Review report:

"Relationship between N:P : Si ratio and phytoplankton community composition in a tropical estuarine mangrove ecosystem"

by A. K. Choudhury and P. Bhadury

General comment:

Sundarbans mangrove is one of the most sensitive ecosystems in response to climate change (either natural or anthropogenic) and also represents a unique ecosystem at the land-ocean boundary of the Bay of Bengal and the major river system in India. Hence any report from this system is a valuable documentation of the changing system. However, the paper is no doubt a novel attempt to address the microfloral diversity in relation to the ideal ratios of macronutrients over the year covering different seasons. Nevertheless, to my knowledge, there are several manuscripts since 1990s reporting phytoplankton diversity from this mangrove system along with physicochemical parameters and hence this study does not report anything new which can enrich the existing literature. The data set still could be valuable with a better and proper presentation.

However, I feel there are several flaws in data presentation, interpretation, discussions and referencing and need thorough corrections to make it publishable. After going through the method and result section, I realize that measurement related error could give a totally wrong impression about the study area. I could not correlate clearly the variation in the macronutrient molar ratios with phytoplankton cell abundance and the molecular data in my opinion is something too different from the title or the objective of this study. I am afraid that this manuscript does not meet up the standard of Biogeosciences, though the objectives are well within the aim of the journals.

Specific comments:

Title:

I do not think the title represents alone the present form of paper and does not follow the way the matter has been discussed in the text. To justify the title, more precise discussion on nutrient stoichiometry is required in relation to changing phytoplankton communities.

Abstract:

"Results show that even though habitat variability does exist at our study area and varied on a spatial and temporal scale, the nutrient concentrations were intricately balanced that never became limited and complemented well with the concept of modified Redfield ratio"

I strictly disagree with this statement from the abstract. In the figures it is shown very clearly that most of the time N:P, N:Si remained much bellow the line of classical Redfield 16:1 and hence despite of the presence of higher nutrient concentrations, the molar ratios are showing

nitrogen limitation which has also been observed in other estuaries in India due to higher phosphate loading relative to nitrogen.

I found several interesting articles from the same system but different stations, describing details of nutrient loading and macronutrient molar rations and also phytoplankton communities. A recent article appeared in marine Chemistry by Ray et al (2014) describes the biogeochemical cycling of nitrogen in the sundarbans mangrove system and it is stated there that nitrogen could be potentially limiting in the mangrove system. This is also consistent with the present observation, however, has not been discussed in this way.

The molecular part of this work is not very clear for me and I do not find a proper link between the microscopic observation and molecular data presented in this manuscript. I am not sure if the molecular is a supportive data for the present study.

"Hence we conclude that the recent concept of functional traits and elemental stoichiometry does not remain restricted to controlled environment of experimental studies only but occur in natural mangrove habitat."

The conclusive remark of the abstract and also of the entire stud in my opinion is extrapolated considering only nutrients are the responsible factors controlling phytoplankton ecophysiology. Usually, for experimental work by altering nutrient ratios would fit to this title more than the field observations. Authors should keep in mind that in the natural dynamic system like a mangrove creek or estuary, with high tidal fluctuation (it goes almost up to 5m) only nutrient ratios alone cannot explain the observed variability. Moreover, meso and microzooplankton grazing (top down control) could be a major controlling factor in this system and there are several references in this regard which have not been considered in this study (Biswas Naha et al., 2014, Marine Pollution Bulletin, DOI: 10.1016/j.marpolbul.2014.04.015.

In this regard, I miss some simple linear correlation with phytoplankton biovoulme and nutrient molar ratios which gives very nice indication if there is any strong relation present in the environment.

Introduction:

The first paragraph of introduction focussed only on "Redfield" or modified "Redfield" ratios with several references and I feel most of the references are based on oceanic data which could be totally different than a tidal creek or estuary. In most of the coastal areas with high nutrient loading Classical Redfield may not be applicable to explain phytoplankton variability.

The second paragraph discusses about nitrogen loading to the coastal environments and lake systems. However, the discussion and references about eutrophication is lakes in this context sounded irrelevant to me. I miss some highly cited popular articles here like several references of Seitzinger, Conley and Smith in the context of nutrient loading and its effect on phytoplankton community. I would suggest the authors to include those references herein.

In the third paragraph the authors talked about dissolved silicate and its role. However, the sentence starts with "Another important nutrient in natural aquatic ecosystems is silicate which is the primary constituent of diatoms, a major constituent of natural phytoplankton assemblages." The sense of this sentence is correct, however, it is better to write in a different way for example "dissolved silicate is a primary need for diatom growth. The rest of the paragraph does not discuss about the role of silicate in diatom growth and effects of deficiency and so on. Rather, it discusses about the variability of N:Si molar ratios which may come afterwards of this paragraph. It is wise to discuss here exactly how any alteration in N:P:Si would impact a natural phytoplankton community.

The next part of discussion tells about the mangrove systems. I feel too less referencing in this part. I think some recent publications from the mangrove systems in this aspect are critically needed in this section. The author should see some recent references like Ray et al (2014) marine chemistry, to show that despite of high nitrogen loading mangrove system can be nitrogen limiting. However, the aquatic ecosystem, within the mangrove can differ from the forest system and additional referencing is required to prove that mangrove waters are nutrient enriched.

I miss some extensive discussion on how N:Si:P affects phytoplankton physiology both in field studies and experimental works which could be related to the objectives at end of this section.

Sample collection:

This is not mentioned anywhere if the sampling was done following high or low tide. In a mangrove creek a sampling during high tide could significantly differ than the sample on the same day during low tide (due to very high tidal fluctuation). Hence it is highly important that the author has followed a tide chat during sample collection. At this place tidal amplitude could be till 5m and hence could introduce significant difference in subsamples collected during different times.

