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

Responses to reviewer #1, #2 and #3.

Response to Referee #1

Anonymous Referee #1

Received and published: 22 November 2016

This is generally a very well written manuscript that investigates the sequential nutrient uptake strategy by phytoplankton within a coastal system to cope with maintain nutrient stoichiometry and favour growth under potentially limiting conditions. The novel use of a flow through system to sample nutrients continuously from a CTD cast allow for a uniquely high sampling resolution. The authors however rely only reporting nutrient concentrations and nutrient ratios without examining other methods for data analysis. This is particularly important for the nutrient incubation experiments that could have calculated nutrient specific growth rates. Throughout the manuscript the authors refer to high levels of primary productivity and phytoplankton growth yet fail to provide any estimates for the Strait of Georgia**. (Addressed below with references)** The demonstration of sequential uptake by phytoplankton to differing nutrient limitation conditions is important in understanding seasonal dynamics of productivity, community succession and nutrient concentrations. The authors mention that different uptake strategies but does suggest explicitly whether the sequential uptake favors either the growth or storage strategy **(addressed below).**

I recommend that this manuscript be accepted; following the address of the minor revisions listed below.

Specific comments:

-- Referee #1:

Page 5, Line 109: Please provide estimates of the biological productivity.

Reply:

Values and a reference have been added.

Daily production up to 5 g C/ m2/day and annual about >300 g C/m2/yr

Harrison, P.J., P.J. Clifford, K. Yin, M. St. John, M.J. Sibbald, L.J. Albright, W.P.

Cochlan and P.A. Thompson. Nutrient and plankton dynamics in the Fraser River plume, Strait of Georgia, British Columbia. Mar. Ecol. Prog. Ser. 70: 291-304 (1991).

Harrison, P.J., T.R. Parsons, F.J.R. Taylor and J.D. Fulton. Review of Biological oceanography of the Strait of Georgia: Pelagic Environment. Can. J. Fish. Aquat. Sci. 40: 1064-1094 (1983).

-- Referee #1:

Page 6, Line 131: This paragraph gives concentrations of Nitrate and Silicate; however the previous paragraph does not give concentrations of Phosphate. If you are going to switch between a conceptual model and measured concentrations, then please be consistent and give measured concentrations for all nutrients discussed.

Reply:

We have deleted the word "concentration" to be consistent.

-- Referee #1:

Page 7, Line 169: What were the detection limits of the nutrients?

Reply:

NO_3 = 0.1 uM, NH_4 = 0.05 uM, PO_4 = 0.05 uM, SiO_4 = 0.01 uM

-- Referee #1:

Page 7, Line 170: Were the field incubations done in the same year? As the figure captions suggest they were performed in different years. There is also no mention of this when you discuss the results of these incubation experiments.

Reply:

The samples were taken in different years, but at the same time of the year. This is noted in the methods now.

--Referee #1:

Page 9, Line 204: What was the silicate concentration at the surface? Inconsistency with the level of detail when reporting nutrient concentrations and nutrient ratios.

Reply:

The dashed lines for silicate on Fig. 5 were very dim, especially on an Apple Mac. We have fixed this problem.

--Referee #1:

Page 9, Line 216: Reference to figure 6. . . This figure is the same as figure 5. Unable to give specific comments on the text without the correct figure to refer to. However, stylistically it would make it easier for the readier if you use the references to the time stamps in the same style as figure 5.

Reply:

Yes, there was a mistake with Fig. 6. Figs. 5 and 6 should be different figures. This has been fixed now. We also fixed the problem of dim dashed lines for silicate.

--Referee #1:

Page 10, Line 230: Was chlorophyll measured? Why was fluorescence not converted to chlorophyll? Increases in fluorescence do not always represent increases in biomass, but can reflect alterations to the photosynthetic apparatus; which in turn is usually driven by the nutritional status of the phytoplankton.

Reply:

Chlorophyll was not measured. An increase in fluorescence usually indicates the increase in biomass in waters, which do not have strong interfering substance such as high concentrations of dissolved organic matter, particularly in the initial incubation phase under sunlight.

--Referee #1:

Page 10, Line 251: If the diamond symbol represents the presence of phosphate, then the ratio of N:Si does not exceed 3:1 at any time point.

Reply:

Corrected. Thank you.

--Referee #1:

Page 11, Line 254: 'highly productive' Once again the authors fail to give any values associated with this type of estimate.

Reply:

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

--Referee #1:

Page 11, Line 272 – 280: This whole section reads like a re-iteration of the results without a closing statement for the reader to take away before moving onto the next section. Consider re-structuring this section.

Reply:

We have revised these sentences into a sentence to summarize the value of the conceptual model to extract information from this sequence of events.

--Referee #1:

Page 12, Line 290: 'increase in cellular content' – An increase in the cellular content of other non-limiting nutrients would only occur if luxury uptake occurs, this is not a direct result of nutrient deficiency. A direct result of nutrient deficiency is changes in intracellular nutrient stoichiometry.

Reply:

We have revised as "Nutrient deficiency results from a decrease in the cellular content of the limiting nutrient and continuous uptake of other non-limiting nutrients."

--Referee #1:

Page 13, Line 324: You discuss how different phytoplankton species will either use the 'growth' 'or storage' strategies; yet here you say that phytoplankton will use 'storage' for non-limiting strategies and 'growth' for limiting nutrients. Which statement is correct? It seems like the author wants to suggest that the old idea of species specific strategies need to be vreised. Suggest a bit more clarification to get this point across to the readers.

Reply:

We have revised this section quite a bit.

--Referee #1:

Page 14, Line 335: Can you please provide a reference for 'internal waves in the open ocean'.

Reply:

a reference paper has been added

Pomar, L., M. Morsilli, P. Hallock, B. Bádenas. 2012. Internal waves, an under-explored source of turbulence events in the sedimentary record. Earth-Science Reviews 111, 56-81.

--Referee #1:

Page 14, Line 335: Reference for 'Phytoplankton in the chlorophyll maximum are generally nutrient sufficient'. I don't necessarily agree with this statement; phytoplankton can exist under steady state nutrient limitation and still exist at the chlorophyll maximum within the water column.

Reply:

Revised as “Phytoplankton in the chlorophyll maximum are frequently exposed to nutrients and …”

--Referee #1:

Page 14, Line 338: How do the phytoplankton sink down? Mixing events? Changes to internal buoyancy?

Reply:

Changes to their internal buoyancy (exchange of heavy ions for lighter ones) and also by clumping since under nutrient deficiency cells produce extracellular carbohydrates that make them sticky and prone to clumping. – Clumping added to the text.

--Referee #1:

Page 14, Line 350: POC/PON ratios are discussed but there is no mention to how they were measured in the methods.

Reply:

Inserted in the methods ---- 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).

Sharp, JH (1974) Improved analysis of particulate organic carbon and nitrogen from seawater. Limnol Oceanogr 19:984-989

--Referee #1:

Figure 1 Caption: I would suggest dropping the text that begins with ‘At T2’. This reads like the discussion of the conceptual profiles that is already mentioned in the introductory text.

Reply:

This figure is important. It will be hard for readers to go back to the text for explanations. Therefore, we think that we prefer to keep this legend.

--Referee #1:

Figure 9A: NH4 is shown on the figure. Not mentioned in the methods or the figure caption.

Reply:

NH4 is now in the methods and the figure legend.

--Referee #1:

Figure 9B: Symbols aren't consistent between panels making it hard to follow. i.e. Top panel, +N+P is open triangles, and then is a closed circle in the bottom panel with open triangles used for +P+Si.

Reply:

The symbols are now fixed.

--Referee #1:

Technical comments: Page 5, Line 111: Space required between 'pynocline.' and 'In the Strait'. Page 7, Line 153: Typo 'florescence'.

Reply:

Corrected. Thank you.

End of reply to referee #1

Response to Referee #2

Anonymous Referee #2

Received and published: 28 November 2016

--Referee #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 presentation 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.

--Referee #2

Suggested revisions
-Redfield is a concept for the open ocean and long-term 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.

--Referee #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.

--Referee #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).

--Referee #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.

--Referee #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.

--Referee #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.

--Referee #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 $NaNO_3$, NH_4Cl, KH_2PO_4, $NaSiO_4$.
Detection limits are as follows.

NO3 = 0.1 uM, NH4 = 0.05 uM, PO4 = 0.05 uM, SiO4 = 0.01 uM

--Referee #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.

--Referee #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 $PO4^{3-}$ was consumed while NO_3^- was taken up", which indicates that turnover of nitrogen did not stop NO_3 uptake so that N:P ratio followed NO_3.

--Referee #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.

--Referee #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 happens one day before fluorescence reaches the maximum.

--Referee #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 $m^{-2}d^{-1}$ in August. (Yin et al. 1997a)"

--Referee #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.

--Referee #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.

--Referee #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.

--Referee #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 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".

--Referee #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.

--Referee #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.

--Referee #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.

--Referee #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.

--Referee #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, NO_3 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 NO_3 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.

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

--Referee #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-day1 phosphate concentraiton) to get N:P ratio on day 1.

--Referee #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.

--Referee #2
-Figure 9C: What does the terminology of +N/+P and +N/+Si mean? - 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.

--Referee #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

--Referee #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).

--Referee #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"?

-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).

--Referee #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#

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

Reply:
We have reduced the number to 1.

--Referee #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.

End of reply to referee #2

Response to Referee #3

Anonymous Referee #3

Received and published: 9 December 2016

Reviewer #3

Yin and Harrison have attempted to prove that there is preferential biological uptake of the most limiting nutrient as soon as the nutrient is added into the system. They provide high resolution nutrient data set and very interesting schematics (conceptual Fig. 1) to prove their claims. I enjoyed reading this manuscript but I still have the following suggestions that can improve the manuscript.

General comments:

1. Research in this manuscript roams around the nutrient uptake ratios. We know that the nutrient uptake and stoichiometry are phytoplankton composition dependent (see Singh et al. 2015; Mills and Arrigo 2010). Authors have not provided any cell abundance microscopic data. I understand this research was conducted long time back but it would still improve the manuscript if authors could provide something on this aspect. They have mentioned a sentence on this in the discussion section (line 317-319) but I suggest them to add some more discussion on this.

Reply:

Thank you. We have added more discussion on phytoplankton assemblage there.

--Referee #3

Specific comments:

Line 38: '3' in 'nitrate' should be made subscript.

Line 103: Fig. 1 in the heading looks a bit odd

Line 111: Give space after full stop

Line 111: N:P ratio of what? of nutrients?

Reply:

Line 38， NO3 is corrected to NO_3^-

Line 103, removed Fig. 1
Line 111, added space
Line 111, corrected as N:P ratio of nutrients

--Referee #3
Line 118: Just average nutrient ratio is not 16N:1P, it is rather when averaged for all the communities together

Reply:
You are right.

--Referee #3
Line 121-122: "The remaining. . .. .phosphate." Which species can take phosphate without taking any nitrate? Diazotrophs? Do they occur in the study area?

Reply:
The idea in this manuscript is to demonstrate that uptake of non-limiting nutrients can be decoupled from the most limiting nutrient. Here it is phytoplankton assemblages that can continue to take up phosphate after nitrate in the ambient water has disappeared.

--Referee #3
Line 175-177: "The incubation flasks. . .. . .16m)." Mention the light intensity at 16 m, at least with compared to the surface value in terms of %. What was the euphotic depth?

Reply:
4 layers neutral screening is about 12.5% light reduction. The euphotic zone could reach down to 20 m.

--Referee #3
Line 184: What is T7? It is not described in the conceptual model.

