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Biogeosciences Discussions

Interactive comment on "Response of *Nodularia spumigena* to pCO_2 – Part I: Growth, production and nitrogen cycling" *by* N. Wannicke et al.

Anonymous Referee #2

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In their manuscript entitled "Response of Nodularia spumigena to pCO2 – Part I: Growth, production and nitrogen cycling" Wannicke et al present data on batch culture experiments at different pCO2 levels. The authors find a positive effect of increasing CO2 on carbon and nitrogen fixation. This finding is interesting especially in view of an earlier study by Czerny et al 2009 who found that increasing CO2 concentrations had a negative effect on carbon and nitrogen metabolism in Nodularia. This study provides a new insight into many questions arising from projected climate change caused by an increase of anthropogenic carbon dioxide. However, I find some major caveats in both experimental as well as interpretive approaches in the manuscript which will be explained in details below and should be considered by the authors in a revised version of the manuscript. The following points highlight experimental caveats: 1- Carbonate chemistry: As already mentioned by two other comments in the open discus-

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sion, the authors do not attain the mentioned glacial (180 ppm), present (380 ppm) and future (780 ppm) concentrations of CO2 with the method employed. Actually at the start of the experiment all cultures have CO2 concentrations above present level. In the manuscript, the authors should use the actual values obtained in the culture vessels and refer to these values as they are (instead of past, present and future). Despite this discrepancy, the observation that growth (C and N fixation) differs with the actual/obtained CO2 concentrations is noteworthy and this should be strengthened in their arguments. 2- Filament abundance and filament-based estimates: Although I am aware that filament counts are easier to obtain and that cell size conversions to biomass have their own disadvantages, to me it does not seem appropriate to compare filament-based rates especially when the authors have observed changes in filament size. Similar assumptions apply for the number of heterocysts per filament which are biased in two ways, first by the decreasing number of heterocysts per filament and second the decreasing size of filaments (one may actually explain the other). The authors argue for this step in order to make comparisons to the literature, however, the majority of the literature provides percentages/relative changes in rates rather than absolute rates. Also, there is only one other study on the effect of increasing CO2 on Nodularia (Czerny et al 2009) which reports on cellular rates rather than filament-based. The conclusions of this study may significantly change when rates are calculated biomass-specific. 3- N2 fixation measurements: The authors used 6-h incubations to measure CO2 and N2 fixation rates. They mention that this would lead to sufficient dissolution of 15N2 gas in the incubation. However, a recent study by Mohr et al 2010 (which they cite) shows that a 6 hour incubation leads to only about 40% of the value used for the calculation. The final isotopic composition of the N2 gas may be sufficiently high to determine significant N2 fixation rates, however, the rates will be underestimated as long as the wrong value is used and the concentration of 15N2 changes over time. The authors should mention this especially as it may explain some of their discrepancies between N2 fixation rates and biomass accumulation. 4- Nitrogen turnover model: The authors developed a model on daily carbon and nitrogen metabolism based solely

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on three (four, including a time zero) measurements which are 3, 6 and 6 days apart from each other. This assumes that rates (fixation, release, growth and so on) do not change within these time periods which does not seem to be a valid assumption. But possibly the authors could provide a more detailed explanation on how their model was established which may resolve this caveat.

The following points highlight interpretive caveats: 1- Inconsistencies: There are several inconsistencies within the manuscript which the authors should resolve. For example, on page 2501 section 4.2, the authors mention that DON and DIN exudation was not directly measurable, however, at the end of the paragraph they provide DON and DIN as a possible explanation for the discrepancy between N2 fixation rates exceeding PON build-up. In my view, it is much more likely that N2 fixation rates on the one day that it was measured was different than the other days. The discrepancy thus results from the extrapolation of a 6-h incubation measurement to a several-day rate and the subsequent comparison to an actual several-day measurement. DON and DIN seems not a likely explanation in the view that both parameters were not detectable. Similarly, in the next section, the authors state that DON/DIN uptake by bacterial contamination was negligible, but, in the next paragraph, provide a "rapid response of the microbial food web" as a possible explanation for the lack of tendencies of organic matter exudation with pCO2. I am not sure whether these inconsistencies are real or are the result of unclear descriptions. However, the authors should clear this up in a revised manuscript. Further, on page 2504, an 80% release rate of total nitrogen fixed is applied to the model, however, this rate was not observed in this study (rather there was no exudation measurable) and is taken from other studies that did not investigate the effect of pCO2. Whether such as exudation rate also applies with increases in CO2 and would thus lead to such a large "extra" input of nitrogen is not supported by the data in this manuscript. There are several other inconsistencies within the results and the discussion sections which the authors should carefully re-evaluate. 2- The nitrogen turnover model: The established model and the corresponding figure do not add any new insights/data to the manuscript and are not explained well. The authors could

