

Interactive comment on "Sequential Nutrient Uptake by Phytoplankton Maintains High Primary Productivity and Balanced Nutrient Stoichiometry" by Kedong Yin and Paul J. Harrison

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-Reviewer 2

The manuscript by Yin and Harrison measured nitrate and phosphate profiles, along with incubation experiments, to explore the ideas of nutrient drawdown in a coastal ecosystem. The title and introduction bring together ideas about the timing of nutrient uptake, the level of primary production, and how those relate to cellular nutrient stoichiometry. These are intriguing ideas and could shed light on a number of important marine processes and the linkages between them. Unfortunately, I found the presen-

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tation of methods and data to be either missing or difficult to follow. The ideas of the introduction didn't necessarily follow the data that was collected. For example, the introduction was mostly about particulate elemental ratios and diversity, but the study itself was about dissolved nutrient ratios of nitrate and phosphorus. No connection was made between these different types of elemental ratios. Because the methods section was missing many details, it was difficult to follow what the experiments were and when they were done; therefore, it was difficult to assess the interpretation of results. I found the conceptual model presented in Figure 1 to mostly add confusion rather than clarification to the results. There were a number of more specific issues found in the bulk of the manuscript, which have been listed below.

#Reply: Thank you for your comments. We have revised the manuscript based on your suggestions and comments.

-Reviewer 2 Suggested revisions -Redfield is a concept for the open ocean and longterm nutrient balance with deep mixing, that specifically does not account for N-fixation or terrestrial inputs. These are not the conditions here. There is no explanation of other nitrogen forms, like ammonium and DON, which are likely important in a coastal system.

#Reply: Redfield ratio is also a concept for phytoplankton nutrient composition. Ammonium concentration was usually small in the Strait of Georgia during summer and was not considered to contribute so much to dissolved inorganic nitrogen. DON is not considered in this conceptual model of sequential nutrient uptake as no evidence indicate rapid uptake of DON.

-Reviewer 2 -Line 62: While the Conley et al. paper is about nutrient limitation and eutrophication control, it says nothing about Redfield, nor does it present any data. It is an opinion piece about coastal management.

#Reply: Redfield ratio has been used to indicate which, N or P, is the most limiting nutrient that should be controlled when managing coastal eutrophication. We have

deleted this citation as our statement is common enough.

-Reviewer 2 -Lines 63-66: what about the work by Martiny and co-authors about global patterns of C:N:P and it's connections to diversity?

#Reply: Yes, we have referred to the paper by Martiny et al. (2013, Nature Geosciences).

-Reviewer 2 Lines 72-75: This sentence was confusing. If the authors are stating that there are no measurements of C:N:P in heterotrophic bacteria, they should take a read through Gunderson et al. (L&O 2002) and Godwin & Cotner (ISME 2015).

#Reply: We have revised the sentence. In the measurements of elemental ratios of C:N:P of organic matter, dead plankton or organic detritus can not be separated from live organisms such as bacteria and phytoplankton. Therefore, when concentrations of these non-living organic matter vary, they contribute to our measurement of elemental ratios, but it is hard to assess their relative contributions.

-Reviewer 2 -Line 138: What about the uptake of ammonium or dissolved organic nitrogen? This would certainly impact both the uptake rates and the overall drawdown of Si:N.

#Reply: Ammonium produced by zooplankton can be taken up and affect drawdown of N:Si, but ammonium is usually very low in the Strait of Georgia during summer and its effect was assumed to be small.

-Reviewer 2 -The methods state that this experiment was done August 6-14, 1991, but a number of other places in the manuscript refer to additional experiments done on other dates (e.g. data shown in Figures 8 and 9). At a minimum, those additional experiments need to be described.

#Reply: The incubation experiments were conducted in different years, but in the same season. We have added the description in Methods.

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-Reviewer 2 -For fluorescence (line 151) and nutrients (lines 165-169), more detail is needed on the standards used and detection limits.

#Reply: Fluorescence has a relative unit, no standardization was made. The standards of nutrients are self-made with chemicals NaNO3, NH4Cl, KH2PO4, NaSiO4. Detection limits are as follows. NO3 = 0.1 uM, NH4 = 0.05 uM, PO4 = 0.05 uM, SiO4 = 0.01 uM

-Reviewer 2 -Line 184: Are T1 and T7 referring to time points, or conceptual models?

#Reply: Yes, they are referring to time points, as shown in the figure legend. However, we have changed T0, T1, ... T6 to C0, C1, C6 in Fig. 1 to avoid the confusion.