Reply:
T7 here refers to the field vertical profile, not to the conceptual model. We have changed T0, T1, … T6 to C0, C1, … C6 in the conceptual model in Fig. 1 to avoid the confusion.

--Referee #3
Line 186: "due to an increase in NO3- in the deep water", what was the source of this high nitrate? What was the station depth?

Reply:
In the Strait of Georgia, deep water has high concentrations of nutrients and is the source of high nitrate. The station depth is over 300 m.

--Referee #3

Line 187: How do the authors know that the silicate is from Fraser River? What is the silicate concentration in the river?

Reply:
The dotted line for SiO4 in the manuscript was very dim on my Apple computer, and you may not see it clearly. SiO4 was minimal at 10 m with higher SiO4 at the surface and at the 20 m. This higher SiO4 is from the Fraser River as the River contains higher SiO4 than the seawater in the Strait of Georgia deep water.

--Referee #3
Line 188: "top of the nutriclines" or "top of the nutriclines at T7"
Line 192: "A strong wind", provide wind speed.
Line 220: '3' in 'nitrate' should be made subscript.

Reply:
All are corrected.

--Referee #3
Line235""both.....undetectable". What could be the reason for this?
In nature, who could still utilize phosphate and silicate without nitrate?

Reply:
Phytoplankton uptake of nutrients can deplete these nutrients to undectable levels.
You are right, phytoplankton can not utilize phosphate and silicate without nitrate, but there is a time lag between their uptake, ie, uptake of 3 nutrients can be decoupled in time. The idea of this paper is to say sequential uptake of these nutrients.

--Referee #3
Line 249: How was the uptake ratio estimated?

Reply:
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-day1 phosphate concentraiton) to get N:P ratio on day 1.

--Referee #3
Line 359: 'this' should be followed by 'study"

Reply:
revised

--Referee #3
Line 356-363: Conclusion seems to be a bit misplaced. A lot of processes have been discussed and presented in the results but the authors have concluded only sequential uptake (which is not very convincing since there are neither any uptake measurements nor any information on community composition)

Reply:
The conclusion has been revised

--Referee #3
References:
Mills, Matthew M, and Kevin R Arrigo (2010) Magnitude of Oceanic Nitrogen Fixation Influenced by the Nutrient Uptake Ratio of Phytoplankton. Nature Geoscience 3(6): 412–416.
Singh, Arvind, SE Baer, Ulf Riebesell, AC Martiny, and MW Lomas (2015) C: N: P Stoichiometry at the Bermuda Atlantic Time-Series Study Station in the North Atlantic Ocean. Biogeosciences 12(21): 6389–6403.
Please also note the supplement to this comment: http://www.biogeosciences-discuss.net/bg-2016-426/bg-2016-426-RC3- supplement.pdf

Reply:
These papers have been cited. Thank you.

End of reply to referee #3

Sequential Nutrient Uptake by Phytoplankton Maintains High Primary Productivity and Balanced Nutrient Stoichiometry

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Running head: sequential nutrient uptake, nutritional strategy, nutrient stoichiometry

Abstract

We hypothesize that phytoplankton have the sequential nutrient uptake strategy to maintain nutrient stoichiometry and high primary productivity in the water column. Phytoplankton take up the most limiting nutrient first until depletion, continue to drawdown non-limiting nutrients and then take up the most limiting nutrient rapidly when it is available. The processes result in the variation of ambient nutrient ratios in the water column around the Redfield ratio. We used high resolution continuous vertical profiles of nutrients, nutrient ratios and on-board ship incubation experiments to test this hypothesis in the Strait of Georgia. At the surface in summer, ambient NO_3^- was depleted with excess PO_4^{3-} and SiO_4^{4-} remaining, and as a result, both N:P and N:Si ratios were low. The two ratios increased to about 10:1 and 0.45:1, respectively, at 20 m. Time series of vertical profiles showed that the leftover PO_4^{3-} continued to be removed, resulting in additional phosphorus storage by phytoplankton. There were various shapes of vertical profiles of N:P and at the nutricline in response to mixing events. A field incubation of seawater also demonstrated the sequential uptake of NO_3^- (the most limiting nutrient) and then PO_4^{3-} and SiO_4^{4-} (the non-limiting nutrients). This sequential uptake strategy allows phytoplankton to acquire additional cellular phosphorus and silicon when they are available and wait for nitrogen to become available through frequent mixing of NO_3^- (or pulsed regenerated NH_4). Thus, phytoplankton subject to the homeostatic stoichiometry of nutrients and are capable of maintaining high productivity by taking advantage of vigorous mixing regimes. 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 and to examine the responses of phytoplankton to nutrients supplied naturally by mixing events. This provided insight into the in situ dynamics of nutrient stoichiometry in the water column and the inferring of the transient status of phytoplankton nutrient stoichiometry in the coastal ocean.

1. Introduction

The stoichiometry of the C:N:P Redfield ratio (Redfield, 1958) remains a central tenet in oceanography as it couples ecosystem processes with ocean biogeochemistry, which is driven by physical processes in oceans. Redfield ratio of C:N:P varies widely across a wide range of environmental conditions. Laboratory cultures of phytoplankton that are in the steady state usually display variable cellular N:P ratios with the nutrient N:P supply ratios (Geider and La Roche, 2002). Recnetly, Martiny et al. (2013) found strong latitudinal patterns of the elemental ratios, which are closely related with ambient levels of nutrients in these waters by making comparative analysis of elemental ratios of organic matter between different latitudes. Even at a fixed site, the Bermuda Atlantic Time-Series Study Station in the North Atlantic Ocean, C: N: P ratio is quite variable (Singh et al. 2015). Four mechanisms have been proposed to explain the variability in C:N:P ratios in marine plankton, as summarized by Weber and Deutsch (2010). The first mechanism emphasizes the relationship between cellular elemental stoichiometry of phytoplankton and ambient nutrient ratios, i.e., the stoichiometry of nutrients in the water column. Based on the average Redfield ratio, this mechanism has been used to infer the most limiting nutrient for phytoplankton and to debate which nutrient, nitrogen or phosphorus, should be managed to control eutrophication effects. The second mechanism suggests that the elemental stoichiometry is taxonomy specific. Diatoms were reported to drawdown nutrients with a low nutrient C:P and N:P ratios (Geider and La Roche, 2002; Elser et al., 2003; Price, 2005), while marine cyanobacteria have higher C:P and N:P ratios (Karl et al., 2001; Bertilsson et al., 2003). Such different uptake ratios of N:P by phytoplankton can influence the magnitude of ocean N-fixation (Mills and Arrigo 2010) Based on the resource allocation theory, the third proposed mechanism is the "growth rate hypothesis", which states that the elemental stoichiometry within a cell is controlled by the biochemical allocation of resources to different growth strategies (Falkowski, 2000; Elser

et al., 2003; Klausmeier et al., 2004). Fast-growing cells may have a lower N:P ratio due to a larger allocation to P-rich assembly machinery of ribosomes (Loladze and Elser, 2011), whereas competitive equilibrium favors a greater allocation to P-poor resource acquisition machinery and therefore, higher N:P ratios. The fourth mechanism is related to the interference from dead plankton or organic detritus with the measurement of elemental composition of organic matter, but such interference cannot be assessed since there is lack of the measurements of non-living organic matters in oceans and coastal waters.

In culture experiments, continuous uptake of non-limiting nutrients has been demonstrated for diatoms under N and Si limitation (Conway et al., 1976; Conway and Harrison, 1977; Harrison et al., 1989). Surge uptake of the limiting nutrient occurs when it is added to the nutrient starved phytoplankton culture, while the uptake of the non-limiting nutrient is slowed or stopped until the diatom has overcome its nutrient debt. Hence, the sequence of which nutrient is taken up first is directly related to the nutrient status of the phytoplankton. It is difficult to assess the nutritional status of phytoplankton in the field, but the application of laboratory results to the interpretation of vertical nutrient profiles can provide information on their nutritional status. To date, there have been no studies of sequential uptake of nutrients in the field using a series of high resolution vertical profiles of nutrients and their application to nutritional status of the phytoplankton.

In this study, we used high resolution continuous vertical profiles of N:P and N:Si ratios to examine how N:P and N:Si ratios respond to the mixing in a highly dynamic coastal water column and the uptake of nutrients. On-board ship incubation experiments were conducted to support the observations of changes in vertical profiles of N:P and N:Si ratios. We constructed seven conceptual profiles to illustrate how a vertical profile of N:P ratios changes with mixing and uptake of nitrogen and phosphorus and how they could indicate the nutritional status of the phytoplankton assemblage. The conceptual model also explains how

N:P ratios respond to mixing, particularly at the nutriclines (nitracline for NO_3^-, phosphacline for PO_4^{3-} and silicacline for SiO_4^{4-}), and indicates which nutrient, NO_3^- or PO_4^{3-}, is taken up first in the water column. To our knowledge, this is the first study to show the dynamics of continuous vertical profiles of N:P and N:Si ratios and to examine the nutritional status of phytoplankton and their response to the supply of nutrients from water column mixing. We believe that our approach can add a new dimension to examining the in situ dynamics of nutrients in the water column and illustrate the ecological role of phytoplankton stoichiometry in phytoplankton competition for nutrients.

1.1. Conceptual Model of Variability in Vertical N:P ratios

The Strait of Georgia (hereafter the Strait) is an inland sea that lies between Vancouver Island and the mainland of British Columbia (LeBlond 1983). It is an ideal area for studying the interactions between mixing, nutrient vertical profiles and phytoplankton nutrient uptake because of its relatively high biomass, frequent wind mixing and shallow (15 m) photic zone. The Strait is biologically productive, reaching as as daily production up to 5 g C m^{-2} day^{-1} and annual about >300 g C m^{-2} yr^{-1} (Harrison et al., 1983, 1991), but inorganic nitrogen is often undetectable in productive seasons in the surface layer. The nutricline sitting within the euphotic zone is often associated with the pycnocline. In the Strait, the ambient N:P ratio of nutrients is ~10:1, similar to other coastal areas (Hecky and Kilham, 1988).

We illustrate the conceptual model of variability in vertical profiles of N:P ratios based on seven (C0 to C6) vertical profiles that we encountered in our field studies and suggest events that likely occurred to produce these nutrient profiles (Fig. 1).

C0: in winter or after a strong wind speed event, the water column is homogeneously mixed, and NO_3^- and PO_4^{3-} are uniformly distributed in the water column. **C1:** with the onset of stratification, NO_3^- and PO_4^{3-} are taken up within the mixed layer. Assuming that the average nutrient uptake ratio is 16N:1P, a N:P uptake ratio that is >10:1 would decrease the

ambient N:P ratio to <10:1. **C2:** the uptake of NO_3^- and PO_4^{3-} proceeds at a N:P ratio >10:1 until NO_3^- is just depleted. At this time the N:P ratio is near 0 and some PO_4^{3-} remains in the water column. **C3:** the remaining PO_4^{3-} is completely taken up and stored as extra/surplus intracellular PO_4^{3-}. **C4:** after cross-pycnocline mixing occurs, the ambient N:P ratio in the newly mixed water should be the same as the ratio in the deep water. As a result, the vertical profile of the N:P ratio will form a right angle on the top part of the nutricline. **C5:** depending on how long the phytoplankton are nutrient limited, their response to the mixed limiting nutrient can be different. When N deficient phytoplankton take up N only, the curve of the N:P ratio parallels the NO_3^- distribution curve and PO_4^{3-} is left behind in the water column. **C6:** on the other hand, if phytoplankton take up PO_4^{3-} before NO_3^- (e.g. if phytoplankton were severely N starved, and there is a lag in NO_3^- uptake), the N:P ratio would be higher at the nutricline than below (Fig. 1).

