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**Biogeosciences Discussions**

# *Interactive comment on* "Influence of elevated $CO_2$ concentrations on cell division and nitrogen fixation rates in the bloom-forming cyanobacterium *Nodularia spumigena*" *by* J. Czerny et al.

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Thank you very much for the many constructive remarks on our manuscript that helped us to improve it substantially.

1. The statement that the surface ocean absorbs 1/4 of current CO2 emissions is not referenced (naturally, since references aren't typically included in an abstract), but to my knowledge this is still a relatively uncertain number and somewhat controversial. Maybe it would be better to start out by simply saying "As CO2 emitted to the atmo-



sphere from human activities dissolves in seawater, it reacts: : :"?

The quantitative statement concerning the oceanic carbon uptake has been erased from the abstract.

2. To comment on page 4282: Isn't Nodularia just one member of a multiple species cyanobacterial consortium that blooms in the Baltic? For readers not intimately acquainted with Baltic cyanobacterial blooms, perhaps this could be mentioned here, along with some of the other co-occurring groups (Anabaena is mentioned in this regard in the discussion). Is Nodularia spumigena the dominant one most of the time? This paragraph implies this but doesn't come right out and say it.

Nodularia often dominates Baltic Sea cyanobacterial blooms and is the most conspicuous of the contributing cyanobacteria as it is the first to accumulate on the surface in periods of calm weather. Anabaena is mostly found inside Nodularia aggregations together with many other heterotrophic and autotrophic eukaryotes. Aphanizomenon forms rather "clean" aggregates, (similar to the tuft type of aggregations formed by Trichodesmium) that are usually more dispersed in the upper water column than Nodularia. Information concerning Anabaena and Aphanizomenon was added to the text.

3. To comment on page 4283: The reason that the cultures were grown in a manner intended to avoid aggregation is well described and justified. Clearly though, as they imply here and in the discussion, a dense surface aggregation might have a completely different response to changing pCO2 due to "microclimate effects". Since both aggregated and dispersed growth seem to be features of this organism's life cycle, it would be especially interesting to do the same experiments with both. At any rate, it is obviously necessary to qualify all of the results obtained here as applying specifically to homogenously mixed cells, and recognize that the story for aggregated surface blooms might be quite different.

We tried to make it clearer that the results can be in situ especially applied to dispersed filaments in a well mixed pre-bloom situation. The pH/[CO2] related physiology

of Nodularia, however, should be described quite well in this study as the setup provides very constant controlled conditions for the cells. With the same physiological equipment Nodularia has to meet the strongly varying chemical demands inside aggregations. We agree that a cross check of this study using a setup where filaments are allowed to aggregate appears to be the logical next step to assess the full ecological relevance of our results. However, for reasons outlined below a culture experiment of this kind is likely to deliver ambiguous results that may prove difficult to be extrapolated to in situ conditions. A: The quantitative sampling methods used here are prone to errors when the biomass contribution is as patchy as in an aggregated Nodularia culture. B: The microclimate conditions a Nodularia culture is exposed to in a stagnating culture bottle are not comparable to natural microclimate conditions. C: Aggregations formed by cultured Nodularia show little similarities to natural aggregations and may differ strongly in there physical properties. D: The microclimate inside an aggregation is characterised by strong chemical inhomogeneity (Ploug, 2008) that makes it impossible to correlated measured variables to a chemical parameter. We are currently working on an alternative approach to elucidate the role of microenvironments in the interaction of filamentous cyanobacteria with their physico-chemical surrounding. A shorter version of this discussion is now included in the manuscript.

4. To comment on M&M page 4283: For a species that often blooms right at the surface, 85 $\mu$mol photons m-2 sec-1 seems like a fairly low irradiance. Is anything known about saturating light levels for growth of this isolate, and is it possible that the cultures were light-limited to a greater or lesser degree?

Saturating light levels are not determined for this isolate of Nodularia spumigena. Nodularia can survive more than 2000 $\mu$mol photons m-2 s-1 when it accumulates at the surface. But in a pre-bloom situation, 85 $\mu$mol photons m-2 s-1 as a depth-integrated light intensity in the mixed upper water layer of the Baltic Sea represent a realistic average light level. As the light intensity is the same in all experimental units an effect of light limitation on the observed treatment differences can be excluded.



