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Comments to article by Yin et al BG 2016-426, E. Marañón, Associate Editor

--Reviewer

Authorship: A new author has been introduced in the revised version of the manuscript. This is irregular and should be justified. This new author is not mentioned in the author contribution section.

Reply:

In the first round, the new author contributed to the conceptual diagram intellectually and drew it, he has plotted the figures. He also searched the updated references. In the second round, he wrote the draft of the responses to reviewers and made the draft of revision.

--Reviewer

The description of field work is incomplete. At the beginning of section 2, when describing the sampling area, the authors must also indicate the sampling dates for all data used in the article. If the sampling took place as part of wider programme, this must be indicated, together with references to published studies that report on other properties of the system during the same study. It would be helpful to have a table with sampling location, station names, and sampling date, instead of including part of this information in the figure legends. This would be particularly helpful to understand the sampling schedule during the time series experiments. The bottle experiments on-deck must also be better explained.

Reply:

We have gone through all data descriptions and figures, and added a new table (Table 1.) to ensure that all sampling information is provided.

We added a couple of sentences in the end of the Method:

Vertical profiles and seawater samples for in-situ incubation which were used in this study were collected at different stations and different sampling times. Water column conditions such as salinity, temperature and fluorescence have been described in the listed publications as shown in Table 1.

--Reviewer

Title: The title states that sequential nutrient uptake maintains high productivity and a balanced nutrient content of phytoplankton, but the validity of these statements is not actually proven by the data. Strictly speaking, productivity (e.g. net phytoplankton biomass accumulation) has not been measured here, nor has the phytoplankton elemental composition. The title should be re-written to make it clear that this is a mechanism that is being proposed (and which certainly is consistent with some of the observations), but not a mechanism that has been observed. I suggest including in the title a phrase along the lines: 'Sequential nutrient uptake as a potential mechanism for phytoplankton to maintain...'

A similar comment can be made in relation to the sentence on lines 271-272.

Reply:

Revised the title as "Sequential Nutrient Uptake as a Potential Mechanism for Phytoplankton to Maintain High Primary Productivity and Balanced Nutrient Stoichiometry".

Revised as "Our results revealed sequential nutrient uptake as a potential mechanism to..."

--Reviewer

Similarly, the linkage between sequential nutrient uptake and the maintenance of phytoplankton stoichiometry near Redfield values (C:N around 7), which the authors make in the last section of the Discussion (lines 366-370), is tenuous at best, given that C:N ratios in particulate matter do not reflect phytoplankton elemental composition alone. These limitations should be explicitly acknowledged.

Reply:

We revised as follows:

In addition, POC:N ratio was slightly higher than 7:1 (Fig. 10) at Stn S1 where nitrogen was more frequently under detection limit than Stns S2 and S3. This might suggest the lack of ambient nitrogen limitation on the cellular nutrient stoichiometry. However, using C:N ratio in particular matter to infer the nutrient limitation has its limitation as particular C:N ratios do not necessarily reflect phytoplankton elemental composition alone, especially in estuarine influenced waters.

Specific comments

--Reviewer

L 29 Insert: 'According to this hypothesis...' (to clarify this is not yet a result)

Reply:

Inserted.

--Reviewer

L31 Re-write: 'These processes would result in...'

Reply:

Revised.

--Reviewer

L38-39 Sentence is awkward, as it seems to refer to vertical profiles at the nutricline. Please re- write.

Reply:

Re-write as "The N:P ratios at the nutricline in vertical profiles responded differently to mixing events.

Line 44 Remove 'and'. The phrase 'subject to the homeostatic stoichiometry' is vague and may be confusing. In fact the work highlights the stoichiometric plasticity of phytoplankton, rather than its fine regulation.

Reply:

Revised:

Thus, phytoplankton are able to maintain high productivity and balance nutrient stoichiometry by taking advantage of vigorous mixing regimes with the capacity of the stoichiometric plasticity.