Similarly, this conceptual model can be applied to N, SiO_4^{4-} and N:Si ratios. The ambient (N:Si) ratio is about 0.5:1 at 20 m in the Strait, with 20 µM NO_3^- and 40 µM SiO_4^{4-}. As the average uptake ratio of N:Si is about 0.7-1:1 (equivalent to Si:N = 1.5-1:1) (Brzezinski, 1985), the N:Si ratio decreases with depth. SiO_4^{4-} is rarely depleted and therefore, the N:Si ratio is mainly determined by the distribution of NO_3^-. The continuous uptake of SiO_4^{4-} without the uptake of NO_3^- can be inferred based on the comparison between the gradient of N:Si and the silicacline. For example, a sharper gradient of the N:Si ratio than the silicacline would indicate the continuous uptake of SiO_4^- without the uptake of NO_3^- as in C5 (Fig. 1)

2. Materials and Methods

2.1. Station Locations

The transect started from station S2, 8 km beyond the Fraser River mouth and under the influence of the river plume and extended 108 km NW to S1 (well beyond the plume) in

the Strait of Georgia (Fig. 2). The station numbers are consistent with previous studies (Yin et al., 1997a).

2.2. Sampling and Data Processing

The sampling was designed to investigate the distribution of nutrients (NO_3^-, PO_4^{3-} and SiO_4^-) and N:P and N:Si ratios associated with mixing processes during August 6-14, 1991. Data at either an anchored station for 24 h, or a transect of a few stations within 10 h was used. At each station, a vertical profile (0-25 m) of temperature, salinity, *in vivo* fluorescence and selected nutrients (NO_3^-+NO_2^-, PO_4^{3-} and SiO_4^-) were obtained. Only vertical profiles of nutrients are presented in this study. Other data (salinity, temperature and fluorescence) are published elsewhere (Yin et al., 1997a). The vertical profiling system has been described in detail by Jones et al. (1991) and Yin et al. (1995a). Basically, a hose connected to a water pump on deck was attached to the CTD probe or S4 (InterOcean®) which has the dual function of a CTD probe and a current meter. Seawater from the pump was connected into the sampling tubing of an AutoAnalyzer® on board ship for *in situ* nutrient measurements, while the CTD probe was lowered slowly into the water at 1 m min^{-1}. Each sampling produced a high resolution continuous vertical profile of physical and biological parameters and thus the relationship between these parameters in the water column can be easily recognized. Data from a vertical profile (a datum point every 3 s) were smoothed over 15 s intervals. This smoothing reduced the fluctuations caused by ship's motion.

2.3. Analysis of Nutrients

All nutrients were determined using a Technicon AutoAnalyzer II. Salinity effects on nutrient analyses were tested on board ship and were found to be small. Therefore, no correction was made for salinity effects. NO_3^-+NO_2^- and PO_4^{3-} were determined following the

176 procedures of Wood et al. (1967) and Hager et al. (1968), respectively. The analysis of SiO_4^-
177 was based on Armstrong et al. (1967) and ammonium analysis followed Parsons et al. (1984). A water
178 sample for particulate organic carbon and nitroeng (POC and PON) was filtered onto a GF/F filter
179 and POC/PON on the filter were analyzed with a Carlo Erba model NA 1500 NCS elemental
180 analyzer, using the dry combustion method described by Sharp (1974).

181 **2.4. Field Incubation Experiments**

182 Niskin bottles (5 L) were used to take seawater samples and the samples were
183 transferred to acid cleaned carboys (10 L). Subsamples of seawater were transferred to
184 transparent polycarbonate flasks (1 L) and placed in Plexiglas tanks. The tanks were kept at
185 the same temperature as the surface water by pumping seawater (from the ship's intake at 3
186 m) through the tank. The incubation flasks were wrapped with 1 or 4 layers of neutral density
187 screening which corresponded to the light intensity from which the samples were taken (1 or
188 16 m). In the nutrient enrichment experiments, NO_3^-, PO_4^{3-} and SiO_4^- were added to the
189 samples, yielding final 20-30, 2-3 and 20-30 µM, respectively. The incubations lasted for 24
190 or 96 h, and subsamples were taken every 3-6 h for measurements of fluorescence and
191 nutrients. The incubation experiments were conducted in different years, but in the same
192 season.

193 **3. Results**
194
195 **3.1. Vertical Profiles of Nutrients and Nutrient Ratios**

196 At S3 near the edge of the Fraser River plume, the profiles documented changes
197 before (T1) and after wind mixing (T7). At T1, both NO_3^- and PO_4^{3-} were low in the surface
198 layer and N:P ratios were low (<2:1) and increased to ~8:1 at 20 m (Fig. 3). At T7, higher N:P
199 ratios of 16-20:1 occurred due to an increase in NO_3^- in the deep water. SiO_4^{4-} was ~30 µM at
200 the surface due to input from the Fraser River, and increased to 37 µM at 20 m (Fig. 3). The
201 N:P ratio curve nearly formed a right angle at the top of the nutriclines at T7 when the

gradient of the nitracline was larger than that of the phosphacline. At T1, the N:Si ratio was near 0 because NO_3^- was near the detection limit, but started to increase along the nitracline at the depth of the SiO_4^- minimum. At T7, N:Si increased more rapidly with the nitracline.

A strong wind speed event occurred on August 7 and the water column was mixed (Yin et al., 1997b). We followed the change in the nutrient profiles and nutrient ratios from S3 near the Fraser River plume, to P4 and P6 and the well beyond the plume to S1. At S3, N:P ratios in the water column were >7:1 when both NO_3^- and PO_4^{3-} were high after wind mixing, with N:Si ratios being <0.5:1 (Fig. 4). As the post-wind bloom of phytoplankton developed along P4-P6 due to the newly supplied nutrients (Yin et al., 1997b), N:P ratio followed the distribution of NO_3^- at P4, and decreased to 0 as NO_3^- was depleted at the surface at P6 (Fig. 4). It was clear that little PO_4^{3-} was consumed while NO_3^- was taken up. At the same time, the silicacline deepened and paralleled the nitracline. At S1, N:P and N:Si ratios formed almost a vertical line. N:P and N:Si ratios were ~8:1 and 0.5:1, respectively, in the deep water (Fig. 4).

The time series (T1, T3, T8 and T11) of Aug 8-9 captured changes over 1 or 2 days after the wind mixing event at S1 that was well beyond the river plume (Fig. 5). At T1, N:P and N:Si ratios were ~9:1 and 0.45:1, respectively, with NO_3^- and PO_4^{3-} being 15 and 1.7 µM, respectively, at the surface. At T3, N:P ratio remained constant at ~9:1, while NO_3^- and PO_4^{3-} decreased by 10 and 1.0 µM, respectively, indicating an uptake N:P ratio of 10:1. In comparison, N:Si ratio decreased from T1 to T3 when SiO_4^- was 35 µM at T1 and decreased by ≥10 µM at T3, producing an uptake N:Si ratio of ~1:1. At T8, N:P ratio followed the NO_3^- distribution as NO_3^- decreased to ~0 µM at the surface while PO_4^{3-} was still ~0.5 µM. This indicated that NO_3^- uptake was more rapid than PO_4^{3-} uptake and hence NO_3^- mainly determined the ambient N:P ratios. The N:Si uptake ratio of ~1:1 continued until T8.

226 However, at T11, the N:P ratio spiked higher in the top 5-10 m of the nutricline, suggesting a
227 more rapid uptake of PO_4^{3-} relative to NO_3^- in the upper portion of the phosphacline (Fig. 5).
228 Changes in the profiles after the wind event on Aug 7 were followed over 5 days (Aug
229 10 – 14) at P5 that was still within the influence of the river plume as evidenced by the higher
230 surface SiO_4^{4-} at the surface (Fig. 6). On Aug 10-11, N:P ratios were higher at the surface
231 where the post-wind induced bloom occurred two days earlier, suggesting that uptake of
232 PO_4^{3-} had caught up with uptake of NO_3^-. The right angle shape of the N:P ratio on Aug 12
233 occurred as the nutriclines became sharper due to entrainment of nutrients. By Aug 13, more
234 NO_3^- was taken up at depth and the N:P ratio followed the deepening of the nitracline and
235 PO_4^{3-} was left behind. On Aug 14, PO_4^{3-} started to decrease. During Aug 10-14, a minimum
236 in SiO_4^{4-} was present at an intermediate depth (5-10 m), coinciding with the top of the
237 nitracline, and the silicacline followed the nitracline below 10 m.

238 **3.2. Changes in Nutrient Ratios During Field Incubations**

239 On deck incubation experiments were used to examine changes in uptake ratios by
240 eliminating any effects due to mixing. Ambient N:P and N:Si ratios were lower at the surface
241 than at depth, indicating higher uptake of NO_3^- at the surface. The indication of a higher
242 uptake ratio of N:P and N:Si was supported by field incubation experiments. During nutrient
243 addition (NO_3^-, PO_4^{3-} and SiO_4^{4-}) bioassays on a sample from 1 m at P3, all nutrients
244 decreased as fluorescence increased (Fig. 7). Ambient N:P and N:Si ratios decreased to
245 almost 0:0 after 96 h, indicating more rapid uptake of NO_3^- than uptake of PO_4^{3-} and SiO_4^{4-}.
246 The temporal decline in the N:P and N:Si ratios resembled the temporal progression during a
247 bloom as illustrated in C0-C3 of the conceptual profiles (Fig. 1) and in the water column (S3,
248 P4, P6) on August 8 (Fig. 4) and during the time series at S1 (Fig. 5). During the incubation,
249 both PO_4^{3-} and SiO_4^{4-} continued to be drawn down after NO_3^- became undetectable (Fig. 7). In
250 an earlier incubation experiment at S3 near the end of the phytoplankton bloom on June 8,

PO_4^{3-} was depleted at 1 m, and both NO_3^- and SiO_4^- continued to disappear with 2 μM NO_3^- and 4 μM SiO_4^{4-} being taken up. However, for the sample taken at 16 m, PO_4^{3-} (~0.5 μM) and SiO_4^{4-} (~5 μM) continued to disappear after 1.25 μM NO_3^- was depleted after 8 h (Fig. 8).

The water sample at S1 on June 4 was incubated for 30 h without an addition of nutrients (Fig. 9-1). The initially low NO_3^-, and PO_4^{3-} remained near depletion levels during the incubation, but SiO_4^{4-} decreased from 9 to <1 μM (Fig. 9-1), which indicated that an additional 8 μM SiO_4^{4-} was taken up in excess in relation to N and P. At the end of 30 h, nutrients were added (Fig. 9-2). Both NO_3^- and PO_4^{3-} rapidly disappeared during the first 6 h, while SiO_4^{4-} decreased little (Fig. 9-2), indicating a sequential uptake of NO_3^- and PO_4^{3-} since 8 μM SiO_4^{4-} was previously taken up as shown in Fig. 9A. The N:P ratio decreased faster after a single addition of NO_3^- or PO_4^{3-} alone than with additions of NO_3^- and PO_4^{3-} together (Fig. 9-3), suggesting an interaction between the uptake of NO_3^- and PO_4^{3-}. The accumulative uptake ratio of NO_3^- to PO_4^{3-} increased with time, especially when only a single nutrient was present. The ratio of N:Si decreased with time, and the accumulative uptake ratio of N:Si exceeded 3:1 in the presence of PO_4^{3-} (Fig. 9-3).

