

Interactive comment on “Response of *Nodularia spumigena* to $p\text{CO}_2$ – Part I: Growth, production and nitrogen cycling” by N. Wannicke et al.

N. Wannicke et al.

nicola.wannicke@io-warnemuende.de

Received and published: 2 June 2012

We would like to thank the two anonymous referees and Czerny for their detailed and constructive comments on our manuscript. Below are the point by point replies to comments and suggestions.

Authors comment to Reviewer # 1

1)The authors suggest that the technique to control the carbonate chemistry might affect the results (acid/ base in Czerny et al or CO_2 bubbling in this study). Hoppe et al 2011, however, showed that both techniques lead to a similar result in growth at least in *E. huxleyi*. There might be other parameter (phosphorus availability or trace metals) which might control the $p\text{CO}_2$ effects in this organism which the authors did

C1619

not account for.

Reply 1) We added the possibility of other effects, rather than the carbon chemistry manipulation to the discussion (subsection 4.1). Nevertheless, differences between the two studies, other than the carbon manipulation, is counterintuitive especially concerning DIP availability, because our study showed a positive CO_2 effect although DIP was already lower in the beginning of our experiment compared to the Czerny study (they were using an artificial growth medium and modified YBCII nutrients with DIP of $5.4 \mu\text{mol L}^{-1}$). If DIP was the variable explaining the different outcome of the two studies the effect observed should have been vice versa (stimulation in the Czerny study, no effect in ours). For this reason, we searched for other differences, focusing on the experimental set-up. Trace metals, vitamins or other growth factors might explain differences, but again the artificial growth medium should be repleted in trace metals and vitamins. Still, unknown stimulating growth factors in the aged seawater used in our study neither be negotiated nor proven. Moreover, light availability and temperature might also affect the outcome of the experiment- this was also added to the discussion. Uncertainties arising with different carbon manipulation methods already occurred in the literature concerning the calcification and productivity of the coccolithophore *Emiliania huxleyi* and conflicting experimental finding of Riebesell et al., e.g. 2000 and Iglesias-Rodriguez et al., e.g 2008. Again here, the major difference was the carbon manipulation method: acid base vs. CO_2 bubbling. On the other hand, Hoppe et al. (2011) highlighted that intraspecific differences might explain most of the inconsistencies. However, *Nodularia* cultures used in the Czerny study were obtained from the IOW culture collection and therefore originated from the same isolate.

2)In order to understand the growth response during the acclimation more information about the “parent” culture would be needed. This could help to understand i.e why $p\text{CO}_2$ at day 0 is above ambient $p\text{CO}_2$ concentrations.

Reply 2) We only have included information concerning the parent culture and the pre-incubation, as well as the corresponding CT, pH and calculated $p\text{CO}_2$. We added this

C1620

information to the description of the experimental set-up. "After three days of acclimation, chlorophyll a concentrations were determined in the three pre-cultures, with 28 $\mu\text{g chl a L}^{-1}$ in the low pCO₂ treatment, 27 $\mu\text{g chl a L}^{-1}$ in the mid and 38 $\mu\text{g chl a L}^{-1}$ in the high pCO₂ scenario in order to inoculate the same quantity to each following replicate bottle. The calculated pCO₂ (for CT and pH) in these pre-cultures were 402 μatm , 422 μatm and 548 μatm for the low, mid and high pCO₂ treatment, respectively."

3) According to the authors, the carbonate chemistry was altered by temporal bubbling for one hour per day. The bubbling was, however, clearly not sufficient enough to yield the aimed pCO₂ concentrations. As a result, the presented carbonate chemistry in the manuscript rather represents the seasonal change of inorganic carbon/ pH in the Baltic Sea (Wesslander et al 2010) than a retrospect to glacial or a projection to enhanced pCO₂ as estimated for the year 2100. The authors should thus refer to their obtained pCO₂ rather than to their target pCO₂.

