding factor?

We can deduce nutrient limitation based on the phytoplankton growth phase at harvest. Cells harvested at exponential phase is more likely to be nutrient replete, while those harvested during stationary phase are nutrient limited. We did not find significant difference in our analysis for light manipulated experiments (Table S1).

It seems more reasonable and conservative to assume that irradiance simply does not have strong effect on P:C?

With our new analysis, we did find noticeable change in P:C with respect to irradiance (Fig. 2). We therefore rephrased the lines 788-795.

42. *Line 420-422: The time range of selected studies seems like a very weak argument. Wouldn't the selection criteria for the studies used in each meta-analysis also have a strong effect on the result. Also couldn't you simply split your analysis between these time ranges to see how it compares to the Yvon-Durocher study? This seems like another speculation that could be very easily examined.*

    With our new dataset, our result is now in a good agreement with Yvon-Durocher study so we rephrased our original sentences (Lines 831-833).

43. *Line 432-434: The sentence here is incomplete or a fragment and should be revised.*

    We removed the word "that" in this sentence (Line 720) to make the meaning clearer.

44. *Line 436: This seems like an erroneous assumption. Couldn't a non-significant effect of iron on stoichiometry also be due to variable and contrasting effects of iron on cellular C and N or reflect the small number of studies examined!?*

    Even with our new larger dataset we still found non-significant effect of iron on N:C (Fig. 2b). We therefore believe that our original assumption that iron availability affects cellular C, N, and P proportionally is not refuted here (Line 769-771).

45. *Line 467-470: Cause and effect seem to be mixed up here. Sea surface warming is driven by air temperature, which in the long-term is driven by radiative forcing (greenhouse effect) rather than visible light. Also changes in incident irradiance at the sea surface are expected to be far smaller than changes in sea surface temperature due to climate change. Surface warming drives stratification, which then results in greater overall light intensity and lower nutrient availability for phytoplankton trapped in a more shallow surface mixed layer. Also some references should be provided in this section.*

    We rephrased the sentence accordingly and cited works by Hutchins and Fu (2017) and Boyd et al. (2015) (Lines 894-898).

46. *Line 474: The word "out" should be placed after "carried"*

    We removed this sentence at it was mostly an irrelevant information.

47. *Line 482-493: This discussion of organic matter decoupling is a bit muddled and unclear. I point out specific problems below. Generally, the value of this paragraph and its connections to the main point of this work are not clear. Is point here simply that P:N:C of cultured phytoplankton analysed here do not directly correspond to ocean particulate matter P:N:C due to the presence of detritus and decomposition?*

    Thank you for clarification and yes, that is exactly our main point. We simplified this paragraph to make our message clear that C:N:P of cultured phytoplankton analyzed here do not directly correspond to ocean particulate matter C:N:P (Lines 991-997).

48. *Line 484: "organic matter accumulation and remineralization". Are implying that detritus plays a role in bulk organic matter P:N:C? If so, this should be stated directly. Amongst the possible causes of decoupling between expected phytoplankton stoichiometry and measured bulk organic matter stoichiometry, detrital material is likely very important and not addressed. Some helpful references:*
    - *Karl D.M., Dobbs F.C. (1998) Molecular Approaches to Microbial Biomass Estimation in the Sea. In: Cooksey K.E. (eds) Molecular Approaches to the Study of the Ocean. Springer, Dordrecht*
    - *Verity, P. G., Williams, S. C., & Hong, Y. (2000). Formation, degradation, and mass: volume ratios of detritus derived from decaying phytoplankton. Marine Ecology Progress Series, 207, 53-68.*

    Yes, we are indeed saying that detritus play role in bulk C:N:P and now included these references above (Lines 994-997).

49. *Line 485-488: This sentence is unclear. One point of Martiny et al 2013a is the increase in C:N (or rather a decrease in N:C) of sinking organic matter (see Figure 4 therein). Aside from that point, it is not clear how sinking organic matter being close to Redfield composition predicts low N:C in phytoplankton.*

    We agree that this sentence was unclear and is now removed.

50. *Line 494-505: The study by Moreno et al. 2018 would be good to include here. It not only supports your point about the value of flexible stoichiometry in global biogeochemical models, it particularly highlights the more flexible P:C of diatoms as an important driver of global patterns*
    - *Moreno, A. R., Hagstrom, G. I., Primeau, F. W., Levin, S. A., & Martiny, A. C. (2018). Marine phytoplankton stoichiometry mediates nonlinear interactions between nutrient supply, temperature, and atmospheric CO2. Biogeosciences (Online), 15(9).*

    Thank you for this suggestion. We now cite Moreno et al. 2018 (Line 1005).

51. *Line 508: This point seems overstated and not in accordance with your results. Didn't you show that irradiance has no clear effect on P:C and only a weak effect on N:C?*

    This original sentence was unclear and is now removed. We do show in our study however that irradiance has a weak, but significant effect for both P:C and N:C (Figures 2 and 4).

52. (*Lines) 510-516: This is an interesting suggestion. You have made other predictions based on your meta-analysis, so you should actually present a prediction using this powerlaw function if you are going to suggest it. Or at least use this function to highlight what terms need to be better constrained and/or what terms should be added (e.g. detrital contribution, decomposition) in order to properly apply a power-law formulation to ocean stoichiometry.*

    Thank you for this suggestion. We did indeed use this power-law function (equation (9)) and we now present our prediction more formally in Table 3.

53. *Line 520: remove the word "on"*

    Removed as suggested (Line 1043).

54. *Line 521: "… evolve under the climate change." is grammatically incorrect or a typo. "under the" could just be changed to "with" or one of many other revisions could be applied*

    Changed from "under the" to "with". (Line 1044).

55. *Line 525: Remove the word "the".*

    Removed as suggested (Line 1047).

References:

56. *Be sure to double-check reference formatting. Reference titles should not be in all caps (a frustrating result of citing articles from Journal of Phycology).*

    Thank you for pointing out. We now have corrected all the reference formats.

Cited literature:

Ågren, G. I.: The C:N:P stoichiometry of autotrophs - Theory and observations, Ecol. Lett., 7(3), 185–191, doi:10.1111/j.1461-0248.2004.00567.x, 2004.

Boyd, P. W., Lennartz, S. T., Glover, D. M. and Doney, S. C.: Biological ramifications of climate-change-mediated oceanic multi-stressors, Nat. Clim. Chang., 5(1), 71–79, doi:10.1038/nclimate2441, 2015.

Collins, S., Boyd, P. W. and Doblin, M. A.: Evolution, Microbes, and Changing Ocean Conditions, Ann. Rev. Mar. Sci., 12(1), annurev-marine-010318-095311, doi:10.1146/annurev-marine-010318-095311, 2020.

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Hutchins, D. A. and Fu, F.-X.: Microorganisms and ocean global change, Nat. Microbiol., 2(6), 17058, doi:10.1038/nmicrobiol.2017.58, 2017.

Laws, E. A. and Bannister, T. T.: Nutrient- and light-limited growth of Thalassiosira fluviatilis in continuous culture, with implications for phytoplankton growth in the ocean, Limnol. Oceanogr., 25(3), 457–473, doi:10.4319/lo.1980.25.3.0457, 1980.

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muelleri to growth under varying light and nitrate : phosphate supply ratios and their influence on critical N: P, Limnol. Oceanogr., 49(6), 2105–2114, doi:10.4319/lo.2004.49.6.2105, 2004.
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Pahlow, M. and Oschlies, A.: Chain model of phytoplankton P, N and light colimitation, Mar. Ecol. Prog. Ser., 376, 69–83, doi:10.3354/meps07748, 2009.
Talmy, D., Blackford, J., Hardman-Mountford, N. J., Dumbrell, A. J. and Geider, R. J.: An optimality model of photoadaptation in contrasting aquatic light regimes, Limnol. Oceanogr., 58(5), 1802–1818, doi:10.4319/lo.2013.58.5.1802, 2013.
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Villar-Argaiz, M., Medina-Sánchez, J. M., Biddanda, B. A. and Carrillo, P.: Predominant Non-additive Effects of Multiple Stressors on Autotroph C:N:P Ratios Propagate in Freshwater and Marine Food Webs, Front. Microbiol., 9(JAN), 69, doi:10.3389/fmicb.2018.00069, 2018.
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A meta-analysis on environmental drivers of marine phytoplankton C:N:P