--Reviewer

Line 47. This second part of the sentence is incorrect: there are many studies showing the results of phytoplankton to natural nutrient pulses supplied by mixing. See for instance Glover et al J Plankton Res (2007) 29 (3): 263-274 for an open-ocean example and also the works of Jonathan Sharples and colleagues for shelf-sea examples.

Reply:

Revised:

To our knowledge, this is the first study to show the in situ dynamics of continuous vertical profiles of N:P and N:Si ratios, which can provide insight into the in situ dynamics of nutrient stoichiometry in the water column and the inference of the transient status of phytoplankton nutrient stoichiometry in the coastal ocean.

--Reviewer

L57 Recently,

L58 Remove 'in these waters'

L69 re-write '...with low C:P and N:P ratios'

Reply:

All corrected.

L81-82 Re-write to make it clear that this assessment, albeit difficult, is not imposible. There are a few examples of direct measurements of elemental ratios in situ both for bacterio- and phytoplankton. See Segura et al Plos One 2016 for a recent example and relevant references: http://dx.doi.org/10.1371/journal.pone.0154050

Reply:

Revised:

The fourth mechanism is related to the interference from dead plankton or organic detritus with the measurement of elemental composition of organic matter, and such interference is difficulty to assess due to lack of the measurements of non-living organic matters in oceans and coastal waters. However, the X-ray microanalysis (XRMA) technique was recently used to produce simultaneous quotas of C, N, O, Mg, Si, P and S in single cell organisms (Segura-Noguera et al. 2016), which will not only help to understand the fourth mechanism, but also understand the variability of stoichiometry of phytoplankton in the oceans.

--Reviewer

L105-105 Related to comment above regarding line 47, here the authors need to be careful when referring to 'nutritional status', which in this study is inferred but never measured, since there are no measurements of phytoplankton elemental ratios. Data of elemental ratios of suspended organic matter (which in any event are difficult to interpret in this context, because of the unkown influence of non-phytoplankton material) are reported in Fig. 10, but they were not obtained during the time-course experiments. The authors need to ackowledge the limitations in their approach, as they are inferring phytoplankton nutrient content (and hence nutritional status) from observations of nutrient concentration in seawater, but the latter can be affected by many other processes in addition to phytoplankton uptake alone.

Reply:

Agree. Revised to:

"to examine responses of phytoplankton to the supply of nutrients from water column mixing".

--Reviewer

L115-116 re-write: reaching a daily production of up to xxx and an anual production of up to xxx

Reply:

Corrected.

--Reviewer

Line 188 Indicate bottles were maintained on-deck.

L 186-187 Actual light attenuation percentages should be indicated.

L 245 Should be 0.0, not 0:0 (which suggests that both nutrients were exhausted)

Reply:

Line 188, revised as, "The incubation bottles were maintained on-deck and lasted for ...".

Line 186-187, revised as: "which corresponded to the light intensity (50-6% of the surface light)"

Line 245, revised as 0.0

--Reviewer

Legend to Fig. 9: The labels +N/+P and +N/+Si are confusing. If, as the legend indicates, they represent the ratio of added N over added P or added N over added Si, why should they change over time? Those ratios refer to the initial nutrient amendment but once the experiment is proceeding, the only ratios one can measure are the actual nutrient ratios in the bottles (indicated by the other 2 lines). So what do the data labeled +N/+P represent?

Reply:

+N stands for "adding N alone" into bottle 1, then we measured concentrations of all three nutrients: NO3, PO4, and SiO4 in this bottle daily during the incubation.

+P stands for "adding P alone" into bottle 2, then we measured concentrations of all three nutrients in bottle 2 daily.

+N/+P means ambient NO3 in bottle 1/ambient PO4 in bottle 2. We measured nutrients in some time intervals, and hence, we had ambient N:P for +N/+P over time.

+N+P stands for "two nutrients in the same bottle".