4. Discussion

The Strait is highly productive, reaching up to 2,700 mg C $m^{-2}d^{-1}$ in August (Yin et al. 1997b). This is due to pulsed nutrient supplies and multiple phytoplankton blooms in the shallow photic zone interacting with wind events (Yin et al. 1997b), and fluctuations in river discharge (Yin et al., 1997a; Yin et al., 1995c). Our results revealed sequential nutrient uptake to optimize nutrient uptake efficiency and generate high primary productivity by phytoplankton by taking advantage of pulsed nutrients in this highly dynamic relatively shallow photic zone.

4.1. Responses of N:P and N:Si ratios to vertical mixing and uptake of nutrients

A vertical profile of N:P and N:Si ratios represents a snapshot of the mixing and the uptake of N, P and Si by phytoplankton in the water column. The depletion zone of the most limiting nutrient in the euphotic zone ends at a depth where the uptake of nutrients just balances the upward flux of nutrients through the nutracline, as indicated in C3 in the conceptual profiles (Fig. 1). Different responses of nutrient uptake to pulsed nutrients by mixing appeared to depend on the previous stability of the water column, the depth of the euphotic zone and nutritional status of phytoplankton. Our observations spanned all seven conceptual profiles (Fig. 1) and indicated the dynamic processes influencing the sequence of nutrient uptake. The change in the profiles of the N:P ratio from S3 to P6 (Fig. 4) displayed the spring bloom-like progression as illustrated in conceptual profiles of C0-C3 (Fig. 1) after the wind mixing event. Various responses illustrated in the conceptual profiles C4, C5 and C6 (Fig. 1) were observed in the observations, including the right angle in the N:P ratio (T7-Fig. 3, P5 Aug 12, Fig. 6), parallel lines between the nitracline and the N:P ratio curve on Aug 12, (Fig. 6), and a spike in the N:P ratio curve at T11 at S1 due to continued uptake of PO_4^{3-} with NO_3^- being depleted during the time period from T1 to T8 (Fig. 5), which was frequently observed on Aug 10 at P5 (Fig. 6).

4.2. Sequential Nutrient Uptake for Balanced Stoichiometry and Nutritional Optimization

Phytoplankton can take advantage of the dynamic mixing regimes and optimize their growth rates by taking up nutrients sequentially. The disappearance of nutrients during the incubation resembled the temporal progression of a bloom as illustrated in C0-C3 of the conceptual profiles (Fig. 1) and in the water column (S3, P4, P6; Fig. 4), or during the time series at S1 (Fig. 5).

Nutrient deficiency results from a decrease in the cellular content of the limiting nutrient and continuous uptake of other non-limiting nutrients. Earlier studies found that N limitation results in excess cellular content of P and Si (Conway and Harrison, 1977; Healey, 1985; Berdalet et al., 1996). Some phytoplankton develop enhanced uptake of the limiting nutrient such as NH_4 and PO_4^{3-} upon its addition after a period of nutrient limitation or starvation and there is an accompanying shut down of the non-limiting nutrient (Conway et al., 1976; Conway and Harrison, 1977; McCarthy and Goldman, 1979). A few hours of enhanced N uptake quickly overcomes the N debt since the enhanced uptake rate is many times faster than the growth rate (Conway et al., 1976). For example. enhanced uptake of phosphorus could double internal P within 5 min to 4 h depending on the degree of P limitation and the pulsed PO_4^{3-} (Healey, 1973). After the nutrient debt has been overcome by enhanced uptake, the uptake of non-limiting nutrients returns to normal after the cell quota of the limiting nutrient is maximal (Collos, 1986). The sequential uptake of a limiting nutrient and then the uptake of both the non-limiting and limiting nutrient is advantageous to allow phytoplankton to maintain maximum growth rates over several cell generations.

4.3. Significance of Sequential Uptake of Nutrients

There are two essential strategies used by phytoplankton to cope with the limiting nutrient (Collos, 1986). One strategy is the 'growth' response where phytoplankton uptake of the limiting nutrient and cellular growth are coupled when the limiting nutrient is available. The other strategy is the "storage" response where phytoplankton have the capability of accumulating large internal nutrient pools, resulting in extensive uncoupling between uptake and growth, and a lag in cell division of up to 24 h following a single addition of the limiting nutrient. The former strategy would have the competitive advantage under frequent pulses of the limiting nutrient, whereas the latter strategy presents an ecological advantage when the nutrient pulsing frequency is lower than cell division rate. A phytoplankton assemblage can

be assumed to contain both strategists in the water column. Phytoplankton species composition in subsurface waters was more or less similar at 3 stations, S1, S2 and S3 considering a span of 100 km across a large salinity gradient (Clifford et al. 1992). Cryptomonads and *Chrysochromulina* spp and *Micromonas pusilla* were dominant at S2, S3 and S1 in cell density (Clifford et al. 1992). The common diatom species included *Chaetoceros spp,* and *Thalassiosira spp*. (Clifford et al. 1992), which are said to use the 'growth' and 'storage' strategies, respectively (Collos 1986). At Stn S2, the chlorophyll maximum at 7 m on August 7 contained 4 times more phytoplankton cells than at the surface (Clifford et al. 1992), and was frequently observed at or associated with the nutricline (Cochlan et al., 1990; Yin et al., 1997 a). Phytoplankton there could use either the 'growth' or 'storage' strategy by different species. The storage strategy of non-limiting nutrients would allow phytoplankton to utilize the limiting nutrient when it is available and thus maximize phytoplankton growth by saving the energy expenditure associated with taking up non-limiting nutrients under limiting irradiance. This may explain why there were various modes or patterns of the N:P ratio at the nutricline, which indicates the different strategies of taking up nutrients sequentially based on the nutritional status of phytoplankton. The sequential uptake strategy allows some phytoplankton species to use the "storage" capacity for non-limiting nutrients and other phytoplankton species to use the "growth" response for the most limiting nutrient when it becomes available by mixing processes.

Sequential uptake of nutrients by phytoplankton can be a fundamental mechanism in maintaining high productivity in the water column where there are frequent mixing events in coastal waters. The sequential uptake strategy largely occurs at the nutraclines near or at the bottom of the photic zone. There is a consistent association between the nutriclines and the chlorophyll maximum in various aquatic environments (Cullen, 2015) and it is also common in the Strait (Harrison et al., 1991). There is a frequent upward flux of nutrients through the

nutricline due to entrainment in the Strait (Yin et al., 1995a, b and c) and by internal waves in the open ocean (Pomar et al. 2012). Phytoplankton in the chlorophyll maximum are generally exposed to nutrients and when these cells are brought up to the surface during entrainment or wind mixing (Yin et al., 1995a), they can quickly photosynthesize (Yin et al., 1995c). When phytoplankton exhaust the most limiting nutrient, their internal nutrient pool decreases and they sink down to the nutriclines, possibly due to the formation of clumps and take up the abundant nutrients there. Thus, the cycle of sequential uptake of limiting and then the non-limiting nutrients may reduce nutrient deficiency in phytoplankton.

Sequential uptake of nutrients can be an important process to maintain the phytoplankton nutrient stoichiometry. Carbon fixation continues after a nutrient becomes deficient (Elrifi and Turpin, 1985; Goldman and Dennett, 1985) and the storage of organic carbon of a higher POC:N ratio is common in phytoplankton (Healey, 1973). When phytoplankton cells with excessive organic carbon due to limitation of a nutrient, sink from the upper euphotic zone to the nutricline where light becomes limiting, uptake of other nutrients occurs by utilizing stored organic carbon, leading to an increase in the cellular N and P quotas. Thus, the ratios of carbon to other nutrients approach optimum stoichiometry. POC:N ratios at Stn S2 and S3 were observed to be between 6:1 and 7:1 in the water column, even though both ambient NO_3^- and PO_4^{3-} were near detection limits (Fig. 10). This demonstrates the lack of ambient nitrogen limitation on the cellular nutrient stoichiometry. Even at Stn S1 where entrainment and mixing were not as strong as at Stns S2 and S3, the POC:N ratio was only slightly higher than 7:1 (Fig. 10).

5. Conclusion

The use of in-situ continuous vertical profiles in this study showes a high variability of ambient N:P and N:Si ratios in the water column, suggesting the dynamics of nutrient uptake ratios, as illustrated in the conceptual model of Fig. 1. The incubation experiments

demonstrated the sequential uptake of nutrients by phytoplankton, which suggests that deficiency of a nutrient that is based on the ambient nutrient ratio could be transient and overcome by the sequential uptake of the most limiting nutrient and non-limiting nutrients. The capacity of sequential uptake of nutrients is an important strategy for phytoplankton to maintain high primary productivity and near optimum cellular nutrient stoichiometry in the water column. The sequential nutrient uptake strategy also offers another mechanism for the explanation of the variability in the nutrient stoichiometry of phytoplankton in the euphotic zone.

Authors contributions

K. Yin collected data and wrote the manuscript.

PJ Harrison supported the research cruise for collection of data and designed the sampling plan.

Competing interests

The authors declare that they have no conflict of interest.

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Figures captions

Figure 1. Conceptual model for sequential nutrient uptake, which is illustrated in vertical profiles of N, P and N:P ratios. C0 to C3 represent a time series of nutrient uptake during bloom development and C4 to C6 indicate subsequent vertical mixing of nutrients and subsequent uptake. The short horizontal line near the middle of the depth axis indicates the euphotic zone depth. N disappears first at C2, and P is left which continues to be taken up at C3. C4 represents mixing of nutrients into the bottom of the photic zone and phytoplankton have not taken up these nutrients yet. At C5, N is taken up first before P, while at C6, P is taken up first before N.

Figure 2. Map of the Strait of Georgia showing the study area and the sampling stations. Note: the Fraser River is located to the right, having two river channels flowing into the Strait of Georgia.

Figure 3. Two vertical profiles (T1=12:15 and T7=06:15) in the time series for August 6-7, 1991 of nutrients at S3. Left panel: NO_3^-, PO_4^{3-} and N:P ratios. Right panel: SiO_4^{4-} and N:Si.

Figure 4. Vertical profiles at S3 near the Fraser River plume to P4 and P6 finally to S1 that was well beyond the plume (108 km away) during August 8, 1991. Left panel: NO_3^-, PO_4^{3-} and N:P ratios. Right panel: SiO_4^{4-} and N:Si ratios.

Figure 5. Selected vertical profiles at S1 during the time series (T1, T3, T8 and T11) of August 8-9, 1991. Left panel: NO_3, PO_4 and N:P ratios. Right panel: SiO_4^{4-} and N:Si ratios.

Figure 6. Vertical profiles in the time series at P5 during August 10-14, 1991. Left panel: NO_3^-, PO_4^{3-} and N:P ratios. Right panel: SiO_4^{4-} and N:Si ratios.

Figure 7. Time course of duplicate in vivo fluorescence, NO_3^-, PO_4^{3-} and SiO_4^{4-}, and N:P and N:Si ratios during an in situ incubation of a water sample taken from 1 m at P3 on August 11 (11:45). NO_3^-, PO_4^{3-} and SiO_4^{4-} were added to the water sample at T=0 before the incubation.

Figure 8. Time course NO_3^-, PO_4^{3-} and SiO_4^- during the field incubation of water samples taken at Stn S3 during June 8, 1989. Top panel: sample taken at 1 m and the incubation was done under 1 layer of screening. Bottom panel: sample taken at 16 m and incubated under 4 layers of screening.