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Abstract. The elemental stoichiometry of marine phytoplankton plays a critical role in the global biogeochemical cycle through its impacts on nutrient cycling, secondary production, and carbon export. Although extensive laboratory experiments have been carried out over the years to assess the influence of different environmental drivers on the elemental composition of phytoplankton, a comprehensive quantitative assessment of the processes is still lacking. Here, we synthesized the responses of P:C and N:C ratios of marine phytoplankton to five major drivers (inorganic phosphorus, inorganic nitrogen, inorganic iron, irradiance, and temperature) by meta-analysis of laboratory experimental data across 366 experiments from 104 journal articles. Our results show that the response of the ratios to changes in macronutrients is consistent across all the studies, where the increase in nutrient availability is positively related to changes in P:C and N:C ratios. We found that eukaryotic phytoplankton are more sensitive to the changes in macronutrients compared to prokaryotes, possibly due to their larger cell size and their abilities to quickly regulate their gene expression patterns required for nutrient uptake. The effect of irradiance was significant and constant across all studies where an increase in irradiance decreased both P:C and N:C. The response to temperature changes was mixed depending on the culture growth mode and the growth phase of phytoplankton at the time of harvest but the weighted mean P:C ratio decreased significantly with warming. Along with other oceanographic conditions of the subtropical gyres (e.g., low macronutrient availability), elevated temperature may explain why P:C is consistently low in subtropical oceans. Iron addition did not systematically change neither P:C or N:C. Overall, our findings highlight the high stoichiometric plasticity of eukaryotes and the importance of macronutrients in determining P:C and N:C ratios, which both provide us insights on how to understand and model plankton diversity and productivity.

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1 Introduction

Elemental stoichiometry of biological production in the surface ocean plays a crucial role in cycling of elements in the global ocean. The elemental ratio between carbon and the key limiting macronutrients, nitrogen (N) and phosphorus (P), in exported organic matter expressed in terms of C:N:P ratio helps determine how much atmospheric carbon is sequestered in the deep ocean with respect to the availability of limiting nutrients. On geologic timescale, N:P ratio reflects the relative availability of nitrate with respect to phosphate, both of which are externally supplied from atmosphere via nitrogen-fixation and/or continents via river supply and lost by denitrification and burial (Broecker, 1982; Lenton and Watson, 2000; Redfield, 1958; Tyrrell, 1999). On shorter timescales the average stoichiometry of exported bulk organic matter reflects elemental stoichiometry of phytoplankton (Bonachela et al., 2016; Garcia et al., 2018; Martiny et al., 2013b) with additional influences of biological diversity and secondary processing of organic matter by zooplankton and heterotrophic bacteria. In the face of global change, understanding and quantifying the mechanisms that leads to variability in C:N:P ratios are crucial in order to have an accurate projection of future climate change.

A key unresolved question is what determines C:N:P of individual phytoplankton. Phytoplankton grow in the upper light-lit layer of the ocean where the amount of inorganic nutrients, light, and temperature vary spatially and temporally. Laboratory studies show that these fluctuations trigger responses at the cellular level, whereby cells modify resource allocation in order to adapt optimally to their ambient environment (Geider and La Roche, 2002). For example, phytoplankton may alter resource allocation between P-rich biosynthetic apparatus, N-rich light-harvesting apparatus, and C-rich energy storage reserves (Moreno and Martiny, 2018). Under a typical future warming scenario, the global ocean is expected to undergo changes in nutrient availability, temperature, and irradiance (Boyd et al., 2010). These changes are likely to have profound effects on physiology of phytoplankton (Finkel et al., 2010; van de Waal et al., 2010) and observations show that competitive phytoplankton species are able to acclimate and adapt to changes in temperature, irradiance, and nutrients on decadal timescales (Irwin et al., 2015). Numerous laboratory and field experiments have been conducted thus far to study the relationship between C:N:P ratio of phytoplankton and environmental drivers. It is however challenging to synthesize those studies and generalize the response of phytoplankton C:N:P to changes in

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environmental drivers. Individual studies employ different sets of statistical analyses to characterize effects of environmental driver(s) on elemental ratios, ranging from a simple t-test to more complex mixed models, which makes interstudy comparisons challenging. In addition, since environmentally induced trait changes are driven by a combination of plasticity (acclimation), adaptation, and life history (Collins et al., 2020; Ward et al., 2019), stoichiometric responses of phytoplankton can be variable even amongst closely related species.

Meta-analysis/systematic-review is a powerful statistical framework for synthesizing and integrating research results obtained from independent studies and for uncovering general trends (Gurevitch et al., 2018). The seminal synthesis by Geider and La Roche (2002) as well the more recent work by Persson et al. (2010) have shown that C:P and N:P could vary up to a factor of 20 between nutrient-replete and nutrient-limited cells. These studies have also shown that C:N ratio is plastic due to nutrient limitation. A meta-analysis study by Hillebrand et al. (2013) highlighted the importance of growth rate in determining elemental stoichiometry showed that both C:P and N:P ratios decrease with increasing growth rate. Yvon-Durocher et al. (2015) investigated the role of temperature in modulating C:N:P. Although their dataset was limited to studies conducted prior to 1996, they have shown a statistically significant relationship between C:P and temperature increase. MacIntyre et al. (2002) and Thrane et al. (2016) have shown that irradiance plays an important role in controlling optimal cellular C:N and N:P ratios. Most recently, Moreno and Martiny (2018) provided a comprehensive summary of how environmental conditions regulate cellular stoichiometry from physiological perspective.

Here, we present results from a systematic literature review and subsequent meta-analysis to quantify how five key environmental drivers affect C:P and C:N ratios of marine phytoplankton. Unlike previous meta-analyses on elemental stoichiometry of phytoplankton that strictly synthesized the effect of a single environmental driver, our study assessed the effects of five drivers, specifically for marine phytoplankton species. Importantly, we use a unique newly defined measure of effect size, a *stoichiometry sensitivity factor* (Tanioka and Matsumoto, 2017), which is a dimensionless parameter that relates a fractional change in P:C or N:C to a fractional change in a particular environmental driver. We compute effect size for each driver-stoichiometry pair from independent studies and subsequently determine the weighted mean P:C and N:C ratios. Further, we compute mean effect size within different subgroups of

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Deleted: It has a number of advantages over narrative review and "vote counting" because it compares the common measure of outcome (effect size) that includes information on both the sign and magnitude of an effect of interest from each study. Effect size from individual studies can be combined across studies to estimate the grand mean effect size and its confidence interval, which are then used to test whether overall effect is statistically significant. In addition, with its comprehensive and rigorous procedure for study inclusion criteria, meta-analysis avoids the pitfall of "cherry-picking" data aimed toward supporting particular hypothesis.

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moderators such as plankton types and growth conditions for detecting any systematic heterogeneity between those subgroups.

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2 Materials and Methods

2.1 Bibliographic search and screening

We systematically screened peer-reviewed publications on monoculture laboratory experiment studies that assessed the effects of dissolved inorganic phosphorus, dissolved inorganic nitrogen, dissolved iron, irradiance, and temperature on P:C and N:C ratios of marine phytoplankton. These five environmental drivers are considered to be the top drivers of open-ocean phytoplankton group in studies (Boyd et al., 2010, 2015). Although CO_2 is another potentially important driver, we did not consider the effects of CO_2 on elemental ratios as previous meta-analysis studies showed that no generalization can be made with respect to the direction of trends in P:C or N:C ratios as a function of CO_2 concentration both in the laboratory-bases experiments (Liu et al., 2010) and mesocosm/field-based experiments (Kim et al., 2018).

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Firstly, we conducted a literature search using Web of Science (last accessed in February 2019) with the sequence of key terms (Table 1). This search yielded 4899 hits. We also closely inspected all the primary studies mentioned in the 8 recent review papers including meta-analyses studies on elemental stoichiometry of phytoplankton in aquatic environment (Flynn et al., 2010; Geider and La Roche, 2002; Hillebrand et al., 2013; Moreno and Martiny, 2018; Persson et al., 2010; Thrane et al., 2016; Villar-Argaiz et al., 2018; Yvon-Durocher et al., 2015). The list is also augmented with data from additional six studies that did not appear in the literature search or in the review papers but were cited elsewhere. Papers were further screened and selected to meet the following criteria: (1) experiments must be carried out in the controlled laboratory environments, where all the environmental factors including temperature, photon flux density, salinity, and any other relevant conditions are controlled; (2) all outdoor experiments such as mesocosm or pond experiments are excluded; (3) experiments must be conducted under unialgal/monoculture settings. However, we note that not all the experiments are carried under strictly axenic condition (i.e., not completely devoid of bacteria and virus); and (4) experiments must be conducted with replicates and must report either standard deviation or standard error. Subsequent

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selection processes based on abstracts, graphs, tables, and full text, and removal of duplicates led to a total of 104 journal articles (Fig. 1).