--Reviewer

Section 2.4 explains the experiment described by figure 7 (in this experiment, all nutrients were added together), but not the experiment described by figure 9, in which multiple treatments were used (including additions of single nutrients). This experiment should be described in the Methods section, and in particular the concentrations of each added nutrient should be indicated.

Reply:

For experiments with additions of a single nutrient alone or multiple nutrients together, a water sample taken at Stn S1 on June 4, 1990. The sample was incubated with no nutrients being added during the first 28 h (pre-incubation); after pre-incubation, nutrients were added in 8 treatments: no additions, NO_3^- alone (+N), PO_4^{3-} alone (+P), SiO_4^- alone (+Si), NO_3^- and PO_4^{3-} together (+N+P), NO_3^- and SiO_4^- (+N+Si), PO_4^{3-} and SiO_4^- (+P+Si) and all three (+N+P+Si). The final concentrations of added NO_3^- , PO_4^{3-} and SiO_4^- was 7-8, 1.3-1.6 and 10-12 µM, respectively.

--Reviewer

Lines 260, 264. To avoid confusion, clarify whether these ratios refer to ambient or uptake ratios. In fact, this applies to the manuscript in general, it should always be specified whether nutrients or nutrient ratios refer to ambient concentrations, uptake, or inferred phytoplankton composition.

Reply:

We have added "ambient" to wherever is applicable.

Line 260-262. This is difficult to follow. It is stated that 'The N:P ratio decreased faster after a single addition of N or P alone than with additions of N and P together (Fig. 9-3)', but in Fig. 9-3 treatments which had only added N or added P are not shown. This again goes back to the problem that it is uncertain what the treatment labeled +N/+P refers to.

Reply:

Again:

+N stands for "adding N alone" into bottle 1, then we measured concentrations of all three nutrients: NO3, PO4, and SiO4 in this bottle daily during the incubation.

+P stands for "adding P alone" into bottle 2, then we measured concentrations of all three nutrients in bottle 2 daily.

+N/+P means ambient NO3 in bottle 1/ambient PO4 in bottle 2. We measured nutrients daily, and hence, we had ambient N:P for +N/+P over days.

+N+P stands for "two nutrients in the same bottle".

We have revised as:

The ambient N:P ratio decreased faster in the samples with a single addition of NO_3^- or PO_4^{3-} alone (+N/+P) than that with additions of NO_3^- and PO_4^{3-} together (+N+P) (Fig. 9-3).

--Reviewer

lines 276-277. Nutrient recycling should also be mentioned here. In particular, P is recycled much faster than N, which in turn is recycled faster than Si.

Reply:

Add the following sentence in the end of the paragraph.

The recycling of nutrients in different preferences such as faster P regeneration than N, which in turn is recycled faster than Si also contributes to the variability of nutrient ratios as shown above.

lines 366-370. As the authors know, elemental ratios of suspended organic matter are affected by the presence of non-phytoplankton material, such as detritus and heterotrophic bacteria. The contribution of these non-phytoplankton components to total POC and PON stocks can change rapidly and is quite difficult to ascertain. In this section, the authors should acknowledge this fact. The observation that C:N of suspended matter is close to Redfield would also be consistent with a high (non-Redfield) C:N in phytoplankton in combination with a substantial contribution of bacterial biomass with a low C:N ratio. The statement 'This demonstrates the lack of ambient nitrogen limitation on the cellular nutrient stoichiometry' is not warranted, as it is based on the assumption that the POC:PON ratios reflects solely the contribution of phytoplankton.

Reply:

Yes, agree. We revised the last sentence as:

In addition, POC:N ratio was slightly higher than 7:1 (Fig. 10) at Stn S1 where nitrogen was more frequently under detection limit than Stns S2 and S3. This might suggest the lack of ambient nitrogen limitation on the cellular nutrient stoichiometry. However, using C:N ratio in particular matter to infer the nutrient limitation has its limitation as particular C:N ratios do not necessarily reflect phytoplankton elemental composition alone, especially in estuarine influenced waters.