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either provide better explanations for this part or combine these data in simpler plots such as rates and molar ratios superimposed in a plot vs. time (and other plots) that allow insights into whether N and C accumulate at a similar rate or whether one or the other may be more or less affected by increasing pCO2. 3- Filament-based rates: As already mentioned above, the filament-based rates do not seem to be appropriate. Many of the "significant" differences in rates or filament quota may be biased by the fact that filament size changed in the different pCO2 treatments. If the authors would plot biomass-specific rates, the treatments would probably look different and may lead to different conclusions. 4- Statistics: In my opinion, some sections of the manuscript are burdened with statistics hiding the actual, important biological findings. For example, the fact that filaments shortened. Although not significant, to me it seems like an important observation which should be covered more.

The following section includes minor scientific and other comments: p 2483, l 9: please clarify whether "This new nitrogen" refers to all of the fixed nitrogen or to the fixed but released nitrogen. If it refers to the latter, it cannot be directly grazed upon. p 2483, l 22: "roughly one-third" is confusing as the authors probably refer to an increase from 280 (pre-industrial) to 380 (present) ppm. However, the intended 180 ppm refer to glacial. Clarify. p 2484, l 3: the correct reference should be Fu et al 2007, not 2008 (the 2008 paper reports on Crocosphaera) p 2484, l 5: see above, the Fu et al 2008 does not report on Trichodesmium p 2484, l 15: starting the paragraph with "Additionally" would imply that there is information before in the same paragraph. Rather start the sentence with s.th. like this: In general, there is scarce knowledge on how.... . p 2485, l 7: "...was isolated from the Baltic Sea by L.Stal and coworkers (NIOO)" rather than "...was isolated by L.Stal and coworkers (NIOO) from the Baltic Sea..." p 2485-2486: the experimental set-up description could be written clearer p 2485, l 12 and 16: 100 and also 200 umol photons mˆ-2 sˆ-1 is rather low for Baltic Sea summer especially when considering that Nodularia is a buoyant organism which may be exposed to much higher irradiances in the summer. The authors should mention this. p 2486, l 6 and 8: it is not clear which reference the authors ate referring to p 2488, l 23: indicate whether

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the samples were stored frozen or in a refrigerator p 2490, l 9: which time window during the day was chosen for the 6-h incubation? Does that cover a maximum C and/or N2 fixation period? p 2490, l 14-19: The authors may elaborate details about the calculation of their model. p 2492, section 3.3: This section should be clarified, some sentences jump from DOC to DON and back to DOC, for example. p 2493, l 10: Tukey's test instead of Turkey's test, change throughout manuscript p 2493, l 16: "this" not "his" p 2495, l 18 and 19: please clarify whether the volume refers to total volume or biovolume. If they refer to total volume, then yes, differences in volumetric rates are significantly different because the biomass in te volumes is different. This refers back to using biomass-specific rates rather than filament-based or bottle-based rates. p 2496, section 3.5: The calculations which are used for the model do not seem clear in this section. p 2497, l 2 and 3: Since there are no error estimates on the percentages, I find it hard to believe that 2 and 4 % will be so significantly different looking at growth rate differences in the double-digits. However, the difference is used to make the eutrophication hypothesis in the next sentence. This appears to be a rather weak statement. p 2505, l 4: diazotrophic cyanobacteria do not rely on dissolved inorganic nitrogen sources (except N2 gas which is usually excluded from this term) Table 1: These authors should state whether the samples were taken before or after the daily aeration of the culture vessels. Figure 5: symbols are all circles and not triangles, squares and diamonds, please change to either those symbols or change the legend. The authors often use the words "therefore" and "henceforward" in a wrong context.

Interactive comment on Biogeosciences Discuss., 9, 2481, 2012.