Reply 3) We agree with the suggestion. We changed the manuscript using the actual obtained pCO₂ according to this suggestion. We added to the method section and result section: "The three target pCO₂ levels were not reached and differ strongly from glacial (180 ppm) and future scenario (780 ppm). Therefore, we re-defined the three pCO₂ level as low (median 315 μatm), medium (median 353 μatm) and high (median 548 μatm) pCO₂ treatments in the course of the manuscript."

4) As the CO₂ aeration was not sufficient, the carbonate chemistry might have been altered additionally by cellular carbon uptake. The authors state that the treatments were different in respect to carbonate chemistry throughout the study. This is true when comparing the acclimations at the same date, however, some acclimations clearly have a similar carbonate chemistry i.e. when looking at calculated pCO₂ from the "380" culture at date 03/29 compared to the "750" culture at date 04/01 or "750 – date 04/13" and "180- date 03-29". The authors also state (page 2498 line 18-19) – "Hence, our approach reproduces the projected change in parameters of the carbonate system expected for the year 2100 by altering DIC at constant TA." As the acclimation did not

C1621

reach a CO₂ concentration projected for the year 2100 this statement is not true. In the revised manuscript, the authors should clarify the whole section about carbonate chemistry manipulation.

Reply 4) We agree with this suggestion. We changed the carbon chemistry paragraph: The pCO₂ targets we aimed at (glacial scenario 180 μatm , present day scenario 380 μatm and future scenario 780 μatm) were not reached. We therefore define the treatments as low pCO₂, medium pCO₂ and high pCO₂ treatment which had a median of 316 μatm , 353 μatm and 549 μatm , respectively. We added on p 2491, line 11 "Ranges of calculated values for the single pCO₂ treatments were for the low treatment 248.5 μatm - 498.6 μatm with a median of 315.7 μatm , for the medium treatment 286.5 μatm - 571.1 μatm with a median of 353.3 μatm and for the high treatment 395.2 μatm - 630.4 μatm with a median of 548.8 μatm . We also changed page 2498 line 18-19 to "Our approach altered DIC at constant TA".

5) It is not clear which data the authors chose to calculate growth rate. It seems that the cultures entered stationary phase already at day 9 with a possible lag phase within the first days. The authors should add information on this in the method and result section.

Reply 5) Growth rates were calculated for the exponential growth phase of 9 days. We added this information to page 2495, line 10.

6) Cultures seemed to be limited by inorganic P most of the time, however, it cells might have been able to partially use DOP instead (POP was stable over the course of the bloom with no PO₄⁻ available, yet DOP decreased). The lower P per filament in the high pCO₂ cells as well as a lower DOP concentration at the end of the experiment in this culture suggest that high pCO₂ leads to a more efficient P usage as well as DOP uptake. The authors might elaborate on this.

Reply 6) Yes, this is true. Cultures were depleted in DIP most of the time and P turnover was more efficient in the high pCO₂ treatment. We can only indicate P cycling in Part I of three companion manuscript, as Part III which is currently in preparation will focus

C1622

on P components and their usage in correlation with pCO₂. We added on p 2497, line 11 “The simulative effect of high CO₂ disappeared during the time course of our experiment from day 9 onwards, along with a complete exhaustion of the inorganic P pool. Nevertheless, the DOP pool was exhausted to a greater proportion in the high pCO₂ treatment along with a higher P concentration per filament indicates a more efficient P usage under high pCO₂. Further investigation of the different DP components and P turnover will be discussed in a third companion manuscript (Unger et al. in prep).”

7)C and N₂ fixation (Fig 6) clearly indicate that the cells were in different growth phases (lag, exponential, stationary phase), as such using average values to calculate the C and N flow in Nodularia (Fig. 7) might introduce errors.

Reply 7) We agree with the reviewers comment. Also according to the critical comments of Reviewer# 2 regarding the model (comment 4 and 7), we decided to delete the model and instead compare production and stoichiometry of POM over the incubation time.

8)It is puzzling that the high pCO₂ cells have more Heterocysts between day 0 and day 3 compared to ambient and low pCO₂ cultures, yet N₂fixation does not reflect this morphological pattern. Please add a possible explanation for this.