2.2 Data Extraction

Data with means and standard deviations of P:C and N:C under varying environmental values provided by the original studies are used directly. GraphClick (Arizona Software, 2010) was used to read off values from graphs when necessary. In cases where N:P and only one of either P:C or N:C is provided, the remaining ratio is determined by either multiplying or dividing by N:P. Similarly, elemental ratios are computed from the measurements of phytoplankton POC, PON, and POP when the ratios are not explicitly given in the original studies.

For nutrient (P, N, or Fe) manipulation studies, we selected two end-members (nutrient limited and nutrient replete) based on the definition given in the original studies. For batch and semi-continuous batch experiments, we compared fractional change in initial concentrations between nutrient replete and limited conditions when calculating stoichiometry sensitivity factor (see section 2.3.2). For continuous (chemostat or turbidostat) nutrient experiments, we used difference in the inflow concentrations of the nutrient replete and limited cultures to determine stoichiometry sensitivity factor. When multiple levels of concentrations are used, we selected two end-member points, one with the lowest growth rate and the other with highest growth rate. When the growth rate was not provided in the original study, we selected two end-member values based on the highest and lowest nutrient uptake rate, chlorophyll concentration, or total concentration level with the underlying assumption that phytoplankton growth is nutrient limited within the range of nutrient levels considered.

For temperature and irradiance manipulations studies, we selected the lowest value and the optimal or saturating value that led to the maximum growth rate for phytoplankton. When growth rate was not explicitly mentioned we selected the lowest and the highest treatment values with the assumption that the phytoplankton is temperature or light limited within the range of values considered.

When more than two factors were manipulated in the same study, multiple experimental units are extracted if and only if each environmental driver was manipulated separately (i.e., conducted in a factorial manner). For example, we extracted total of 4 experimental units from a 2-by-2 factorial study

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on temperature and nutrient: (1) comparing nutrient limited vs. replete treatment at low temperature; (2) same as in (1) at high temperature; (3) comparing low vs. high temperature response at nutrient limited condition; and (4) as in (3) at nutriment replete condition. An experimental unit refers to a controlled experiment of the same phytoplankton species or clade between control and treatment groups while all the other environmental factors are kept constant. If experiments reported multiple measurements over time, only the final value was extracted.

We also extracted for each experimental unit phytoplankton functional type (i.e., [Diatoms, Coccolithophores, Dinoflagellates, other Eukaryotes, non-diazotrophic Cyanobacteria, Diazotrophs], Eukaryotes vs. Prokaryotes, cold-water vs. temperate species), growth mode (i.e., batch vs. semi-continuous vs. continuous), growth phase at harvest for batch/semi-continuous experiments (i.e., lag, exponential, stationary, decline), N form [NO_3^-, NH_4^+, NO_3^- + NH_4^+, N_2], and light regime (i.e., continuous vs. periodic light). Cold-water species is operationally defined if the control temperature (for P, N, Fe, or I manipulated experiments) or the maximum treatment temperature (for T manipulated experiments) was less than the threshold temperature of 10 °C. Attempted but ultimately discarded moderators for subsequent analysis mainly due to the lack of sample size include salinity, axenic nature of the culture, and the number of generations required for acclimation before the start of the experiment.

Our final dataset consists of 241 experimental units of P:C and 366 experimental units of N:C from 104 journal articles encompassing 7 taxonomic phyla (Bacillariophyta, Chlorophyta, Cryptophyta, Cyanobacteria, Haptophyta, Miozoa, and Ochrophyta), and 6 plankton functional types (Diatoms, Coccolithophores, Dinoflagellates, other Eukaryotes, non-diazotrophic Cyanobacteria, and Diazotrophs) and are available in the Zenodo data repository (http://doi.org/10.5281/zenodo.3723121).

2.3 Statistical analysis

We used two different measures of effect size for this study. One is a commonly used natural logarithm-transformed response ratios, ln(RR) (Hedges et al., 1999) and the other is the stoichiometry sensitivity factor (Tanioka and Matsumoto, 2017). By using two separate measures, we can give a more robust

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prediction on how elemental stoichiometry varies with a change in given environmental driver. All statistical analyses were performed with R v3.5.2 (R Core Team, 2018).

2.3.1 Response ratios

The natural logarithm-transformed response ratios ln(RR) of individual experimental unit and its variance (v) was calculated following Lajeunesse (2015):

$$\ln(RR) = \ln\left(\frac{Y_t}{Y_c}\right) + \frac{1}{2}\left[\frac{S_t^2}{N_t * Y_t^2} - \frac{S_c^2}{N_c * Y_c^2}\right] \quad (1)$$

$$v = \frac{S_t^2}{N_t * Y_t^2} + \frac{S_c^2}{N_c * Y_c^2} + \frac{1}{2}\left[\frac{S_t^4}{N_t^2 * Y_t^4} - \frac{S_c^2}{N_c^2 * Y_c^4}\right] \quad (2)$$

Y denotes mean P:C or N:C, S the standard deviation of that mean, and N is the sample size for the treatment (subscript t) and the control (subscript c) groups. We removed any experimental unit with a studentized residual value of ln(RR) exceeding the absolute value of 3 as an outlier (Viechtbauer and Cheung, 2010).

2.3.2. Stoichiometry sensitivity factor

The second effect size is the newly defined stoichiometry sensitivity factor s_X^Y (Tanioka and Matsumoto, 2017), which relates a fractional change in an elemental stoichiometry (response variable Y) to a fractional change in environmental driver (variable X):

$$s_X^Y = \frac{(Y_t - Y_c)/Y_c}{(X_t - X_c)/X_c} \quad (3)$$

We estimate variance of s_X^Y from the simple error propagation of equation (3) by assuming that the uncertainties associated with the environmental driver X is negligible compared to the errors associated with Y:

$$v_X^Y = \left(\frac{(Y_t - Y_c)/Y_c}{(X_t - X_c)/X_c}\right)^2 \left[\frac{S_t^2/N_t + S_c^2/N_c}{(Y_t - Y_c)^2} + \frac{S_c^2}{N_c * Y_c^2}\right] \quad (4)$$

In essence, the magnitude of s-factor is a measure of how sensitive Y (P:C or N:C) is to a change in stressor level X, and the sign indicates whether Y changes in the same direction as X (positive sign) or in the opposite direction to X (negative sign). The s-factor allows for different kinds of response: a linear response of Y with respect to X ($s_X^Y = 1$), a near hyperbolic response that saturates at high X ($0 < s_X^Y <$

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1), a logarithmic growth ($1 < s_X^Y$), a decay ($0 > s_X^Y$), and the null response ($s_X^Y = 0$). This s-factor metric is conceptually similar to the homeostasis coefficient H (Persson et al., 2010), which relates fractional change in resource nutrient stoichiometry to fractional change in organism's nutrient stoichiometry.

Importantly, an advantage of using s_X^Y as effect size is that its magnitude is a direct, quantitative measure of the strength of environmental driver over the range of values examined. In contrast, ln(RR) only compares the effect of stressor on two end point values (control and treatment) without taking changes in the stressor into an account. Further, we can directly compare the strength of s_X^Y across different pairs of X and Y as it is non-dimensional. For convenience, we use the term "s-factor" in the rest of this paper when describing s_X^Y in a generic sense.

We used the same set of experimental units used in calculating ln(RR) to calculate s-factors (i.e., any outliers are carried over). However, we did not calculate s-factors for iron because the fractional change in dissolved iron concentration, often spanning multiple orders of magnitude, are substantially larger compared to the fractional change in P:C or N:C ratios leading to extremely low s-factor. For temperature-manipulated experiments, we converted degrees Celsius into absolute temperature scale Kelvin. We used photon-flux density (PSD) measured in µmol photons m^{-2} s^{-1} for irradiance and µM for inorganic phosphorus and nitrogen experiments.