-Reviewer 2 -Line 199: clear how? Lack of change in ambient dissolved nutrient concentrations does not necessarily imply lack of uptake. It could just as easily be fast turnover rates.

#Reply: Yes, you are right. In this case here, we stated: "little PO43- was consumed while NO3- was taken up", which indicates that turnover of nitrogen did not stop NO3 uptake so that N:P ratio followed NO3.

-Reviewer 2 -Line 225-226: Further explanation is necessary to understand which experiments were considered "on-deck" and how that relates to the conceptual model, which is all about mixing events.

#Reply: The incubation experiments conducted on board the ship were considered to be "on-deck" experiments. These experiments show that sequential nutrient uptake happens in seawater and confirm our observations of vertical profiles of N:P and N:Si ratios which are related to the conceptual model.

-Reviewer 2 -Line 230: Fluorescence does not equal biomass.

#Reply: Yes, you are right. Here we used it for an indication of when we could stop incubation. We found that the disappearance of the most limiting nutrient usually hap-

pens one day before fluorescence reaches the maximum.

-Reviewer 2 -Lines 257-258: there is no data shown on primary production, and thus this statement is difficult to evaluate.

#Reply: Revised as "The Strait of Georgia is highly productive, reaching up to 2,700 mg C m-2d-1 in August. (Yin et al. 1997a)"

-Reviewer 2 -Lines 269-280: The logic here is quite hard to follow, as each sentence is long and refer to multiple panels of different figures, with limited explanation and/or the use of vague terms (i.e "sitting on top" or "parallel lines").

#Reply: We have revised the section to simplify the discussion.

-Reviewer 2 -Line 316-317: What is the evidence for higher phytoplankton cell counts? -Line 318-319: This statement needs to be referenced and further explained.

#Reply: We have made references for the sentence, and also revised this paragraph based on another reviewer.

-Reviewer 2 -Line 335-336: It's not clear how open ocean internal waves are relevant to this discussion.

#Reply: In the open oceans, there are usually a permanent feature of the subsurface chlorophyll maximum. Phytoplankton there could use the sequential nutrient uptake strategy to maintain growth. Therefore, we would like to imply that our concept of sequential nutrient uptake is widely applicable.

-Reviewer 2 -Lines 338-339: Either in this manuscript or in the literature, what evidence is there that phytoplankton are changing position in the water column in the pursuit of nutrients? The work by Bienfang and colleagues in the early '80s would indicate that physiological nutrient status does not directly correlate to sinking rates.

#Reply: Our evidence mainly come from the vertical movement of the chlorophyll maximum. For example, in Yin et al. (1997a), we observed that the chlorophyll maximum

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was at the surface on Aug 10 and moved down to form the subsurface chorophyll maximum couples of days later. We think that this is due to phytoplankton sinking. We have revised the sentence to ".. their internal nutrient pool decreases and they sink down to the nutriclines, possibly due to the formation of clumps".

-Reviewer 2 -Line 350: POC and PON were not discussed in the methods or results, but introduced in the discussion and figures. In addition, from looking at Figure 10, it would seem that POC:PON ratio simply did not change, which could be due to any number of reasons, the most likely one being that C:N is a function of cell size and not limitation or luxury uptake. Besides, the introduction spells out all the reasons particulate ratios may be an unreliable measure of cellular nutrient stoichiometry.

#Reply: The method for POC and PON analysis has been added. POC and PON in a water sample was filtered onto a GF/F filter and analyzed with a Carlo Erba model NA 1500 NCS elemental analyzer, using the dry combustion method described by Sharp (1974). In laboratory cultures of phytoplankton, N limitation often leads to higher C:N ratio. In this study, we mainly focus on variability of ambient nutrient ratios, and little change in POC:PON simply shows that sequential uptake of nutrients can maintain phytoplankton stoichiometry.

-Reviewer 2 -Lines 355-363: The conclusions don't appear to be related to the primary points in the manuscript.

#Reply: We have revised the conclusion.

-Reviewer 2 -Figure 2: an inset of a larger area (zoom out) might be helpful for readers not familiar with this area. Also, the Fraser River location should be highlighted (it's a bit hard to see) and the approximate plume area/distance/direction should be indicated, as it is mentioned multiple times (e.g. lines 143, 183, 215, Figure 4, etc.) as having an influence on the sampling and results.

#Reply: This manuscript is mainly conceptual and the location of the study area is not

too important. We have added a "Note" in the figure legend to point out the Fraser River.

-Reviewer 2 -Figures 5 and 6 look like copies of each other. Are the two different stations really exactly the same at all time points? Either way, what is this time series? It was not explained in the methods.