Reply 8) This sentence is misleading. Heterocyst number was statistically higher in the high pCO₂ treatment on day 3. We rephrased this sentence to: “On day 3 there was a significant higher number of heterocysts per filament in the high pCO₂ treatment compared to the mid pCO₂ (p=0.005, n= 50) and to the low pCO₂ treatment (p= 0.0001, n= 50). “ Nitrogen fixation rates on day 3 were by a factor of 4 to 6 higher than measured on the other sampling days. This corresponds to an overall higher number of heterocysts per filament. Moreover, the morphological pattern corresponds to the increase in N₂ fixation on day 3 in correlation to pCO₂ with 28% higher N₂ fixation in the high pCO₂ treatment compared to the mid and by 53% compared to the low pCO₂ treatment.

C1623

References M. D. Iglesias-Rodriguez et al., Science 320, 336 (2008) U. Riebesell et al., Nature 407, 364 (2000) J. Unger, N. Wannicke, Endres, A. Engel, M. Voss M. Nausch (in prep.) Response of Nodularia spumigena to pCO₂- Part III- Turnover of different phosphorous compounds

Authors comment to Reviewer # 2

1)Carbonate chemistry: As already mentioned by two other comments in the open discussion, 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.

Reply 1) The three target pCO₂ levels were not reached and differ strongly from glacial and future scenario. Therefore, we re-defined the three pCO₂ level as low (180 ppm), medium (380 ppm) and high (780 ppm) pCO₂ treatments in the course of the manuscript.

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,

C1624

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.

Reply 2) We completely agree with the reviewers comment. We have re-calculated filament-based uptake rates to biomass-based ones. Normalisation was done to particulate organic carbon, taken from the measurement by flash-combustion and IRMS analysis. These values refer to the actual carbon and nitrogen biomass on a filter and do not rely on cell size conversions to biomass. Czerny et al. 2009 also presents abundance based uptake rates, although not normalised to filaments, but to individual cell number- these are also not biomass specific. Carbon specific uptake rates in this study were added to the result section, discussion and Figures (6).

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.

Reply 3) The debate whether to use the N₂ fixation measurement method according to Mohr et al. 2010 or Montoya et al. 1996 is still ongoing and was recently discussed in a workshop in Kiel, Germany with pros and cons for each one of them. We have used the Montoya method in our lab before and published results on Nodularia N₂ fixation and exudation rates with incubation periods of only 2 hour. According to Mohr's hypothesis, those N₂ uptake rates should theoretically be much too low and lead to imbalances in the calculated C and N uptake rates. However, we found no such imbalances in C and N build up (Wannicke et al. 2009). Moreover, a recent Diploma thesis in our

C1625

lab found a good agreement between the two methods (Fabian 2012). Although 6 hours seem to be on the lower limit of incubation time, ensuring sufficient ¹⁵N gas dissolution, as Mohr et al. 2010 presented in their paper, we would rather assume an underestimation of our N₂ fixation rates, as the reviewer also mentioned. However, we found an overestimation of N₂ fixation relative to the PON build-up, because N₂ fixation exceeded PON build up, not vice versa.

4) Nitrogen turnover model: The authors developed a model on daily carbon and nitrogen metabolism based solely 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.

Reply 4) We decided to delete this model to avoid misleading extrapolations. See also comment # 7.

The following points highlight interpretive caveats:

5)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

C1626

are the result of unclear descriptions. However, the authors should clear this up in a revised manuscript.

Reply 5) We suppose the assumed inconsistencies emerged due to unclear descriptions in the manuscript. However, we agree that extrapolation of rates measurements to daily rates are error-prone. Comparing these rates with DON and DIN measurements made for several days may be even more inaccurate. We have deleted the turnover model in order to avoid misleading extrapolations we cannot prove. Page 2501, line 17 we added "Still one has to keep in mind, that N₂ fixation was measured for a period of 6 hours at the sampling day, while accumulation of PON was determined for a longer period of 3 and 6 days, which might include errors in the rate extrapolation and makes it inadequate for comparison."

6) 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 an 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.