2.3.4. Meta-analysis and weighted mean responses

We calculated weighted mean ln(RR) ($\overline{\ln(RR)}$) and s-factor ($\overline{s_X^Y}$) using the mixed-effects model with the R package *metafor* (Viechtbauer, 2010). The weighted mean (M) and its variance (V) are calculated as:

$$M = \frac{\sum_{j=1}^{k} w_j M_j}{\sum_{j=1}^{k} w_j} \quad (5)$$

$$V = \frac{1}{\sum_{j=1}^{k} w_j} \quad (6)$$

where k is the total number of experimental units, M_j is effect size (ln(RR) or s_X^Y) in experimental unit j, and W_j is the weighting factor which is inverse of the variance (Hedges et al., 1999). The 95% confidence interval for the weighted mean was computed as

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Stoichiometry s-factor and its standard error for each individual experiment unit are obtained by carrying out linear regression on the log-transformed X and Y. When using temperature as the environmental driver, we converted degrees Celsius into absolute temperature scale Kelvin. In analyzing iron manipulation experiments, we computed stoichiometry s-factor with respect to change in biologically available free dissolved inorganic iron concentration (Fe'). We estimated Fe' from total dissolved iron concertation, temperature, irradiance, and pH (Sunda and Huntsman, 2003) when iron availability in the original research is provided in terms of total dissolved iron concentration instead of Fe'. For calculating s-factors for PO_4 ($s_{PO_4}^{P:C}$ and $s_{PO_4}^{N:C}$), we only selected experiments where NO_3 concentrations are kept constant. The same was true for calculating dependency on NO_3 ($s_{NO_3}^{P:C}$ and $s_{NO_3}^{N:C}$). We defined s-factors separately ($s_{NP}^{P:C}$ and $s_{NP}^{N:C}$) for studies where both PO_4 and NO_3 are manipulated simultaneously to adjust the N:P supply ratio. ¶
→We summarized s-factors by a random-effects model meta-analysis to determine the weighted mean s-factor using the *metafor* R package (Viechtbauer, 2010). For each environmental driver-stoichiometry pair, we conducted an overall meta-analysis across all the studies (where n ≥ 5) as well as meta-analysis within 3 plankton functional types (PFT) as a categorical moderator. To calculate the PFT averaged s-factors, we fitted separate random-effects model within each level of PFT. A Wald-type test (Viechtbauer, 2010) was used to test whether mean s-factors for PFTs are statistically different from each other. PFTs classified in our study are: (1) diatoms (Bacillariophyta); (2) eukaryotes excluding diatoms; and (3) cyanobacteria. This classification is chosen in order to give a relatively balanced distribution of studies and power across moderator categories. Similar classification of PFTs are commonly employed in the global ocean biochemical models (e.g., Dunne et al., 2013; Ilyina et al., 2013; Moore et al., 2004). All the statistical analyses were performed with R v3.5.2 (R Core Team, 2018) and the codes of the functions used to run all the analyses are available in [4]

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$$CI = M \pm 1.96 \times \sqrt{V} \quad (7)$$

In the subsequent sections of this paper, the values of $\overline{\ln(RR)}$ are back-transformed and represented as percent change:

$$\left(e^{\overline{\ln(RR)}} - 1\right) \times 100\% \quad (8)$$

and considered statistically significant if 95% CIs do not overlap with zero.

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2.3.5. Testing the effect of moderators

We determined the effects of moderators by *rma* function of *metafor* package which is an omnibus test of between-moderator heterogeneity based on χ^2 distribution (Liang et al., 2020). Moderators we tested are PFT, N form, growth mode, growth phase at extraction, and light regime (continuous vs. periodic). The effect of moderator is considered significant when P-value is less than 0.05. We use the weighted mean s-factors in determining the effects of moderators except for iron experiments where we used response ratios instead.

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3 Results

Phosphate addition increases both the mean P:C (235% [95% CI: 169%, 322%]) and N:C (23% [13%, 34%]) significantly (Fig. 2b). Mean stoichiometric sensitivity factor of P:C ($s_P^{P:C}$) with respect to change in phosphate is 0.21 [0.12, 0.29] (Table 2) which means that on average P:C ratio of phytoplankton changes by 0.21% for every 1% increase in PO_4 concentration. The effect of phosphate on N:C is an order of magnitude smaller but also statistically significant and positively correlated ($s_P^{N:C}$ = 0.023 [0.004, 0.042]). Eukaryotic phytoplankton have significantly larger $s_P^{P:C}$ than prokaryotes ($P < 0.05$, Fig. 3a) and the diatoms and coccolithophores especially have noticeably large $s_P^{P:C}$ (Fig. S1a, Table S1). In addition, phytoplankton grown under chemostat experiments have significantly larger stoichiometric sensitivity compared to those grown under batch or chemostat condition (Fig. 3b, $P < 0.001$). There was no between-moderator heterogeneity in $s_P^{N:C}$ (Table S1).

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The response of N:C to changes in inorganic nitrogen is similar to the response of P:C to PO_4 changes where an increase in inorganic nitrogen raises N:C on average by 70% [49%, 93%] (Fig. 2b) with the positive overall mean s-factor $s_N^{N:C}$ of 0.14 [0.08, 0.20] (Table 2). Again, eukaryotic phytoplankton

have higher stoichiometric sensitivity than prokaryotes (Fig. 3a, $P < 0.01$). Nitrogen addition does not affect the weighted mean P:C (Fig. 2). Surprisingly however, phytoplankton grown with the culture made of up nitrate and ammonia have significantly larger $s_N^{P:C}$ compared to those grown with nitrate only, ammonia only, or those under semi-diazotrophic condition (Fig. S2, Table S1). The small sample size however precludes us from making any firm conclusions.

Increase in iron availability does not lead to significant changes in both P:C and N:C (Fig. 2b). In addition, the effects of any moderatos are not statistically significant (Table S1). Although diazotrophs that utilize N_2 as its nitrogen source have significantly large response compared to other PFTs (-20% [-36%, 1%]) (Table S1), their stoichiometric response is not quite statistically significant.

Increase in light availability significantly decreases both P:C (-21% [-38%, -0.4%]) and N:C (-18% [-23%, -12%]) with overall negative s-factors ($s_I^{P:C} = -0.034$ [-0.062, -0.007], $s_I^{N:C} = -0.024$ [-0.034, -0.013]). Although the magnitudes of both the response ratios and s-factors are small compared to those of macronutrients, the responses across PFTs are consistent (Fig. S1c, S1f, Table S1). Phytoplankton grown under chemostat or batch condition have significantly more negative $s_I^{N:C}$ compared to those grown under semi-continuous environment (Fig. 3b, $P < 0.01$). In addition, plankton grown under periodic light cycle have significantly lower $s_I^{N:C}$ compared to those grown under continuous light (Fig. 3d, $P < 0.05$).

The response of P:C to warming is significant where on average P:C decreases by 15% [-24%, -5%] with negative mean s-factor of $s_T^{P:C} = -3.6$ [-6.8, -0.4]) (Fig. 2a, b). The large magnitude of s-factor compared to that of other drivers reflects the fact that the fractional change in temperature (measured in kelvins) is considerably smaller than the fractional change in P:C. There is a significant variability due to growth mode where batch culture and chemostat culture experiments respectively have more negative s-factors for P:C and N:C (Fig. 3b, $P < 0.05$). In addition, phytoplankton extracted during exponential have noticeably more negative s-factors than those extracted during stationary growth phase (Fig. 3c) for both P:C ($P < 0.001$) and N:C ($P < 0.05$). The difference in mean response s-factor ratio amongst PFTs and between cold vs. temperate species is not statistically significant (Fig. S1e, Table S1). Response of N:C is mixed and the weighted mean effect sizes are therefore not statistically significant.

4 Discussion

4.1 Basic framework

One of the fundamental tenets of chemical oceanography is the Redfield Ratio, which implies that phytoplankton cells achieve a constant cellular C:N:P ratio at the well-known molar ratio of 106:16:1 (Redfield et al., 1963). Constant C:N:P is achieved for algal cells growing under steady state conditions where the balance is achieved between uptake of elements and assimilation into cellular functional pool (Berman-Frank and Dubinsky, 1999; Klausmeier et al., 2004). Under such conditions, the growth rate of all cellular constituents averaged over one generation is the same, whether it is the carbon-specific, nitrogen (protein)-specific, or phosphorus (DNA)-specific growth rates (Falkowski and Raven, 2007). In the real ocean however, balanced growth is not always achieved due to short-term and long-term changes in physical conditions of ocean. (Moore et al., 2013; Moore and Doney, 2007). For example, the deficiency of essential nutrients limits the formation of building blocks of new cells (e.g., N for proteins, P for nucleic acids and ATP), light limitation slows carbon assimilation (i.e. making of carbohydrates and reductants), and low temperature slows down the essential cellular transport and enzymatic reactions for growth (Madigan et al., 2006). A good example of unbalanced growth is phytoplankton bloom in the spring where the transient changes in surface temperature, irradiance and nutrient supply rate alter the growth rate and elemental stoichiometry of phytoplankton (Polimene et al., 2015; Talarmin et al., 2016). In addition, future environmental variabilities caused by climate change are expected to cause temporal shift in phytoplankton C:N:P on longer timescales (Kwiatkowski et al., 2018b, 2019; Tanioka and Matsumoto, 2017).

