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).

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) correctly categorizing taxonomic affiliation of some 'diatoms' in the data, 2) discussing limitation of the analysis, 3) providing more details of the database, and 4) considering other functional groups (i.e. categorical modifiers). We will address 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 will correct these misclassifications in the new database and will re-run 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 will follow the reviewer suggestion to analyze the data with a finer classification for the non-diatoms. In the revised version, we will use more specific moderators. Tentative groups are 1) diatoms, 2) coccolithophores, 3) dinoflagellates, 4) other eukaryotes, 5) prokaryotes, and 6) diazotrophs.

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 already included the basic details of the growth conditions mentioned here (temperature, light-dark cycle, and light level). We will add details on salinity, culture medium (natural or artificial seawater), acclimation (# of generations), optimality (temperature and salinity), and growth mode (batch, semi-continuous, and chemostat).

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 will mention 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 will modify lines 31-32 accordingly.

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 will redefine new categorial classes: 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 selected studies where inflow nitrate concentration (for chemostat and semi-continuous experiments) or initial nitrate concentration in the fresh media (for batch experiments) are manipulated but other forms of inorganic nitrogen are kept constant. It is beyond the scope of this to consider if other sources of N have the same or different impacts on N:C. We will mention this point in the modified manuscript.

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 is not in fact statistically significant for N:C (Table 2). We will rephrase our conclusion 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 commonly true for other nutrients. We will therefore remove this sentence altogether.

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

Since this sentence is vague and not well supported, we will remove 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)?*

    Although information on light-dark cycle are in our database already, we did not analyze the effect of light-dark cycle or the total daily dose on C:N:P. We will discuss more extensively limitations of our meta-analysis in this regard (i.e., the fact that not all the factors associated with irradiance are included).

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. Indeed, N availability has stronger influence in low latitudes. We will rephrase this sentence to make the meaning clearer.

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 will therefore remove 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).*

    We will carry out analysis on dataset by Yvon-Durocher study to test whether timescale difference is indeed the only reason that leads to a different conclusion. If this is not the case, there must be other reasons (difference in the selection of the effect size) for explaining the divergence between ours and Yvon-Durocher's result. We would be providing possible explanations if necessary.

    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, which will analyze original experimental studies that have not yet been conducted.

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 will modify the sentence 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.

Quigg, A., Finkel, Z. V., Irwin, A. J., Rosenthal, Y., Ho, T.-Y., Reinfelder, J. R., Schofield, O., Morel, F. M. M. and Falkowski, P. G.: The evolutionary inheritance of elemental stoichiometry in marine phytoplankton., Nature, 425(6955), 291–4, doi:10.1038/nature01953, 2003.

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.