Reply 6) The 80% exudation rate was used to build up a simplified model of production and turnover as it might take place in the Baltic Sea in future times and is used to indicate implications as it is stated in the headline. We were not able to observe DON release in our study, mainly because of the sampling interval chosen which was too long. We added on page 2504 line 28 a calculation using 50% exudation and stated "although to date it is not known whether DON exudation will be affected by an increase in pCO₂ itself". As long as no detailed study is available on exudation showing lower or higher DOM release, we can only present estimated values.

7) The nitrogen turnover model: The established model and the corresponding figure

C1627

do not add any new insights/data to the manuscript and are not explained well. The authors could 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.

Reply 7) Similar to comment # 5, we have deleted the model and instead adapted the suggestion made here to combine the data in a simpler plot showing rates and molar ratios superimposed in one plot (Figure 7).

8) 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.

Reply 8) We consent with the reviewer. Biomass specific uptake rates were calculated and plotted instead of filament based rates (Fig. 6). We also replaced uptake rates in the statistical tests and in the result section.

9) 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.

Reply 9) We agree with this opinion and have deleted some of the statistics including the ANCOVA which was replaced by t-test or ANOVA. Instead, we discussed the shortening of filaments and heterocyst number in more detail on page 2497 line 11 ff.

The following section includes minor scientific and other comments: 10) 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.

C1628

Reply 10) We agree that this sentence is incorrect. It should refer to all of the fixed nitrogen. We change this sentence to: "Diazotrophical fixed nitrogen can be transferred to lower food web levels via the dissolved fraction (Ohlendorf et al., 2000) and to higher trophic levels by grazing directly on cyanobacteria or indirectly via the microbial loop (Engström-Ost et al., 2011)"

11) 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.

Reply 11) We changed this sentence to: "At present day an atmospheric partial pressure of CO₂ (pCO₂) of 380 ppm prevails being elevated by 27% compared to the pre-industrial times with 280 ppm."

12) p 2484, l 3: the correct reference should be Fu et al 2007, not 2008 (the 2008 paper reports on Crocosphaera)

Reply 12) Change done.

13) p 2484, l 5: see above, the Fu et al 2008 does not report on Trichodesmium

Reply 13) Change done. This reference was deleted.

14) 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: : . .

Reply 14) Change done. We re-phrased this sentences starting with: "In general, there is scarce knowledge on how. . ."

15) p2485, 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: : :"

Reply 15) Change done.

C1629

16) p 2485- 2486: the experimental set-up description could be written clearer

Reply 16) We have restructured the paragraph to make the experimental set-up clearer. We divided the experimental set-up into three parts: firstly, the preparation of aged seawater, secondly, culturing of the parent culture and it's acclimation to the pCO₂ treatments and thirdly, the actual experimental run.

17) p 2485, l 12 and 16: 100 and also 200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ 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.

Reply 17) We have added information on the general light intensity cyanobacteria would be experiencing in the Baltic Sea in summer time in comparison to our experimental light availability. "The chosen light intensity is rather low compared to light intensities cyanobacteria usually experiencing in summer time in the Baltic Sea with approximately 1000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. Still higher light intensities than 200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ were not achievable using the fluorescent lighting and would have led to an increase in experimental temperature."

18) p 2486, l 6 and 8: it is not clear which reference the authors are referring to

Reply 18) We changed this sentence to: "Three pCO₂ treatment targets were selected to achieve 180 μatm representing glacial conditions, 380 μatm representing present day and 780 μatm representing year 2100 conditions (Boer et al., 2000)."

19) p 2488, l 23: indicate whether the samples were stored frozen or in a refrigerator

Reply 19) We added: "...stored at 2-5°C in a refrigerator."

20) 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?

Reply 20) Incubation started around 9 am and lasted till around 3 pm. This covers

C1630

maximum C fixation and is a period of steepest N₂ fixation increase during the day (See Wannicke et al 2009, "Release of fixed N₂ and C as dissolved compounds by *Trichodesmium erythreum* and *Nodularia spumigena* under the influence of high light and high nutrient (P)" *Aquat Microb Ecol*, Vol. 57: 175–189, 2009)

21) p 2490, l 14-19: The authors may elaborate details about the calculation of their model.