The degrees to which phytoplankton C:N:P ratios are affected by stresses depend both on the cellular stress response mechanisms and the magnitude of the environmental change as well as temporal variability of environmental drivers. Most types of stress responses can be divided into a stress-specific, primary response and a general secondary response (Brembu et al., 2017). The stress-specific responses are strong, robust and consistently observed across photosynthetic organisms, while secondary responses are variable amongst different organisms. Primary and secondary responses are closely related to acclimation (plasticity response) and adaptation (evolutionary response) respectively. In essence, acclimation refers to environmentally induced trait change of an organism in the absence of any genetic

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change, while adaptation involves genetic changes driven by natural selection (Collins et al., 2020). Since primary responses do not involve genetic adjustment or natural selection, the responses are fast and often commonly shared amongst different marine phytoplankton. For example, changing the nutrient uptake affinity of a lineage within a generation in response to changing nutrient supply is a commonly seen trait across all phytoplankton groups. On the other hand, secondary response depends both on the environmental condition and genotype (Brembu et al., 2017). The secondary responses take longer time (usually up to few hundred generations) and there is typically no single, unique response even when referring to a single species or functional group and a specific environmental driver (Collins et al., 2020). In the subsections below, we discuss any possible underlying cellular mechanisms responsible for producing changes in C:N:P ratios (see Fig. 4 for schematic illustration).

4.2 Macronutrients (Phosphate and Nitrate)

Overall, we observe a consistent trend across all studies where P:C and N:C increases with increase in the supply of dissolved inorganic phosphorus and nitrogen respectively (Fig. 2). Since the changes in X:C and the supply of element X are positively related, $s_P^{P:C}$ and $s_N^{N:C}$ are both positive. Observations of phosphate/nitrate against particulate organic matter P:C and N:C across the global ocean indeed broadly follow this general trend (Galbraith and Martiny, 2015; Tanioka and Matsumoto, 2017).

Phytoplankton can temporally store excess nutrient intracellularly until the rate of carbon assimilation catches up to achieve steady-state balanced growth. Excess phosphorus for example can be stored mainly as polyphosphate (Dyhrman, 2016) and excess nitrate can be stored primarily as protein and free amino acids (Liefer et al., 2019; Sterner and Elser, 2002). Phytoplankton can consume these internal stores of nutrients (e.g., polyphosphates under P limitation) while maintaining the same level of carbon fixation, when the uptake of the nutrients does not meet its demand for growth (Cembella et al., 1984). In addition, phytoplankton can reduce their number of ribosomes and RNA content under P limitation as RNA typically accounts for 50% of non-storage phosphorus (Hessen et al., 2017; Lin et al., 2016) which would conserve phosphorus for other uses in a cell, resulting in lower P:C ratios. Similarly, cells can reduce synthesis of N-rich protein content under N limitation resulting in lower N:C ratio (Grosse et al., 2017; Liefer et al., 2019). These transient processes controlling the intracellular content of

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P or N (but not C content as much) likely result in positive correlation between P:C and N:C with macronutrient concentrations.

Although $s_P^{P:C}$ and $s_N^{N:C}$ are consistently positive across all the studies, they are noticeably higher for eukaryotic phytoplankton than for prokaryotes (Fig. 3a). There are several hypotheses for explaining this trend. One of the most plausible hypotheses is related to the size and storage capacity difference amongst phytoplankton groups (Edwards et al., 2012; Lomas et al., 2014). Since eukaryotes are generally larger and possess more storage capacity, they are capable of greater luxury uptake and accumulation of internal P and N reserves when the nutrient is in excess (Talmy et al., 2014; Tozzi et al., 2004). When nutrients are scarce, large cell size of eukaryotes allow them to increase their carbon content considerably by accumulating excess carbon as polysaccharides and lipids (Liefer et al., 2019; Lin et al., 2016). Another plausible hypothesis concerns variability in acclimation/adaptation strategy at the genetic level (Dyhrman, 2016). Recent studies suggest that different phytoplankton groups exhibit different levels of transcriptional responsiveness and have dissimilar strategies for using nitrate (Lampe et al., 2019) and phosphate (Martiny et al., 2019). For example, diatoms have superior abilities to uptake and store nutrients by being able to quickly regulate their gene expression patterns required for nutrient uptake compared to other phytoplankton groups (Cáceres et al., 2019; Lampe et al., 2018, 2019). These hypotheses provide plausible explanations for why eukaryotes have elevated stoichiometry sensitivity to macronutrients compared to prokaryotes.

4.3 Iron

Iron is used in key biochemical processes such as electron transport, respiration, protein synthesis, and N fixation (Marchetti and Maldonado, 2016; Twining and Baines, 2013). Many of the iron-dependent processes are required for harvesting energy and biochemical intermediates. As energy acquisition is equivalent to light acquisition in phototrophs, it makes sense that % changes in stoichiometry for iron are similar in sign and magnitude as for light (Fig. 2b). Although the effect of increasing iron on N:C is similar in sign and magnitude to that of light, we found unlike irradiance increase that increasing iron availability does not lead to a significant change in mean N:C (Figure 2b). This suggests smaller than expected changes in the carbon or the nitrogen content (e.g., compounds such as porphyrin and

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Deleted: A previous meta-analysis study showed that cellular N:P ratio of phytoplankton is significantly positively correlated with N:P supply ratio of nutrients (Persson et al., 2010), providing a picture that essentially "algae are what they eat". As cellular N:P is effectively a ratio between cellular N:C and P:C, our analysis is consistent with this picture because the mean plasticity of P:C is greater than that of N:C (i.e. the magnitude of $s_{NP}^{P:C}$ is significantly greater than that of $s_{NP}^{N:C}$ with the opposite sign; Fig. 2c). We would expect $s_{NP}^{P:C}$ and $s_{NP}^{N:C}$ to be more equal in magnitude if cellular N:P ratio was more homeostatic. Cellular N content generally covaries with cellular protein contents (Leonardos and Geider, 2004; Liang et al., 2019), while cellular P content covaries with macromolecular pools of RNA, DNA, and phospholipids (Liefer et al., 2019). Large stoichiometry sensitivity of P:C over N:C suggest N-uptake and protein synthesis change does not keep pace completely with P-uptake and synthesis of P-rich molecules. This pattern of larger stoichiometric flexibility of P:C over N:C with respect to nutrient availability has also been observed globally in the marine environment (Galbraith and Martiny, 2015) consistent with our meta-analysis result. ¶

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phycobiliprotein that are essential for light harvesting) under Fe limitation (Falkowski and Raven, 2007; Twining and Baines, 2013). Alternatively, Fe availability may be affecting cellular C, N, and P more or less proportionally for all phytoplankton leading to constant P:C and N:C (Greene et al., 1991; van Oijen et al., 2004; La Roche et al., 1993; Takeda, 1998). We also did not find noticeable heterogeneities in P:C and N:C amongst different moderators. In the future study, we could combine cellular C:N:P information with other measures of phytoplankton physiology (e.g., chlorophyll fluorescence, Fv/Fm ratio) in order to provide a more coherent, mechanistic picture on how changes in iron availability affect their physiology.

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4.4 Irradiance

Light availability affects the photoacclimation strategy of phytoplankton and subsequently the cellular allocation of volume between N-rich light-harvesting apparatus, P-rich biosynthetic apparatus, and C-rich energy storage reserves (Falkowski and LaRoche, 1991; Moreno and Martiny, 2018). At a fixed growth rate, high irradiance should downregulate production of N-rich light harvesting proteins and pigments in order to minimize the risk of photooxidative stress. Excess carbon fixed under high irradiance condition is stored as C-rich storage compounds such as lipids and polysaccharides (Berman-Frank and Dubinsky, 1999). As a result, N:C is expected to decrease under high light. In contrast, under low light condition, macromolecular composition should favor N-rich light harvesting apparatus over C-rich storage reserves, thus elevating N:C. This line of reasoning would predict negative relationship for the effect of irradiance increase on N:C, which is borne out in our meta-analysis (Fig. 2).

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Similarly, P quota should be affected by change in irradiance if P is the main limiting nutrient (Moreno and Martiny, 2018). Under P limitation, P:C is expected to decrease at increased light level because the total supply of inorganic phosphorus will not be able to keep up with the increase in photosynthetic carbon fixation, leading to decoupled uptake of C and P (Hessen et al., 2002, 2008). Conversely, P:C is expected to increase at lower irradiance because carbon fixation decreases while phosphorus uptake remains constant (Urabe and Sterner, 1996). As we did observe such P:C responses with statistically significant negative s-factor (Fig. 2), we can infer that most of the experiments were likely to have been P-limited, although such information is not necessarily given in the original studies.