Figure 9. Time course of NO_3^-, PO_4^{3-}, and SiO_4^{4-} during the field incubation of a water sample taken at Stn S1 on June 4, 1990. Fig. 9-1) pre-incubation: no nutrients were added to the sample during the first 28 h; Fig. 9-2) after pre-incubation, nutrients were added in 8 treatments: no additions, NO_3^- alone (+N), PO_4^{3-} alone (P), SiO_4^{4-} alone (+Si), NO_3^- and PO_4^{3-} together (+N+P), NO_3^- and SiO_4^{4-} (+N+Si), PO_4^{3-} and SiO_4^{4-} (+P+Si) and all three (+N+P+Si); Fig. 9-3) ambient and uptake nutrient ratios calculated from the time course in (Fig. 9-2). The sign "+" means "added". +N/+P and +N/+Si indicate the ratio of the added N alone over the added P alone and over the added Si alone, respectively. 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-day1 phosphate concentraiton) to get N:P ratio on day 1.

Figure 10. Vertical profiles of particulate organic C:N ratios at stations Stn S2, S3 and S1 along the increasing distance from the river during August 20-23, 1990.

Fig. 1

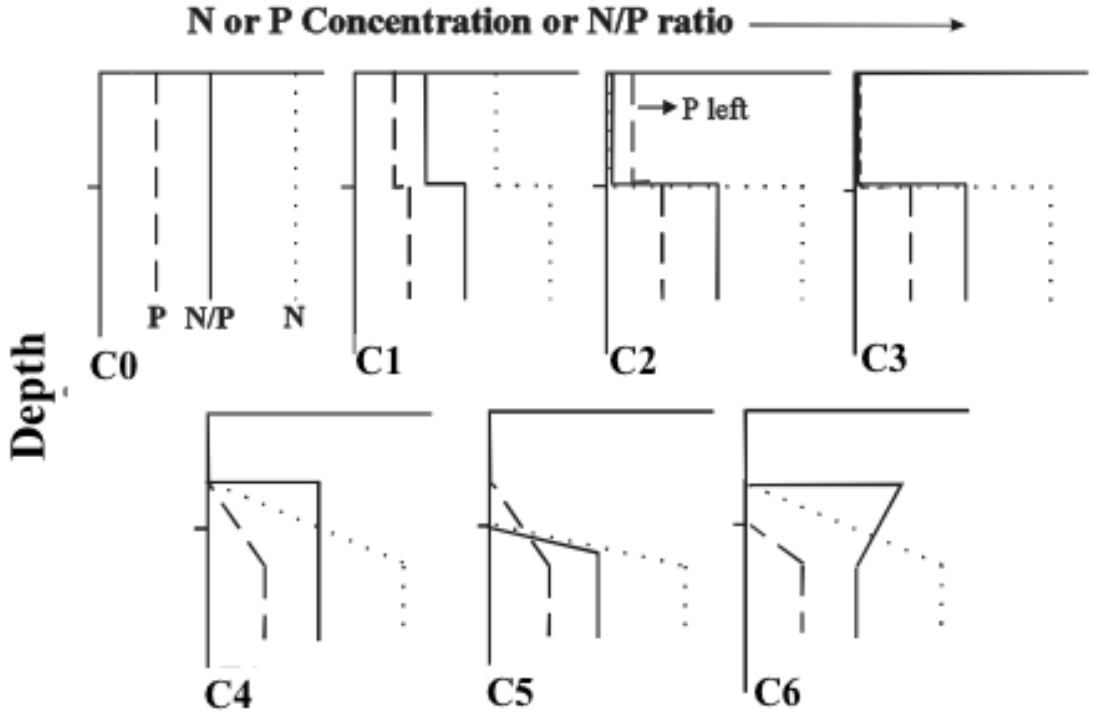

N or P Concentration or N/P ratio
Depth
P
N/P
N
C0
C1
P left
C2
C3
C4
C5
C6

Fig. 2

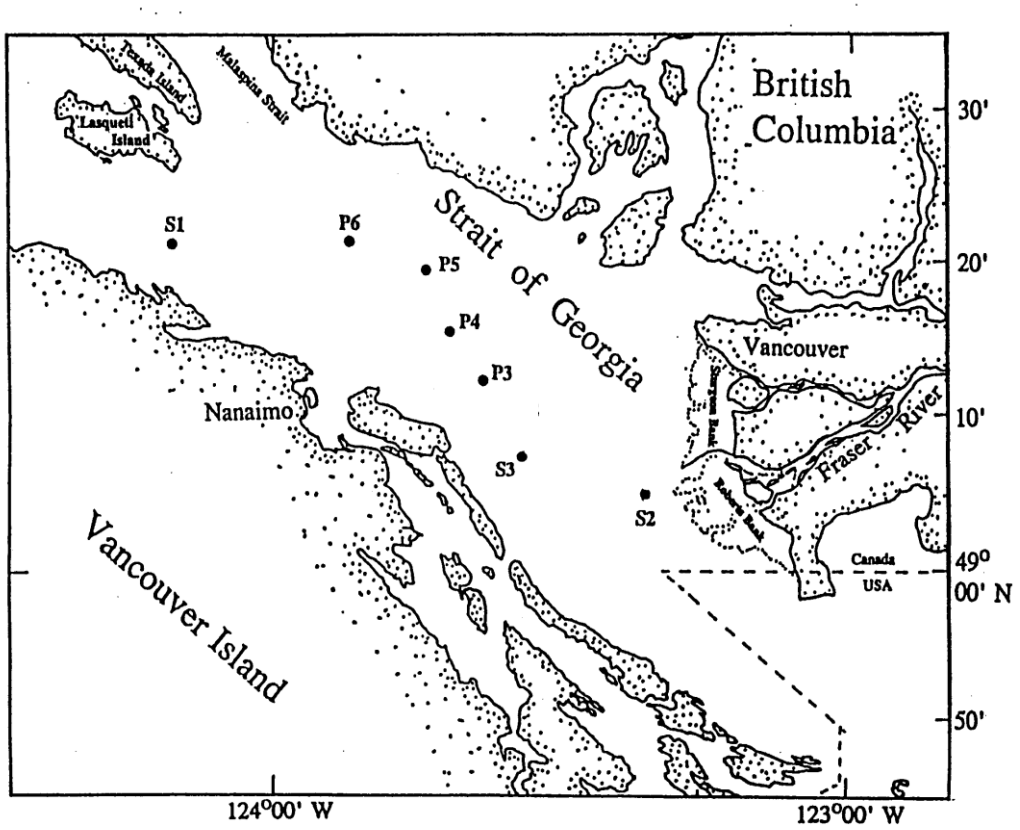

Texada Island
Malaspina Strait
Lasqueti Island
British Columbia
30'
S1
P6
P5
Strait of Georgia
20'
P4
Vancouver
P3
Nanaimo
Fraser River
10'
S3
S2
Vancouver Island
Canada
USA
49°
00' N
50'
124°00' W
123°00' W