Reply 21) See answer# 5 and #7) We have deleted the model.

22) p 2492, section 3.3: This section should be clarified, some sentences jump from DOC to DON and back to DOC, for example.

Reply 22) We have clarified this section, considering DOC, DON and DOP consecutively.

23) p 2493, l10: Tukey's test instead of Turkey's test, change throughout manuscript

Reply 23) Change done.

24) p 2493, l 16: "this" not "his"

Reply 34) Change done

25) 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 the volumes is different. This refers back to using biomass-specific rates rather than filament-based or bottle-based rates.

Reply 25) We replaced volumetric and filament based uptake rates by biomass specific rates (based on POC).

26) p 2496, section 3.5: The calculations which are used for the model do not seem clear in this section.

Reply 26) See authors comment #5 and #7.

C1631

27) 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.

Reply 27) We also have added the error estimates to the percentage here for the recalculated biomass specific uptake rates and deleted the sentence page 2497, line 3/4, because of its weakness. Also percentage differences for growth rates were corrected. Growth rates calculated for the days 0 to 9 were highest in the high pCO₂ scenario ($508 \pm 89 \mu\text{atm}$) being elevated by $40 \pm 25 \%$ relative to the mid pCO₂ scenario ($398 \pm 104 \mu\text{atm}$) ($p \leq 0.05$, $n=12$) and by even $84 \pm 38 \%$ relative to the low pCO₂ treatment ($340 \pm 80 \mu\text{atm}$) ($p=0.001$, $n=12$). In the same time period (0-9 days) biomass specific C fixation in the high pCO₂ treatment increased by $9 \pm 44 \%$ compared to the mid pCO₂ scenario and by $60 \pm 60 \%$ relative to the low pCO₂ scenario with high standard deviations due to an increase in uptake from day 0 to 3 and a decrease in rates again at day 9. N₂ fixation in the high pCO₂ treatment relative to the mid and low scenario was elevated by $67 \pm 50\%$ and $93 \pm 35\%$, respectively.

28) p 2505, l 4: diazotrophic cyanobacteria do not rely on dissolved inorganic nitrogen sources (except N₂ gas which is usually excluded from this term)

Reply 28) We added to this line to clarify: "Since diazotrophic cyanobacteria can exploit elemental N₂, as well as organic N sources, they do not solely rely on dissolved inorganic nitrogen sources like nitrate and ammonia."

29) Table 1: These authors should state whether the samples were taken before or after the daily aeration of the culture vessels.

Reply 29) We added on p 2486, line 28: "Samples were taken between 8 and 9 am, before daily aeration." Daily aeration took place at 2 pm.

30) Figure 5: symbols are all circles and not triangles, squares and diamonds, please

C1632

change to either those symbols or change the legend.

Reply 30) We changed the legend to the actual symbols (circles).

31) The authors often use the words “therefore” and “henceforward” in a wrong context.

Reply 31) In order to prevent misunderstandings, we deleted or replaced these adverbs. p 2484, line 24: we changed to “Hence” p 2486, line 14: We deleted “Therefore” p 2492, line 11: We changed “Henceforward” to “From thereon” p 2493, line 23: We changed “Henceforward” to “From thereon”

References

Jenny Fabian. Growth and production of a Baltic Sea cyanobacteria bloom in relation to pCO₂. 2012 Diploma thesis, University of Rostock Mohr, W., Wallace, D. W. R., Grosskopf, T., and LaRoche, J.: Methodological underestimation of oceanic nitrogen fixation rates, PLoS ONE, 5, e12583, 2010. Wannicke, N., Koch, B., and M, V.: Release of fixed N₂ and C as dissolved compounds by *Trichodesmium erythreum* and *Nodularia spumigena* under the influence of high light and high nutrient (P), *Aquat. Microb. Ecol.*, 57, 175-189, 2009.