Why the atmospheric temperature was reported in not clear since no meteorological data or gas flux was calculated.

The water Nutrients were also analysed after collecting 125ml water samples and fixed with formalin. I wonder was there any duplicate done for nutrient analysis? For all 5 nutrients it is only possible to measure with replicated if they are done on an autoanalyzer which requires very less samples. Otherwise for all 5 nutrients 125ml waters samples are definitely not sufficient with a replicate. There is no error bar provided in the graphs for each data point.

The water for nutrient analysis was filtered through a nitrocellulose paper and I am really doubtful that it may leach significant quantity of nitrate while filtering against pressure. The same problem occurs while using a GF/F filter paper which leaches silicate the filtered water. It is recommended to use polycarbonate membrane filter to avoid such contamination.

Nothing has been mentioned about the cleaning of the bottles: acid clean or similar, since this may also introduce significant error in nutrient analysis.

This is surprising that for phytoplankton analysis only 125ml water sample was collected which is too less I feel. Especially in a turbid estuary 125 ml samples definitely would not give a reproducible count.

How water samples were collected for pH measurements and how was it carried or analysed has not been mentioned in the manuscript. What was used to preserve the pH samples and which kind of vial/container was used for pH water sample measurements?

I am still sceptical about addition of the molecular data with this microscopic analysis and I am not sure if the present from of the manuscript is giving a clear idea following the title of the manuscript. I feel it is confusing for the authors. I am afraid that I may not be the right person to judge this part of the manuscript and I would suggest the editor to send the same for an expert with molecular taxonomy expertise.

Results:
Data presentation:

First thing which surprises me is the naming of the stations are as station 1 and station 3 instead of 1and 2. Is there is clarification for this?

Overall data representation is very poor. Most of the tables are not in the format of international journals. For example in table 3 (showing diversity, evenness index and relative %) values are given almost up to 6th decimal point which is not right. Moreover the international formatting for table has also not been followed and position of a particular value should be according to its decimal position. The same would be applied for table 4 where the bio-volume and surface area has been given in mm^3 with several decimal places and it would convenient to produce in μm^3 with more presentable numbers.

Resolution of figures is very poor. The name of the "Y" axes cannot be read in any of the cases. Moreover, the title of the "Y" axes stating "concentration (μM)' the first letter should be of capital letter and "μ" has been written as "u". I feel very confusing the way the molar ratios have been presented for two stations. Font size used for figure caption, title and series title are all different in different figures and most of then start with small letter which is incorrect. It is suggested to re-draw all figures with definite big font size and higher resolution.

It was not clear why physicochemical parameters were given together with the study site. This could be also presented separately. It would be interesting to see a monthly variation figure for dissolved oxygen too.

For some data points error bars are given and some data points show very high spread of the data set. For example in Fig. 2d also shows a data point for the month of August. Nutrient analysis must be done in triplicate from each sample for such kind of investigations; otherwise it can introduce large variation.

pH values are very high than it should be. With a salinity values of <25psu, consistent pH values till 8.6 is very much unusual. For example during January a salinity value of 16psu

cannot be associated with a pH of 8.6. There is definitely something wrong in pH measurements.

I am not convinced with the trend of dissolved oxygen given here season wise. This is opposite to what is reported usually for Indian tropical systems. Moreover, sampling without following the high tide may also introduce similar error. During low tide the turbid water usually shows low DO values relative to the clearer high tidewater. The trend presented here with minimum DO values during monsoon and maximum during pre-monsoon is tough to interpret. Since with dilution of seawater during monsoon, dissolution of oxygen increases, and the reverse in premonsoon (higher saline water prevents dissolution of oxygen in water). It is not really clear why the trend was opposite.

Ammonia concentrations sounded too high to me. I suggest the authors to check the reference by Biswas et al (2005) "Estimation of ammonia exchange at the land–ocean boundary condition of Sundarban mangrove, northeast coast of Bay of Bengal, India" Atmospheric Environment, 39 (2005) 4489–4499. Ammonia concentrations in mangrove waters cannot be such high as has been reported here. Usually it remains bellow 1-2μmol L^{-1} and would be readily taken up by the biota. In Feb the value given is almost 7 μmol L^{-1} which is definitely not possible. This can only occur in a polluted estuary with very low dissolved oxygen value and associated with high nitrite (NO2) values. With dissolved oxygen values above 6mg L^{-1} it is unusual to get 7 μmol L^{-1} ammonia. This value can significantly impact the observed Redfield ratios and may introduce serious error.

Nitrite values have not been given. It is also not mentioned if the measure nitrate value was a combination of nitrate+nitriete.

For measuring ammonia what kind of water was used to prepare the reagent was also not mentioned. Nutrients measurement is a major part of this manuscript and none of the nutrient measurements have been described in details.

Nowhere in the manuscript phytoplankton cell number per lit has been given. The organic carbon content derived from applying formula works well in the laboratory culture experiment and I am not sure this is a good indicator in the natural system without measuring POC/PON in elemental analyzer. Specially, in a turbid system phytoplankton minimize carbon content to maximize their chlorophyll to capture maximum light.

Chlorophyll content was also not measured during this study and I think should be included for such kind of measurements.

After going through the method and result section, I realize that measurement related error could give a totally wrong impression about the data presented here. I could not correlate clearly the variation in the macronutrient molar ratios with phytoplankton cell abundance and the molecular data in my opinion is something too different from the title or the objective of this study. I am afraid that this manuscript does not meet up the standard of Biogeosciences, though the objectives are well within the aim of the journals.