Fig. 3

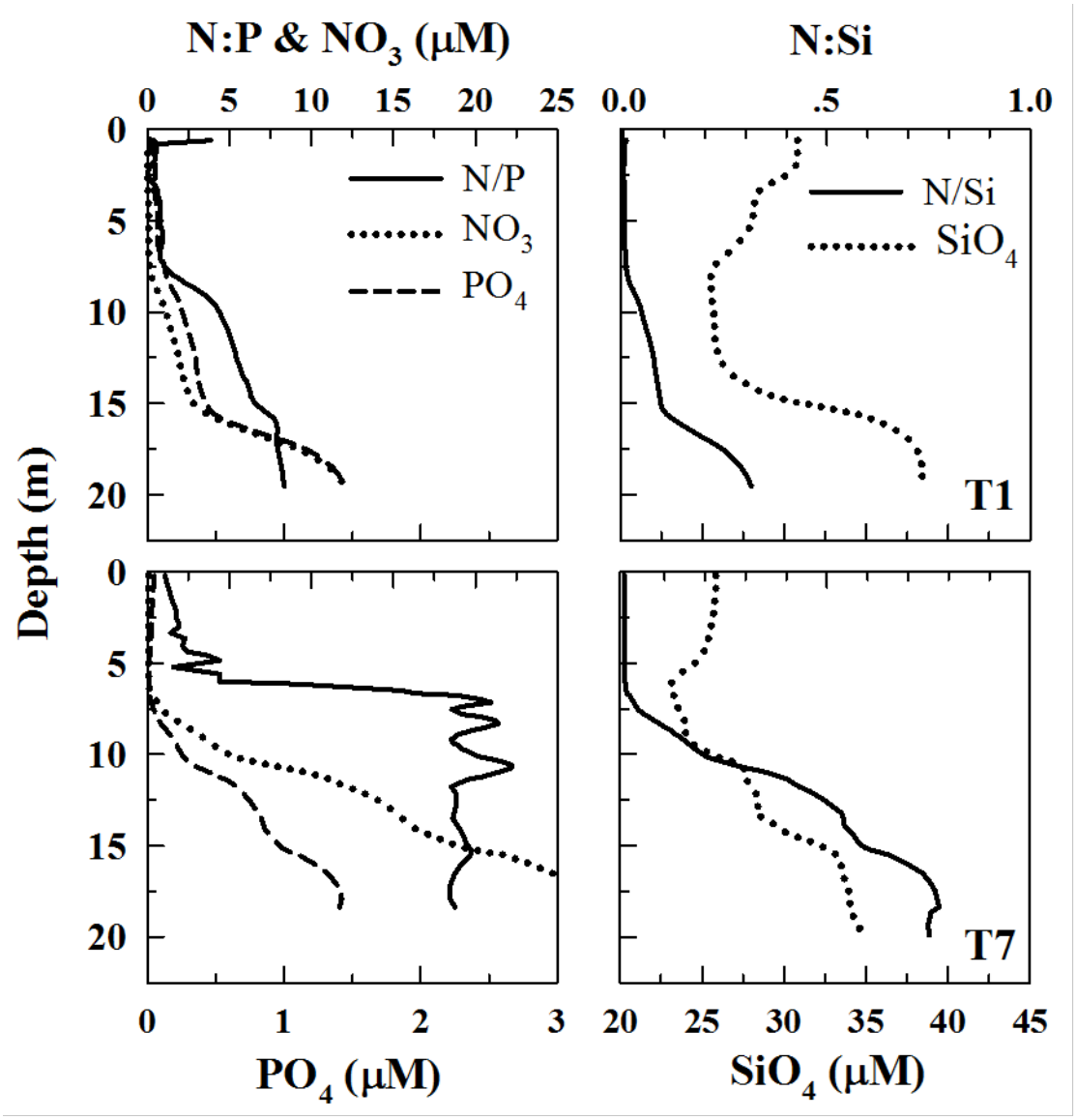


Fig. 4

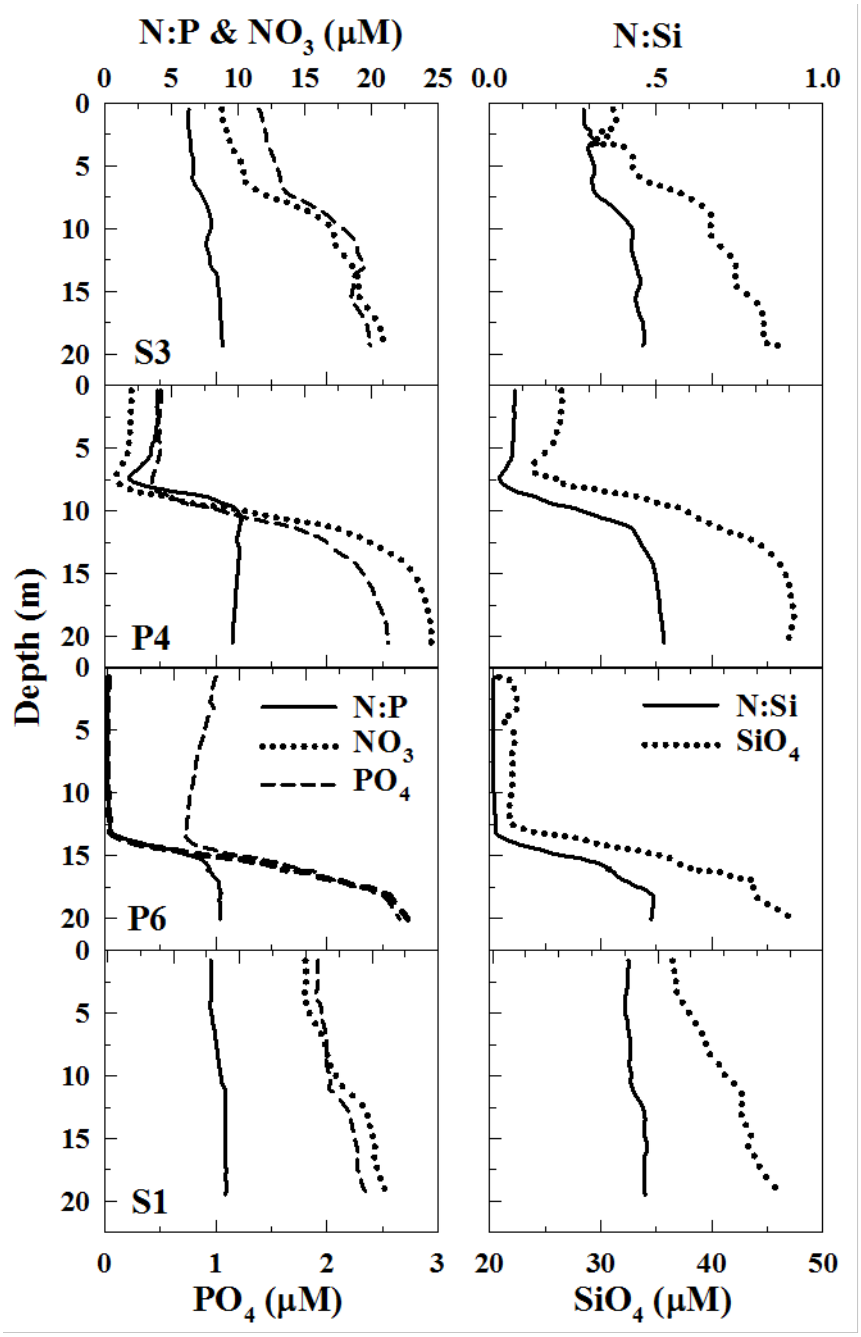

N:P & NO3 (µM)
N:Si
Depth (m)
S3
P4
P6
S1
N:P
NO3
PO4
N:Si
SiO4
PO4 (µM)
SiO4 (µM)

Fig. 5

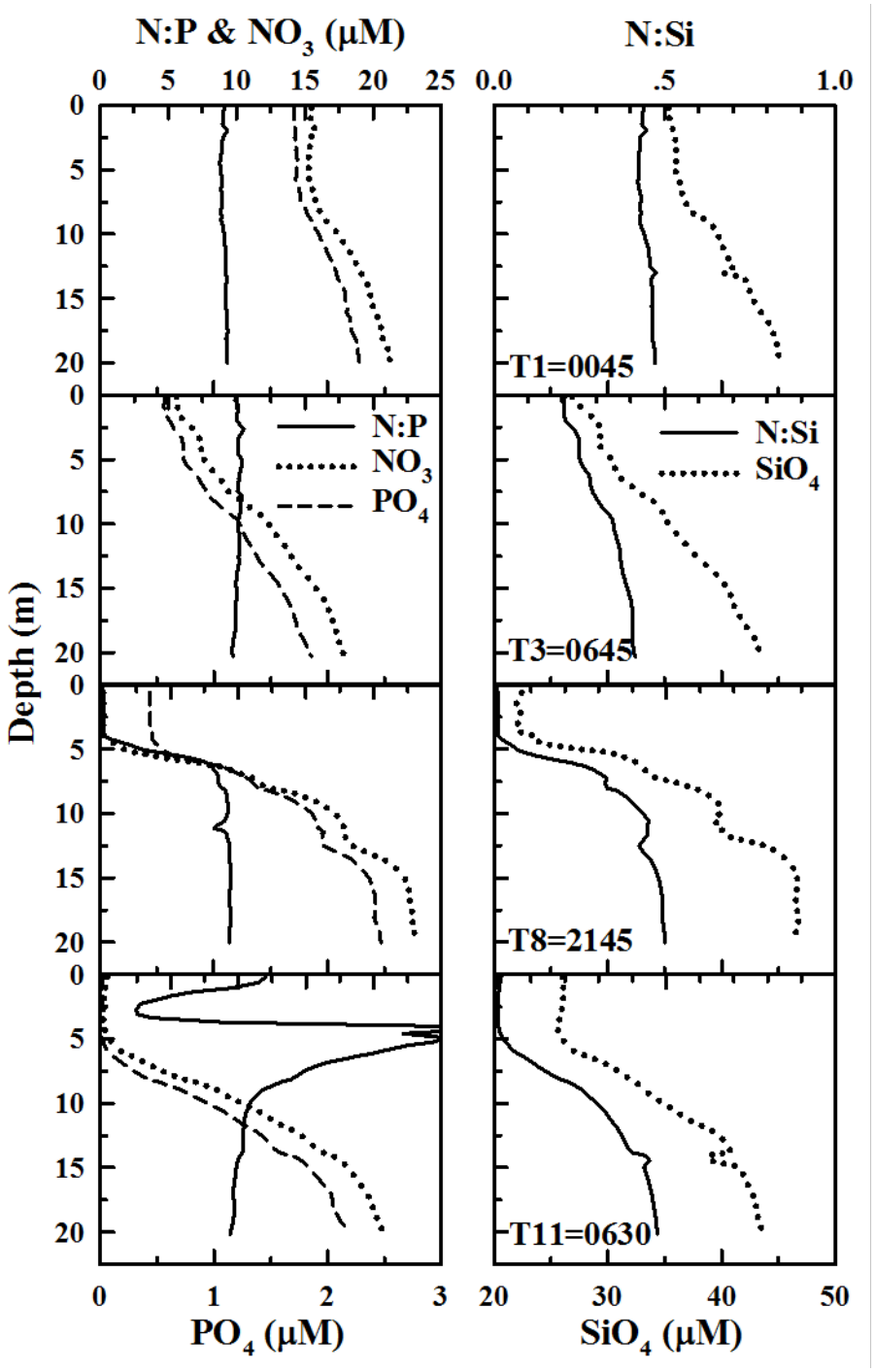

N:P & NO3 (µM)
N:Si
Depth (m)
N:P
NO3
PO4
N:Si
SiO4
T1=0045
T3=0645
T8=2145
T11=0630
PO4 (µM)
SiO4 (µM)

Fig. 6

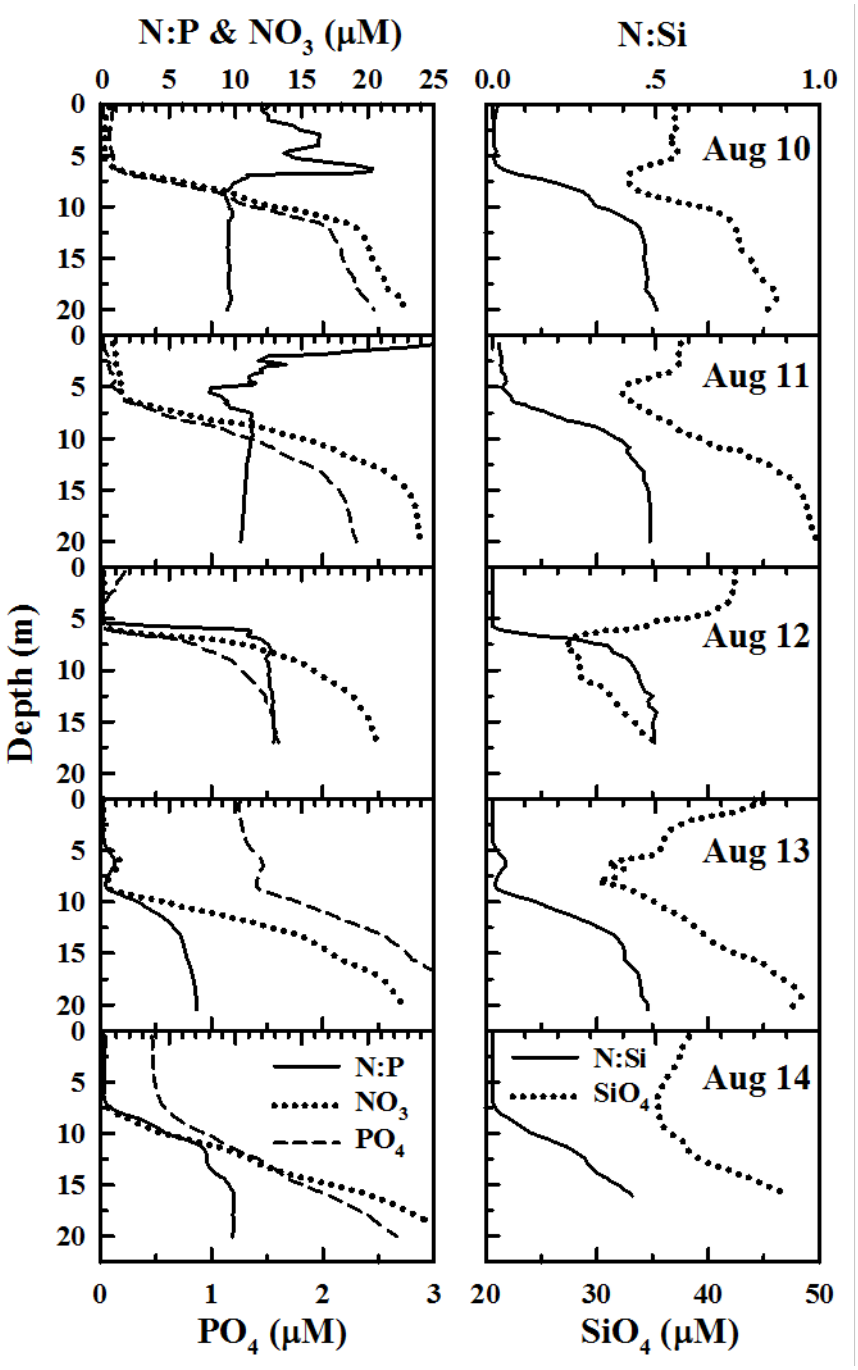

N:P & NO3 (µM)
N:Si
Depth (m)
Aug 10
Aug 11
Aug 12
Aug 13
Aug 14
N:P
NO3
PO4
N:Si
SiO4
PO4 (µM)
SiO4 (µM)