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The magnitude of the weighted mean s-factors for both P:C and N:C however are small and the heterogeneity amongst PFTs are not discernible. This result agrees with a previous study which compiled experimental data prior to 1997 (MacIntyre et al., 2002). It is possible however that s-factors obtained in our meta-analysis are underestimated as there are several factors that may mute the effect of irradiance on N:C ratio of phytoplankton. For example, increase in nitrogen requirement for Rubisco (Li et al., 2015) and nutrient uptake machinery (Ågren, 2004) at high irradiance could be partly offset the reduction in N content resulting from the down regulation of light harvesting apparatus. In addition, multiple studies have noted increase in the protein demand (e.g., D1 protein) for repairing damaged light harvesting apparatus at high irradiance (Demmig-Adams and Adams, 1992; Li et al., 2015; Talmy et al., 2013) which also works in favor of stabilizing N content. Furthermore, we may have underestimated our s-factor if the high end member irradiance were above the optimal light level. This is a fundamental limitation of s-factor determination as the original studies do not measure the true optimal irradiance across the range of irradiance values but simply report an arbitrary value that is either "high" or "light replete".

Interestingly, we observed larger stoichiometric shifts in nutrient replete batch and chemostat culture compared to those cultures conducted under semi-continuous setting (Fig. 3b). In addition, we found that experiments conducted under periodic daily light cycle have larger negative s-factors compared to those experiments carried under continuous light (Fig. 3d). This is consistent with the global observation (Martiny et al., 2013a) and model studies (Arteaga et al., 2014; Talmy et al., 2014, 2016) which have shown that both the magnitude and temporal variability of N:C of phytoplankton are higher in the nutrient-rich, light-limited polar/subpolar regions than in the light-replete subtropics.

4.5 Temperature

We found that P:C ratio decreases as temperature increases while N:C remains relatively unchanged. Our result is consistent with a previous meta-analysis (Yvon-Durocher et al., 2015) that showed decrease in phytoplankton P:C under both laboratory and field settings. Moreover, our study and the study by Yvon-Durocher et al. both support the idea that P:C is more flexible than N:C with respect to change in

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Deleted: methodological factors that may mute the effect of irradiance on N:C ratio of phytoplankton. Firstly, not all studies were carried under nutrient (nitrate) limited condition, hence the downregulation of N-rich light harvesting apparatus was not needed to maintain growth. Secondly, the growth rate was not controlled in all the studies. Ideally, chemostat/turbidostat experiments are most suited for isolating the effect of environmental driver as it allows direct manipulation of growth rate. This is because any change in cellular nutrient:C ratio can be attributed to a specific environmental driver rather than to changes in specific growth rate (Hessen et al., 2002). However, for practical and economic reasons, batch and semi-continuous culture are more commonly used (La Roche et al., 2010). Thirdly, we did not consider the effect of light regimes (i.e. the length of light and dark hours) and diel changes on N:C. Longer light period leads to a more stable N:C over the course of the day as the amount of carbon fixed remains relatively constant, while experiments with longer dark hours leads to larger diel change in N:C (Lopez et al., 2016; Mohr et al., 2010; Ng and Liu, 2015; Talmy et al., 2014). We speculate that the lack of diel changes may have muted the underlying photoacclimation responses. Despite these experimental limitations, consistency in the s-factors across all studies indicates irradiance measured by photon flux density is one of the key determinants for N:C. This is

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temperature, which suggest that intracellular P content is more sensitive to change in temperature than intracellular N content.

Although the underling mechanism for explaining lower P:C at higher temperature is not fully understood, there are currently three main hypotheses (Paul et al., 2015): (1) increase in metabolic stimulation of inorganic carbon uptake over phosphorus uptake; (2) increase in nutrient use efficiency which enables greater carbon fixation for given nutrient availability; and (3) "translation compensation theory," which predicts that less P-rich ribosomes are required for protein synthesis and growth as the translation process becomes kinetically more efficient (McKew et al., 2015; Toseland et al., 2013; Woods et al., 2003; Xu et al., 2014; Zhu et al., 2017).

Differences in s-factors amongst PFTs was not statistically significant and none of the PFT displayed statistically significant response in isolation. In other words, we did not see any PFT-specific adaptive/evolutionary response to warming (Schaum et al., 2018; Taucher et al., 2015). However, we observed noticeable variability due to the difference in culture growth mode (Fig. 3b) and growth phase at extraction (Fig. 3c). The latter factor is particularly noticeable for P:C, where phytoplankton extracted during nutrient-replete exponential growth phase have significantly more negative stoichiometric flexibility with larger magnitude compared to those extracted during nutrient-deplete stationary phase. This is consistent with multiple recent studies which suggest that the effect of temperature on growth and metabolic rates are greater when plankton are not nutrient and/or light limited (Aranguren-Gassis et al., 2019; Marañón et al., 2018; Roleda et al., 2013). This leads us to hypothesize that change in P:C ratio due to ongoing warming will be more noticeable in the nutrient rich polar regions especially given the fact that temperature is already increasing at a startling rate due to polar amplification (Post et al., 2019).

4.6 Limitations and caveats

In the real ocean, none of the environmental changes discussed will likely occur in isolation because changes in irradiance, temperature, and nutrient availability are often linked. For example, an increase in sea surface temperature enhances the vertical stratification of the water column, which leads to greater levels of irradiance and nutrient limitation for phytoplankton trapped in a more shallow mixed layer (Boyd et al., 2015; Hutchins and Fu, 2017). Indeed, a meta-analysis on the pair-wise effects of environmental

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4.4 Temperature¶

For microorganisms, temperature is arguably the most important environmental factor affecting growth and survival (Madigan et al., 2017). Temperature controls the kinetic responses such as enzyme activity, cell division, and nutrient uptake which all are thought to occur at higher rates with elevated temperatures (Hessen et al., 2017). Also, temperature can alter macromolecular composition, rate of protein synthesis, and storage of elements (Moreno and Martiny, 2018). Phytoplankton are able to efficiently grow over a range of temperatures around the optimal growth temperature but their growth at substantially different temperatures can lead to photodamage (Huner et al., 2008), inhibition of protein synthesis (Li et al., 2019), or the decline in photosynthetic efficiency (Falk et al., 2006). As a result, a growth curve of phytoplankton is unimodal (Boyd et al., 2013; Zhu et al., 2017) with increasing growth rate from the minimum temperature to the optimum temperature and decreasing growth rate towards the maximum temperature (Madigan et al., 2017). ¶

Broadly, there are two kinds of species, a thermal specialist whose growth rate rapidly drops off as temperature exceeds the optimal temperature, and a thermal generalist whose growth rate remains constant over a wide range of temperatures (Collins et al., 2020). Since the P:C and growth rate are intricately linked (Sterner and Elser, 2002), our meta-analysis suggests that cyanobacteria are ... [5]

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drivers on elemental stoichiometry of phytoplankton has shown that the interactions of two environmental stressors can impose predominantly non-additive effects to C:N:P of phytoplankton so that the overall effect of multiple stressors is more than simply the sum of its parts (Villar-Argaiz et al., 2018). In addition to the individual phytoplankton stoichiometry, the bulk organic matter stoichiometry also reflects the phytoplankton community composition (Bonachela et al., 2016; Weber and Deutsch, 2010) as well as the stoichiometry of detrital material. Processes such as decomposition (Karl and Dobbs, 1998; Verity et al., 2000; Zakem and Levine, 2019), viral shunt (Jover et al., 2014), and preferential remineralization of phytoplankton macromolecules (Frigstad et al., 2011; Grabowski et al., 2019; Kreus et al., 2015) can also decouple phytoplankton C:N:P from the bulk organic matter C:N:P.

4.7 Implications for global ocean biogeochemistry

Recent global biogeochemical models are starting to incorporate a more realistic representation of plankton physiology, which includes flexible phytoplankton C:N:P (e.g., Buchanan et al., 2018). Modeling studies with flexible phytoplankton stoichiometry have demonstrated that proliferation of C-rich phytoplankton under future climate scenario has the potential to buffer expected future decline in carbon export and net primary productivity caused by increased stratification (Kwiatkowski et al., 2018a; Moreno et al., 2018; Tanioka and Matsumoto, 2017). This buffering effect cannot be simulated by biogeochemical models with fixed phytoplankton C:N:P.