Authors comment to Czerny

1) In the Czerny et al. study, low density semi-continuous batch cultures were grown in artificial YBC II media providing inorganic phosphorus, vitamins and trace metals in surplus. This approach offers a high level of reproducibility and measured effects can be attributed to CO₂ / pH treatments with a high certainty. However, investigating only exponential growth, results can at best be applied to natural pre-bloom conditions when growth is not limited by nutrients and cannot be applied to high density bloom situations under multiple growth limitation.

Reply 1) We agree with Czerny that investigating only exponential growth has some constraints when extrapolating the obtained results to field situations. However, we needed to refer to this study in detail because it is the only one using heterocystous

C1633

cyanobacteria. We aimed, to detect general patterns, disregarding influencing factors when possible. Using aged seawater like in our study and conditions cyanobacteria might experience in the field (temperature, low DIP conditions, except light, which was too low in our study compared to summer situations) with very low supply of DIP reflects field situations. Overall, we did not, attempted to investigate a high density bloom situations under multiple growth limitation. To do so, we would rather apply a field sampling approach using a multiple specie set-up.

2) Experiments simulating such conditions, like the present study, are urgently needed, but are technically more difficult to control. A more sophisticated experimental setup (chemostat) instead of simple batch cultures is generally recommended when CO₂ effects are studied under nutrient limiting conditions. In batch cultures, the physiological state of the stock culture can have substantial influence on the lag phase after inoculation.

Reply 2) We agree that a chemostat set up is more useful when studying cultures which are nutrient depleted. Since our study was done with a large group of scientists requiring large volumes we could not use chemostats. Moreover, to keep the Chemostat set up axenic is quite difficult over 15 days. In Addition, cultures do not exude DOM under chemostat conditions. A batch set-up would have been necessary either way to monitor DOM development. Cultures were acclimated to the experimental pCO₂, as well as all other treatment bottles filled with aged seawater. The parent culture was in a stationary growth phase when beginning the acclimation period.

3) An acclimation of batch cultures to treatment conditions by a minimum of 5 generations at exponential growth should therefore be standard procedure in culture experiments. General guidelines for culture experiments are given in LaRoche et al. (2011) and work cited herein. It appears that the pre-culture media used in Wannicke et al. were not equilibrated with gas mixtures prior to inoculation, but were only bubbled during the three days of acclimation. It is important to know when and if treatment levels were established in the pre-cultures so that found results can be classified as short

C1634

term or long term response.

Reply 3) The comment is quite correct. However, we did not attempt to reproduce long-term response as our investigation time of 15 days was short. Equilibration with our target pCO₂ did not work during the experimental time, due to the biological production and consumption of CO₂. Nevertheless, as Reviewer # 2 already stated in comment #1 “Despite this discrepancy, the observation that growth (C and N fixation) differs with the actual/obtained CO₂ concentrations “. We have corrected the Method and Results section regarding our obtained versus the target pCO₂ values, but the actual tendency in stimulation at higher pCO₂ still is statistically significant.

4)Information about phosphate concentration, pCO₂, pH and growth rate of the pre-cultures is missing. Experimental batch cultures were started with low amounts of inorganic phosphorus. After three days, initially supplied phosphate (0.35mol kg⁻¹) was obviously depleted and therefore another 0.35mol kg⁻¹ of phosphate was added. Overall, growth rates in this experiment were low (0.1-0.2 d⁻¹) relative to *Nodularia* cultures growing under nutrient replete conditions (=0.319 /d-1 in Wannicke et al., 2009); 0.3-0.5 d⁻¹ in Czerny et al., 2009). It is not reported whether or when cultures entered the stationary growth phase. Moreover, it should be mentioned which cultures were already in a decaying state (indicated by decreasing cell numbers P 2493 L 9) during the last phase and whether they are included in the analyses.