#Reply: Yes, there was a mistake. Now we have used the correct figures.

-Reviewer 2 -Figure 7: The time-series results were not explained in the methods. How was this experiment performed? What is the bottom of the axis in the NO3-(middle panel)? It looks like NO3- goes to zero. Was the in vivo fluorescence measure calibrated to a chlorophyll standard, or was it all relative? How do the authors explain a potential lag in uptake of N and P? How would this relate to mixing events, which are presumably short-term?

#Reply: The time series results were referred to in lines 227-235. The method for the incubation experiment has been described in the Methods and also in the figure legend. The bottom axis for 3 panels is the same, incubation time. Yes, NO3 does go to zero. Fluorescence was not converted to chlorophyll as chl was not measured. Time lags in incubation experiments are usually associated with low biomass. However, in this case, we made 4 times sampling within 10 hours and there appeared to be little time lag as both NO3 and PO4 responded as a decrease within 10 hours. The relation between mixing events and the responses of phytoplankton in nutrient uptake can be coupled with or without time lags depending on phytoplankton nutritional status.

-Reviewer 2 -Figure 8: Is this station S3? There is no station 3 in the map in Figure 2. Why was this experiment done more than two years before the rest of the experiment? Why wasn't it explained in the methods?

#Reply: Yes, it is S3. We conducted quite a few experiments during 1989-1992 and used this experiment to demonstrate continuous uptake of NO3 with little P at 1 m

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sample and continuous uptake of PO4 and SiO4 after NO3 depletion. We gave explanations in the figure legend.

-Reviewer 2 -Figure 9: Most of the figure blurb needs to be in the methods. Additionally, exactly how the uptake ratios were calculated, and those results, need to be added to the manuscript. Why was this experiment done more than a year before the other experiments described herein?

#Reply: We have added the figure blurb in the figure legend and described how N:P ratio was calculated, explained why the experiments were conducted in different years. The uptake ratio was directly calculated from the decreasing concentrations over time during the incubation of seawater samples, e.g., using (day 2- day 1 nitrate concentration) /(day 2-day 1 phosphate concentration) to get N:P ratio on day 1.

-Reviewer 2 -Figure 9B: This figure contains the first mention of ammonium. How (i.e. what method) was it measured?

#Reply: Yes, we have added the method for ammonium into the Method.

-Reviewer 2 -Figure 9C: What does the terminology of +N/+P and +N/+Si mean?âÅÍ-Why was this sampling done the year prior to what was explained in the methods?

#Reply: We have fixed these in the figure legend. The sign "+" means "added" and "+N/+P " means, the single added N over single added P.

-Reviewer 2 Technical revisions -Line 57: what is the "stoichiometry of the water column"? Are the authors referring to the dissolved NO3-:PO4 ratio?

#Reply: Revised as stoichiometry of nutrients

-Reviewer 2 -Line 58-59: do the authors mean homeostatic when they say "variable"? That would make the sentence make more sense. Also, is there a reference for this relationship?

#Reply: Eventually, N:P ratio is homeostatic and hence, we have added this word in

the abstract, but here we meant that cellular N:P ratios vary with the nutrient supply N:P ratio. We have added a reference (Geider and La Roche 2002).

-Reviewer 2 -Line 66: typo. . . should read "mechanism proposed is the. . ." -Line 93: This should probably say that it is a "conceptual model". -Line 101: Did the authors mean to say "competition"?âĂÍ -Line 106: give a reference to Figure 2.

#Reply: Line 66: Revised: the proposed mechanism Line 93: Yes, added "conceptual" Line 101: replaced completion with competition Line 106: We have added a reference by LeBlond (1983).

-Reviewer 2 -Lines 113-120: It was confusing to see the conceptual models named T#, because that makes me think of a time-series. In fact, later in the paper (e.g. line 184), this same notation is used for time-series experiments.

#Reply: We have changed T# in Fig. 1 to C#

-Reviewer 2 -Line 144-145: One citation should be enough to explain station numbers.

#Reply: We have reduced the number to 1.

-Reviewer 2 -Why are there three figures that comprise Figure 9 given subscripts. This is a bit confusing, as lettering typically implies panels, not separate figures.

#Reply: We have revised the figure legend for Fig. 9, as Fig. 9-1, 9-2 and 9-3. Interactive comment on Biogeosciences Discuss., doi:10.5194/bg-2016-426, 2016.

End of reply

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Interactive comment on Biogeosciences Discuss., doi:10.5194/bg-2016-426, 2016.