One way to model the dependencies of multiple environmental drivers (e.g., P, N, irradiance, and temperature) on C:N:P of marine phytoplankton is the power-law formulation by Tanioka and Matsumoto (2017):

$$[X:C] = [X:C]_0 \left(\frac{[PO_4]}{[PO_4]_0}\right)^{s_{PO_4}^{X:C}} \left(\frac{[NO_3]}{[NO_3]_0}\right)^{s_{NO_3}^{X:C}} \left(\frac{I}{I_0}\right)^{s_I^{X:C}} \left(\frac{T}{T_0}\right)^{s_T^{X:C}} \quad (X = P \text{ or } N) \quad (9)$$

where subscript "0" indicates reference values. The s-factors obtained from this meta-analysis are the exponents of equation (9) for different PFTs. Within the context of the power law formulation, our results would indicate, for example, that eukaryotic phytoplankton would have the largest plasticity in P:C and N:C compared to prokaryotes with respect to the change in nutrient availability. Under future warming,

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Iron is used in key biochemical processes such as electron transport, respiration, protein synthesis, and N fixation (Marchetti and Maldonado, 2016; Twining and Baines, 2013). Many of the iron-dependent processes are required for harvesting energy and biochemical intermediates. As energy acquisition is equivalent to light acquisition in phototrophs, it makes sense that s-factors for iron are similar in the signs and magnitudes to those of light.

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high s-factors of eukaryotes may thus play an important role in buffering the expected future decline in carbon export and net primary productivity (Kemp and Villareal, 2013).

We can give a first-order estimate of how much the elemental stoichiometry of marine phytoplankton may change in the future using equation (9) given a typical projection of the change in the key environmental drivers and the estimates of the s-factors (Table 3; Fig. 4). Global climate models generally predict a decline in macronutrients and increase in temperature and irradiance as a result of surface warming, increased vertical stratification and reduced mixed layer depth (Bopp et al., 2013; Boyd et al., 2015). With large projected declines in macronutrients (-28.0% for phosphate, -18.7% for nitrate) we can predict increase in C:P and C:N by ~10 units (molar ratio) and ~0.2 units, respectively, assuming the mean biomass-weighted particulate organic matter C:N:P of 146:20:1 as the present-day value (Martiny et al., 2013b). Further increase in C:P is expected due to temperature increase of around 1% (~3K). The total C:P change ranges from +6 ~ +25 taking into account all the uncertainties associated with the s-factors. For C:N, we estimate an overall increase by 0.1~0.4 units largely driven by decrease in nitrogen availability. The effect of change in irradiance is noticeably smaller (Table 3). In summary, this simple calculation highlights potentially a large shift for C:N:P, whose change is predominantly driven by reduction in macronutrients and temperature increase.

5. Conclusions

Our meta-analysis represents an important bottom-up approach in predicting how elemental stoichiometry of phytoplankton may evolve with the climate change. We conclude that macronutrient availability is the most significant and shared environmental driver of C:N:P. Changes in C:N:P by macronutrients are driven by primary/plasticity responses commonly shared across phytoplankton. Our analysis shows that eukaryotic phytoplankton have higher stoichiometric plasticity compared to prokaryotes. Eukaryotes' large stoichiometric flexibility and high intrinsic growth rate can explain their unexpectedly high diversity (Malviya et al., 2016) and large contribution to carbon export globally even in oligotrophic regions (Agusti et al., 2015; Nelson and Brzezinski, 1997). The effects of temperature on C:P is also significant suggesting that future ocean with elevated temperature and increased stratification will favor production of carbon-rich organic matter. Future laboratory-based studies focused on exploring the effects of multiple

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Deleted: For example, irradiance, temperature, and nutrient availability are often linked because the change in light availabilit ... [13]

Moved up [10]: Indeed, a meta-analysis on the pair-wise effects of environmental drivers on elemental stoichiometry of

Deleted: In addition, a recent multi-driver study carried for eight different drivers has shown that only a few dominant drivers can [14]

Moved up [11]: reflects the phytoplankton community composition (Bonachela et al., 2016; Weber and Deutsch, 2010) as

Deleted: organic matter accumulation and remineralization, which can be decoupled from the organic matter production ratio (Schulz ... [15]

Moved up [12]: . This buffering effect cannot be simulated by biogeochemical models with fixed phytoplankton C:N:P. ¶

Deleted: Many of the global models with flexible C:N:P currently employ simple linear models where elemental stoichiometries are ... [16]

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Moved up [14]: The s-factors obtained from this meta-analysis are the exponents of Equation (2) for different PFTs.

Deleted: Within the context of the power law formulation, our results would indicate, for example, that diatoms would have the [17]

Moved up [15]: may thus play an important role in buffering the expected future decline in carbon export and net primary productivity

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environmental drivers would interactively alter the elemental composition of phytoplankton would be needed for a complete understanding. In addition, a further investigation on how change in environmental drivers affect stoichiometry of heterotrophs and zooplankton will be useful in filling the gaps to gain more mechanistic views on how these drivers affect the whole marine ecosystem.

Data availability: All the data and codes used in the meta-analysis are available in Zenodo data repository (http://doi.org/10.5281/zenodo.3723121).

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Author contributions: TT and KM designed the study. TT carried out the literature review, data selection, analysis, and created figures. Both TT and KM wrote the manuscript.

Competing interests: The authors declare no conflict of interest.

Acknowledgements: This research was supported by a grant from the US National Science Foundation (OCE-1827948). TT acknowledges support from University of Minnesota Doctoral Dissertation Fellowship. KM acknowledges sabbatical support by the Leverhulme Trust Visiting Professorship and the University of Oxford. We thank Carolyn Bishoff, Julia Kelly, and Amy Riegelman from University of Minnesota Library for helping out literature search and data selection. We also thank James Cotner for providing us feedback on the manuscript.

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Captions for figures

Figure 1. Flow chart showing (1) the preliminary selection criteria and (2) the refined selection criteria used for determining s-factors. Numbers (k values) correspond to the number of journal articles. See Supplementary Information (Appendix S1) for a full list of studies included in the meta-analysis.

Figure 2. Summary plot showing weighted mean responses of P:C and N:C using (a) Stoichiometry sensitivity factor, and (b) % changes between control and treatment. Numbers next to the plots in (b) correspond to the number of experimental units and the numbers are identical in (a). Numbers in the outside column are the weighted means. $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$, ns: not significant. Note that x-axis is different for temperature experiments in (a).

Figure 3. Summary plot showing statistically significant effects of moderators. (a) Eukaryotes vs Prokaryotes, (b) Growth mode, (c) Growth phase at harvest, (d) Light regime. $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$

Figure 4. Illustration of how the five environmental drivers under a typical future climate scenario affect the cellular allocation of volume between P-rich (red), N-rich (blue), and C-rich (orange) pools. The values for projected changes in C:P and C:N between 1981-2000 and 2081-2100 are given in Table 3.

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Figure 2: S-factors for P:C and N:C with respect to changes in (a) Phosphate, (b) Nitrate, and (c) Nitrate/Phosphate for individual experimental units and different phytoplankton functional types (PFTs). Mean values for PFT are indicated by filled diamond. Mean across all PFTs are indicated by open diamonds. Error bars represent the 95% confidence intervals. "N/A" signifies that the total experimental units were less than five for a given driver-stoichiometry pair in order to carry out a meta-analysis. ¶
Figure 3: S-factors for P:C and N:C with respect to changes in irradiance for individual experimental units and different PFTs. Legend and error bars are as Figure 2. ¶
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Figure 4: S-factors for P:C and N:C with respect to changes in temperature for individual experimental units and different PFTs. Legend and error bars are as Figure 2. ¶
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Figure 5: S-factors for P:C and N:C with respect to changes in iron for individual experimental units and different PFTs. Legend and error bars are as Figure 2. ¶ ... [19]
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Tables

Key search terms
(TS=(phytoplankton OR algae OR microalgae OR diatom OR coccolithophore* OR cyanobacteri* OR diazotroph*) AND TS=(stoichiometr* OR "chemical composition" OR "element* composition" OR "nutritional quality" OR "nutrient composition" OR "nutrient content" OR "nutrient ratio*" OR C:N OR C:P OR N:P OR P:C OR N:C OR "cellular stoichiometr*" OR C:N:P OR "element* ratio*" OR "food qualit*" OR "nutrient concentration" OR "carbon budget") AND TS = (phosph* OR "phosph* limit*" OR nitr* OR "nitr* limit*" OR iron OR "iron limit*" OR nutrient OR "nutrient limit*" OR "nutrient supply" OR "nutrient availabilit*" OR "supply ratio*" OR eutrophication OR fertili* OR enrichment OR temperature OR warming OR light OR irradiance OR "light limit*") AND TS = (marine or sea or ocean OR seawater OR aquatic)).