Fig. 7

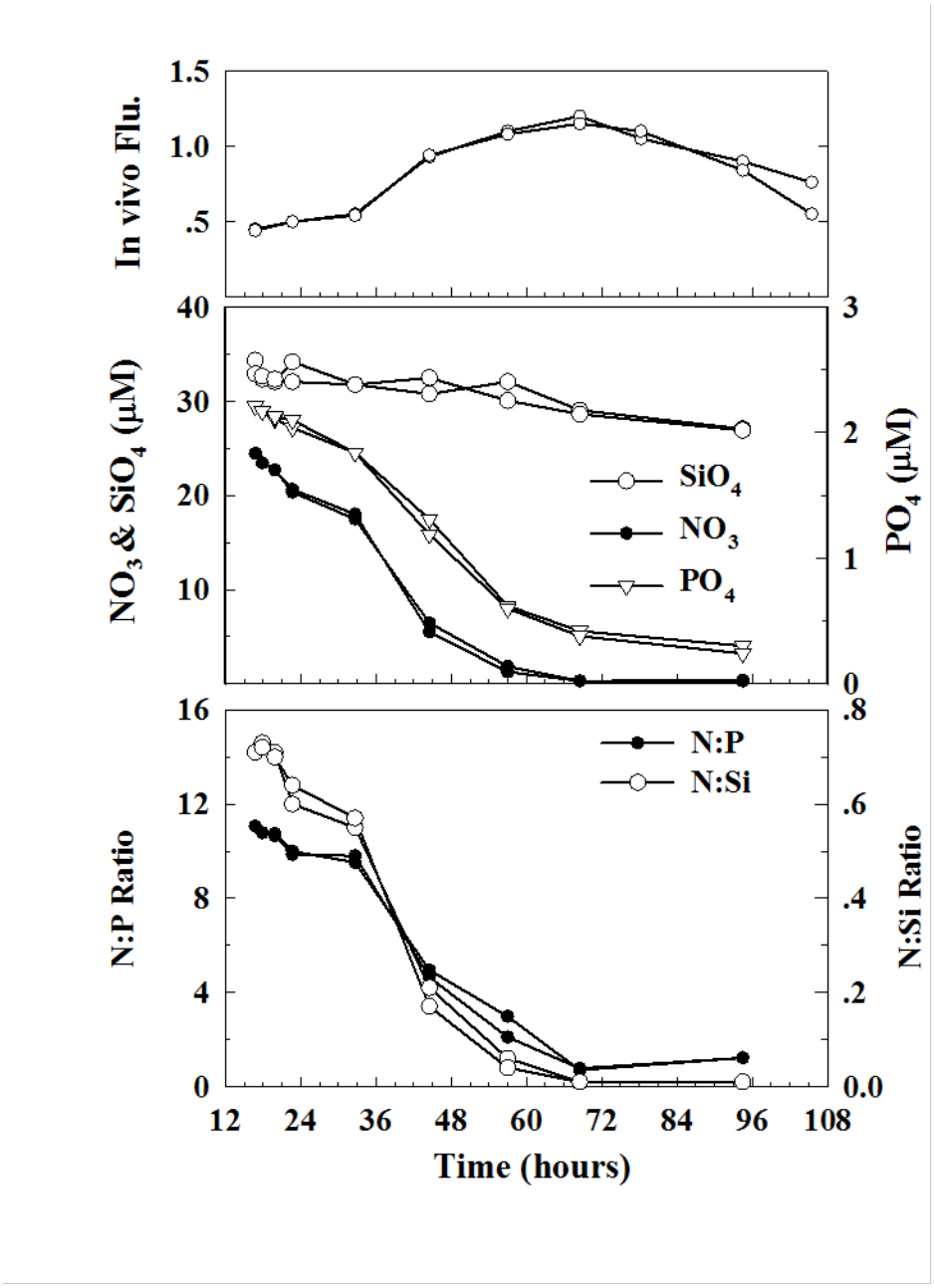

In vivo Flu.
1.5
1.0
.5
0
NO3 & SiO4 (µM)
40
30
20
10
0
PO4 (µM)
3
2
1
0
SiO4
NO3
PO4
N:P Ratio
16
12
8
4
0
N:Si Ratio
.8
.6
.4
.2
0.0
N:P
N:Si
12
24
36
48
60
72
84
96
108
Time (hours)

Fig. 8

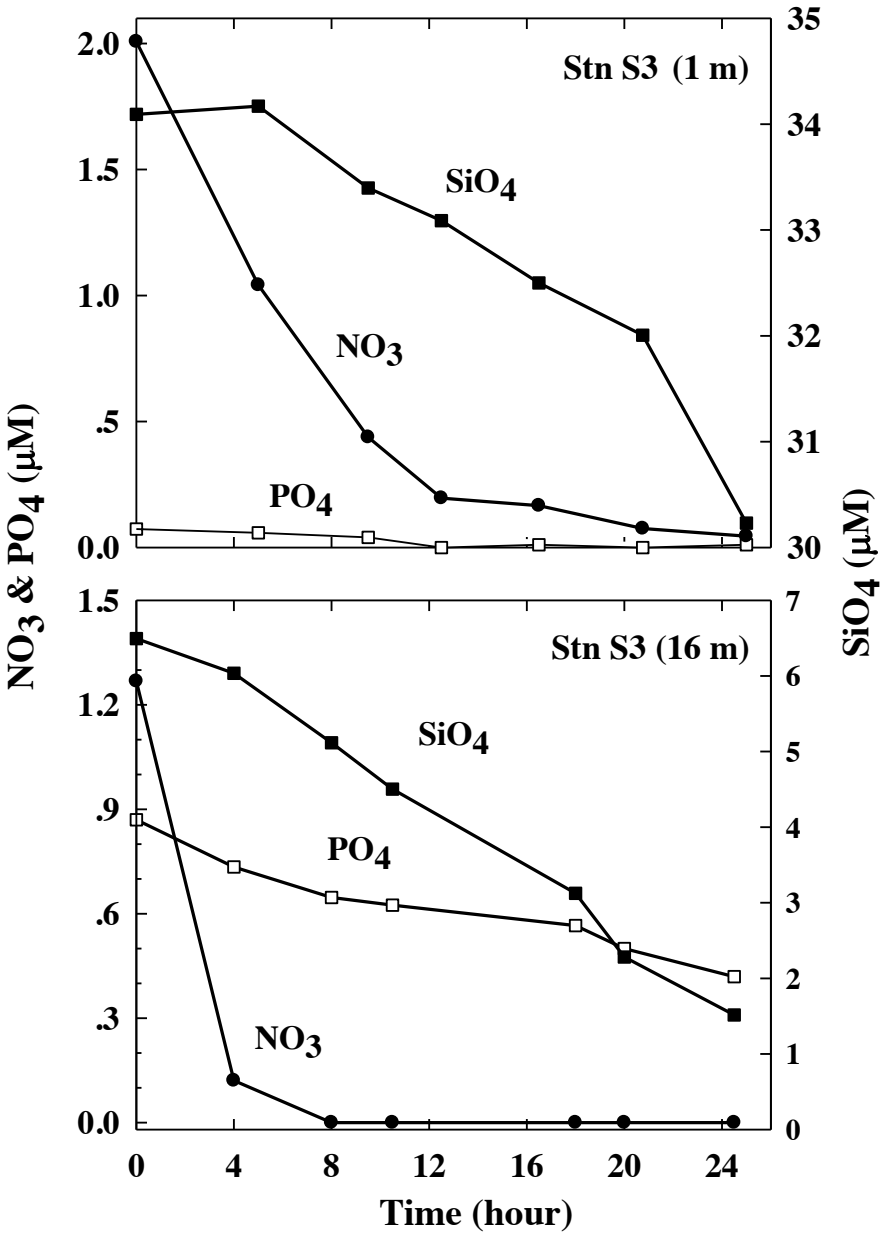

Stn S3 (1 m)
SiO4
NO3
PO4
Stn S3 (16 m)
SiO4
PO4
NO3
NO3 & PO4 (µM)
SiO4 (µM)
Time (hour)
0
4
8
12
16
20
24
2.0
1.5
1.0
.5
0.0
35
34
33
32
31
30
1.2
.9
.6
.3
7
6
5
4
3
2
1

Fig. 9-1

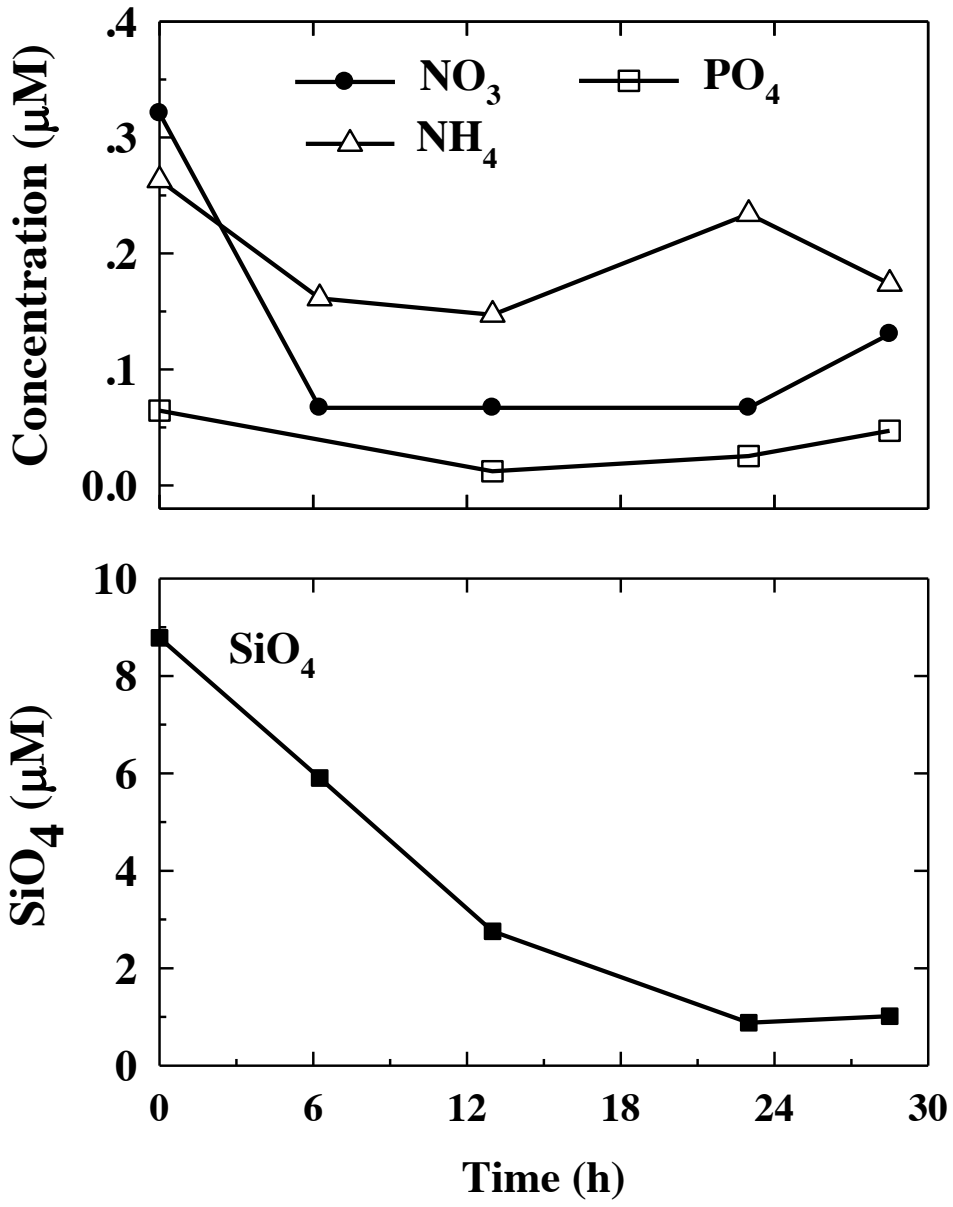

Concentration (μM)
.4
.3
.2
.1
0.0
NO3
PO4
NH4
SiO4 (μM)
10
8
6
4
2
0
SiO4
0
6
12
18
24
30
Time (h)