Reply 4) We have added the requested information regarding the parent culture and pre-culturing in subsection 2.1. We added: “One week before the start of the acclimation period, the parent culture was still in a stationary growth phase and removed from the walk-in incubation chamber to a climate controlled room.” And:“ Thirdly, after three days of acclimation, chlorophyll a concentrations were determined in the three pre-cultures, with 28 μg chl a l⁻¹ in the low pCO₂ treatment, 27 μg chl a l⁻¹ in the mid and 38 μg chl a l⁻¹ in the high pCO₂ scenario in order to inoculate the same quantity to each following replicate bottle. The calculated pCO₂ (from CT and pH) in these pre-cultures were 402 μatm, 422 μatm and 548 μatm for the low, mid and high pCO₂

C1635

treatment, respectively.” It is self-explanatory that growth rates under nutrient depleted conditions do not reach growth rates obtained in studies using a full growth media like F/2 (Wannicke et al 2009) and YBCII with still repleted P conditions of 5 μM (Czerny et al 2009). Looking at the change in abundance it is visible that cells had a lag phase of 3 days and reached stationary growth after 15 days. Filament abundance then entered a stationary phase and considering standard deviation of abundance there was no decaying phase with significant reduction in abundance at day 15. Growth rate determination only included exponential growth phase from day 0 to day 9- see page 2495, line 10.

5)Another issue of the present study is the range, precision and documentation of the pCO₂ and pH treatment levels. The “Guide to best practices for ocean acidification research and data reporting” is referenced by Wannicke et al. for the choice of gas mixtures used to manipulate carbonate chemistry. Supplied CO₂ mixing ratios 180, 380, and 750 ppm are used as treatment levels by Wannicke et al., but measured values (Table 1) range between 480 and 590 atm CO₂ at the start of the experiment. Obviously, equilibration was not achieved after the three days of bubbling. Pure CO₂, gas mixtures or acid can be used to adjust CO₂ treatments (Schulz et al., 2009), but it is mandatory to refer to achieved treatment levels when describing measured responses. It has to be stressed that treatment range is overall small and not directly comparable to present summer conditions in the Baltic (Thomas and Schneider, 1999) or to CO₂ scenarios by the IPCC (Meehl et al., 2007). As common summer surface pCO₂ concentrations in the Baltic Sea are usually below the applied concentrations, it is also misleading to refer to the treatments as “past”, “present” and “future”.

Reply 5) We agree with this comment. We have changed the carbon chemistry section according to the suggestion. We changed past, present and future pCO₂ treatments to low, mid and high pCO₂ treatments and added median and mean value of the actual pCO₂ values we obtained in each treatment bottle. See also author comment to Reviewer# 1, comments #3 and #4.

C1636

6) Determining carbonate chemistry equilibrium using potentiometric pH measurement on the NBS scale results in high measurement uncertainty (Dickson, 2011). Therefore, it should be stated in the manuscript whether standard deviations presented in Table 1 are measurement uncertainties or differences in equilibration of the treatment replicates.

Reply 6) As indicated in the table caption the standard deviations represent the differences in equilibration of the treatment replicates.

7) If replicate bottles considerably differ in pCO₂ / treatments overlap, it would probably be better to use regression analyses evaluating each experimental unit at its individual treatment level instead of using ANCOVA, requiring replication of treatments.

Reply 7) In the original manuscript multiple regression analysis was already applied page 2490, line 25 & 26. ANCOVA was deleted in the manuscript, as it was correctly mentioned by Czerny et al. using an ANCOVA would ignore the actual pCO₂ level. We used t-test or ANOVA to compare mean values between the three set-ups.

References Czerny, J., Ramos, J. B. e., and Riebesell, U.: Influence of elevated CO₂ concentrations on cell division and nitrogen fixation rates in the bloom-forming cyanobacterium *Nodularia spumigena*, *BG.*, 6, 1865-1875, 2009. Wannicke, N., Koch, B., and M, V.: Release of fixed N₂ and C as dissolved compounds by *Trichodesmium erythreum* and *Nodularia spumigena* under the influence of high light and high nutrient (P), *Aquat. Microb. Ecol.*, 57, 175-189, 2009.

Please also note the supplement to this comment:

<http://www.biogeosciences-discuss.net/9/C1619/2012/bgd-9-C1619-2012-supplement.pdf>

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