Table 1. Key word search terms used for literature search (Web of Science, February 2019). In the search field, "TS" refers to a field tag for "topic" and "*" is a wildcard search operator.

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		Stoichiometry sensitivity factor				Log-response ratio			
Drivers	n	$\overline{s_X^Y}$	ci.lb	ci.ub	sig.	$\overline{\ln(RR)}$	ci.lb	ci.ub	sig.
Phosphorus									
P:C	54	0.21	0.12	0.29	***	1.21	0.99	1.44	***
N:C	52	0.023	0.0041	0.042	*	0.21	0.12	0.29	***
Nitrogen									
P:C	32	0.0073	-0.0053	0.020	ns	0.09	-0.070	0.25	ns
N:C	60(1)	0.14	0.082	0.20	***	0.53	0.40	0.66	***
Fe									
P:C	37					0.0090	-0.14	0.16	ns
N:C	65					-0.019	-0.094	0.055	ns
Irradiance									
P:C	35	-0.0034	-0.062	-0.0070	*	-0.24	-0.47	-0.0034	*
N:C	94	-0.0224	-0.034	-0.013	***	-0.20	-0.26	-0.13	***
Temperature									
P:C	83	-3.6	-6.8	-0.35	*	-0.16	-0.27	-0.053	**
N:C	96	-0.42	-1.90	1.07	ns	-0.014	-0.061	0.033	ns

Table 2. Summary of the meta-analysis using the stoichiometry sensitivity factor and natural logarithm-transformed response ratio. *n*, number of experimental units (numbers in bracket = number of outlier studies). ; $\overline{s_X^Y}$, weighted mean stoichiometry sensitivity factor with environmental driver X and response variable Y; $\overline{\ln(RR)}$, weighted mean value of the natural logarithm-transformed response ratio; ci.lb, lower boundary of 95% CI; ci.ub, upper boundary of 95% CI; sig., significance of the mean weighted effect size; ns, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Any experiments with studentized residual value of ln(RR) exceeding 3 was removed as outliers. Red bold texts highlight statistically significant environmental driver using both effect sizes.

Change in stoichiometry	Change in Environmental Drivers					
	P↓ (-28%)	N↓ (-18.7%)	I↑ (+0.7%)	T↑ (+9%)	Fe↑ (+6.5%)	Combined
Δ (C:P) (molar)	+10.4 (5.9-14.6)	/	+0.03 (0.01-0.06)	+3.7 (0.4-7.1)	/	+16 (6-25)
Δ (C:N) (molar)	+0.06 (0.01-0.10)	+0.22 (0.12-0.31)	<0.01	/	/	+0.3 (0.1-0.4)

Table 3. Projected changes in C:P (molar) and C:N (molar) between 1981-2000 and 2081-2100 given model-based projected changes in environmental drivers from Boyd et al. (2015). Changes in C:N and C:P are calculated separately for each driver with s-factors from Table 2 combined with reference C:N:P of 148:20:1, a global biomass-weighted mean ratio of particulate organic matter (Martiny et al., 2013b). Ranges are derived from propagating uncertainties for the weighted mean s-factors in Table 2. We used Equation (9) in the main text for estimating the combined effect of multiple drivers.

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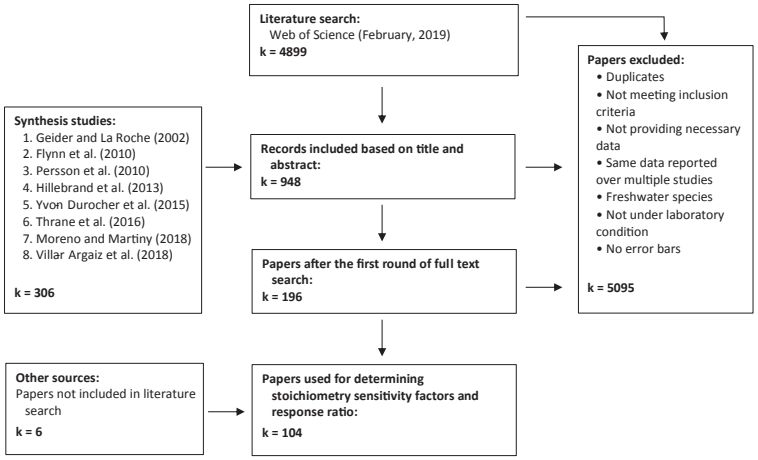
Figure 1



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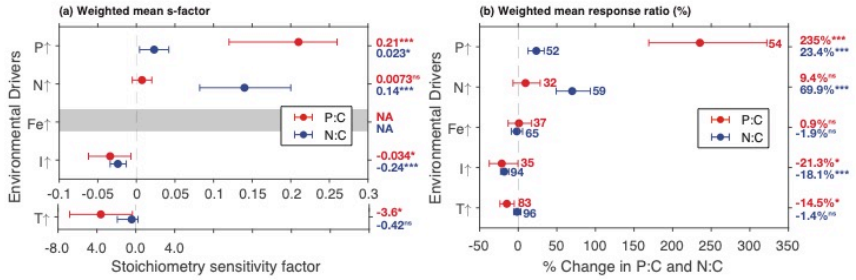
990 **Figure 2**



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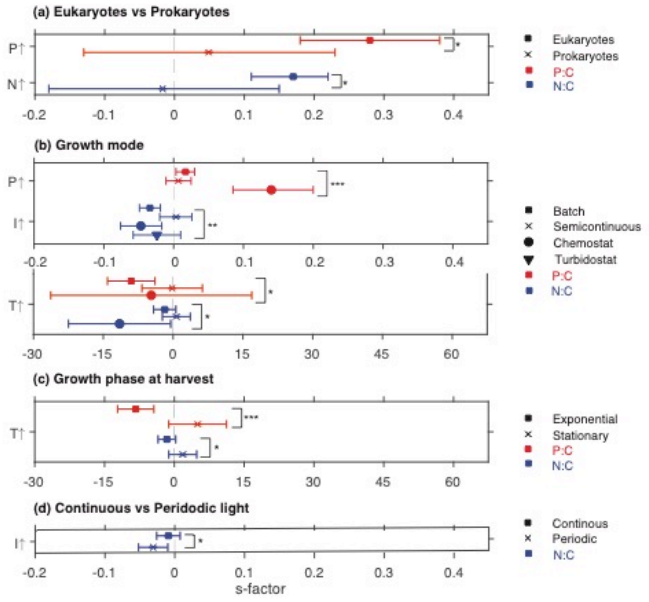

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Figure 4

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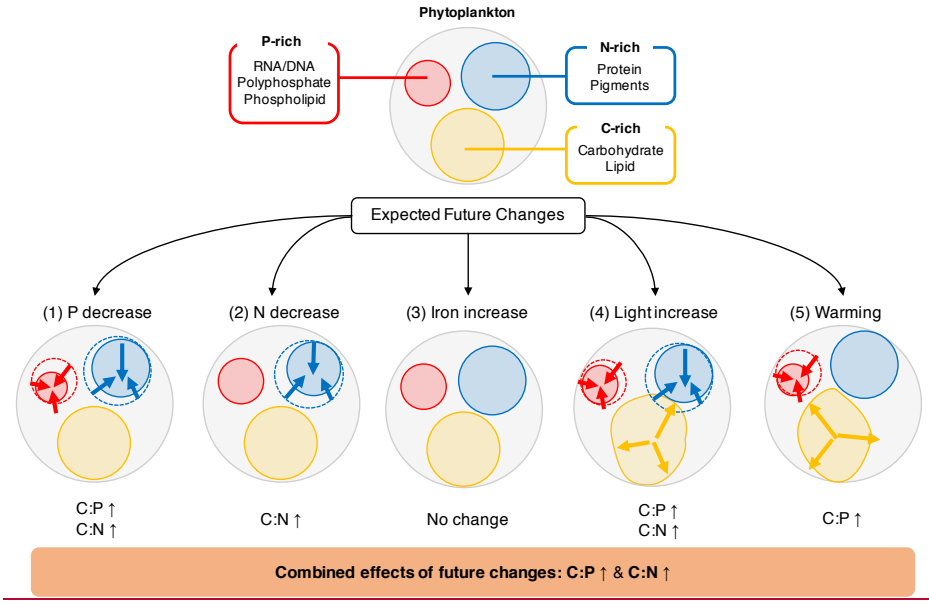

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