Fig. 9-2

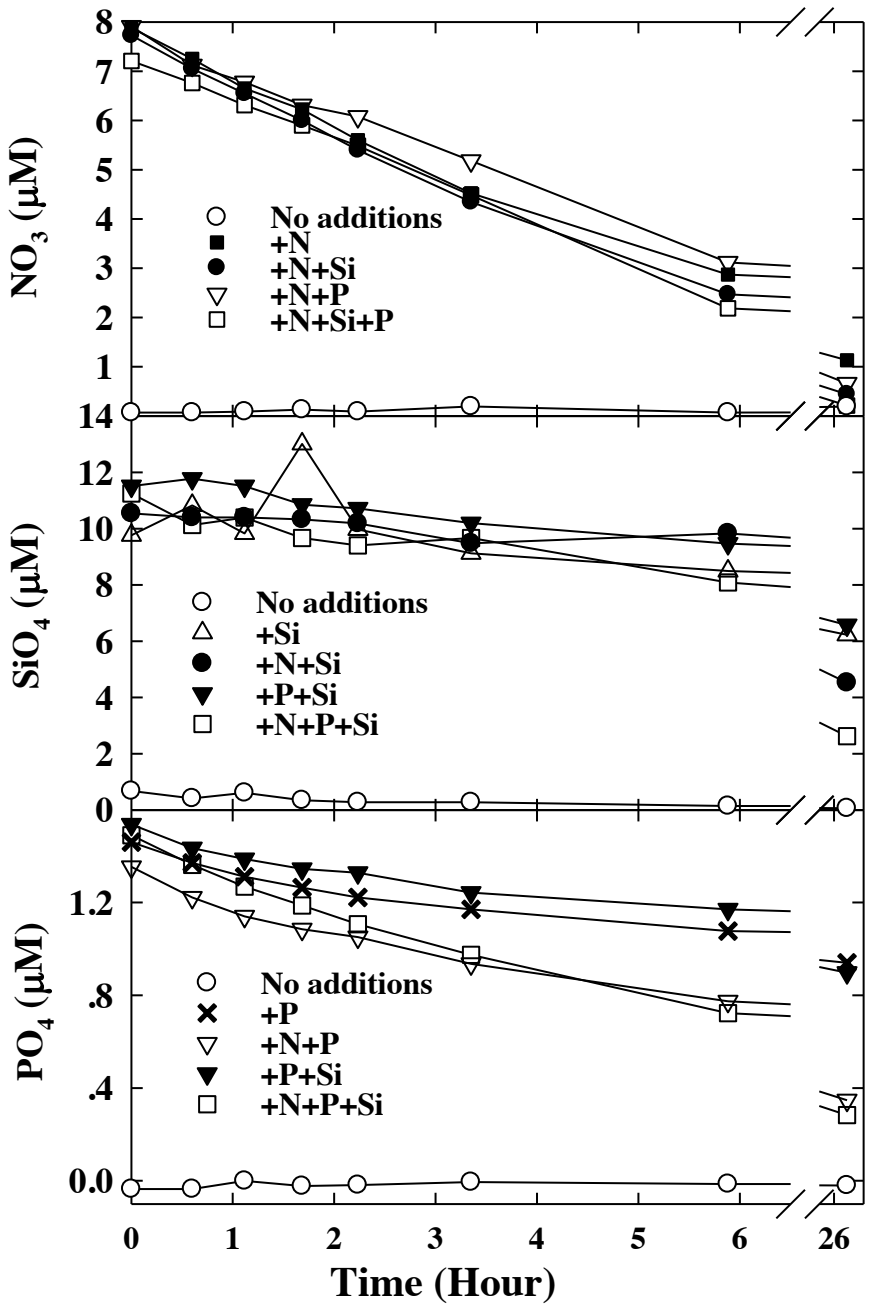

NO3 (µM)
No additions
+N
+N+Si
+N+P
+N+Si+P
SiO4 (µM)
No additions
+Si
+N+Si
+P+Si
+N+P+Si
PO4 (µM)
No additions
+P
+N+P
+P+Si
+N+P+Si
Time (Hour)

Fig. 9-3

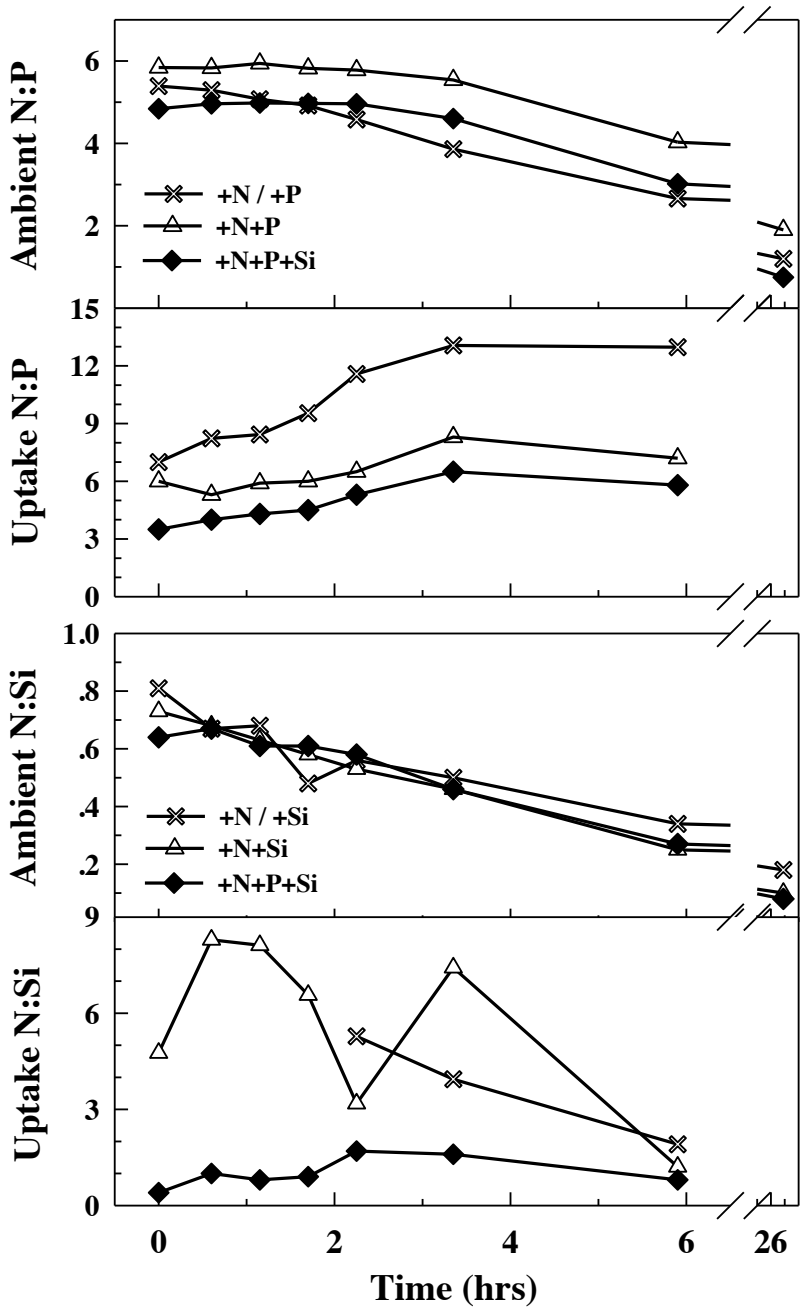
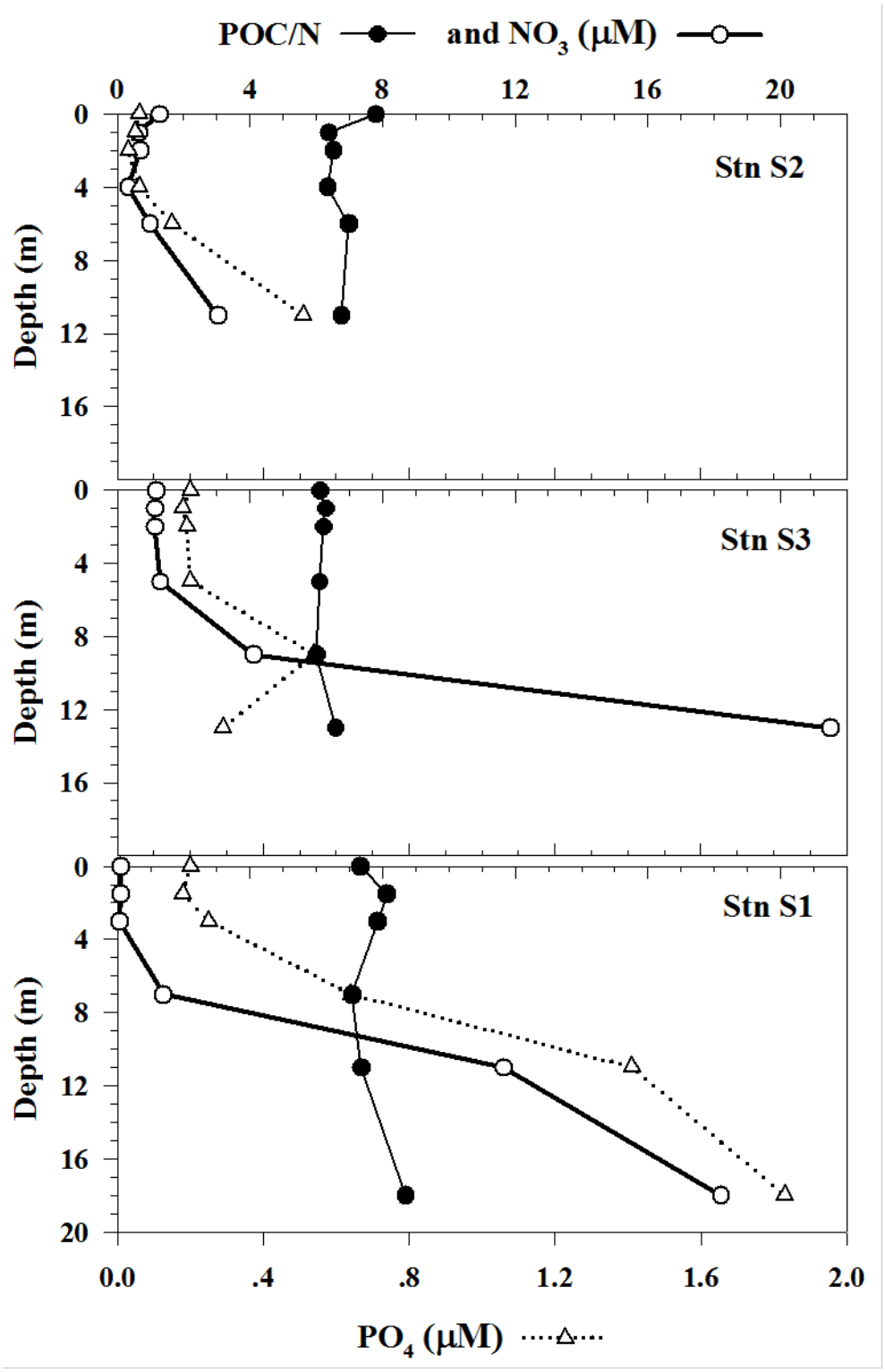

Ambient N:P
Uptake N:P
Ambient N:Si
Uptake N:Si
+N / +P
+N+P
+N+P+Si
+N / +Si
+N+Si
+N+P+Si
Time (hrs)

Fig. 10


POC/N
and NO3 (µM)
0
4
8
12
16
20
Stn S2
Stn S3
Stn S1
Depth (m)
0
4
8
12
16
20
0.0
.4
.8
1.2
1.6
2.0
PO4 (µM)