5. The authors chose to manipulate pCO2 using acid/base additions rather than bubbling. This is fine, but this text says that TAlk was measured at the start of the experiment, whereas the data and legend in Table 1 say it was measured at the end but not the beginning. This should be clarified, and perhaps something included in the text to specifically recognize that TAlk in acid manipulated seawater does not realistically mimic TAlk in gas-equilibrated seawater. It is also not completely clear whether any attempt was made to maintain the initial target pCO2 levels through further acid additions during the 7 day growth period of the experiment. It seems that perhaps this was not the case, and pCO2 was allowed to vary as the culture grew, but this is not entirely obvious from the text here.

The acid/base manipulation technique was chosen here because it is the most simple and precise way for CO2 manipulation in closed bottles without headspace. As the pCO2 range applied in this experiment is relatively small, the discrepancy in carbon ion speciation compared to a method where DIC is changed while TAlk is kept constant is negligible. The change in [HCO3-] is the only difference between the two manipulation approaches. As [HCO3-] shows a relative small change compared to its total concentration and is available in surplus in all experimental units, there should not be an effect of [HCO3-] on the measured trends. We measured DIC and alkalinity from the batch of artificial seawater that was prepared for the experiment. Based on these measurements we manipulated TAlk to reach the target pCO2/pH treatments. The bottles were kept closed during the whole 7 days of incubation, during which [CO2] drawdown by photosynthesis, as it appears during natural bloom development, was allowed. In the end of the experiment DIC and TAlk was measured from every experimental unit. The mean of the DIC values measured at the beginning and in the end together with the TAlk measured in the end were used to calculate the mean treatment pCO2/pH conditions in the single experimental units. We made this clear in the text.

6. Results, page 4288: A minor comment- I would suggest that the chlorophyll a results in Fig 1b be referred to on the previous page, when presenting the rest of figure

1, rather than here.

The order of the presented results was changed accordingly.

7. Results, page 4288: The rates of cellular carbon and production calculated from cell quotas and growth rates need to be qualified as being net production rates. Calculations from changes in cell number can't account for carbon lost to respiration and exudation, of course.

Production rates are now qualified as net production rates.

8. Results, page 4288: If cell carbon and phosphorus quotas increase by a third or so at high pCO2, but cell volume is unchanged, doesn't this imply a quite substantial increase in cellular density? How else can you have cells of the same size, but containing a lot more C and P? This result is an odd one- are there precedents in the literature for this? Maybe this puzzling observation deserves some consideration in the discussion section.

Yes this is right. As there was no measurable change in cell size there has to be a substantial increase in cell density. Intracellular pools of carbohydrates i.e. cyanophycin are known to affect density and thus regulate buoyancy of Baltic cyanobacteria as well as Trichodesmium. Examples are reviewed by (Sellner 1997; Allen 1984). There should be an effect on cell density by the treatment. Unfortunately, we did not directly measure cell density or determine storage substances. The implication on cell density is now included in the discussion.

9. Discussion, page 4290: The text here says the "accumulation of cellular nitrogen was less pronounced". Actually, there was no significant increase in the cellular N quota at all, correct?

Yes correct. This was indeed incorrectly formulated. It was changed in the revised version of the manuscript.

10. Discussion, page 4290: I like the explanation that this stoichiometry effect could be



due to reduced N transfer from the heterocysts to the vegetative cells, and a good case for the possible pH sensitivity of this process is made on the next page. However, I can think of another possible explanation for increases in C and P but not in N in the cells. Could there have been an enhanced loss of fixed N as exuded material (ammonium or possibly DON) at high pCO2? Some of our results from Hutchins et al 2007 suggested this for Trichodesmium, at least indirectly through comparisons of N fixation measured by acetylene reduction ("gross" rates) and 15N assimilation ("net" rates). This seems like a possible alternate explanation to the hypothesis presented here about altered N transfer from heterocysts.

An enhanced loss of fixed N was actually part of our hypothesis of defective transfer of amino acids. We now try to make it more explicit in the text.

11. Discussion, page 4294: The obvious question arises, are the trends observed in this study general among heterocystous cyanobacteria? Apparently the authors have a paper in prep on Anabaena, of which they say somewhat obscurely "there will be a different reaction to rising CO2". Without stealing the whole story from this upcoming paper, can't the authors here come out and say whether Anabaena is stimulated by higher pCO2 or not? It is not too useful to readers to simply imply coyly that there is another response among similar species, without even generally indicating what it is. If Anabaena does show a different (positive?) reaction to increasing pCO2, doesn't this potentially argue against the pH effects on heterocyst/vegetative N transfer model they discuss extensively earlier in the text? If this model is correct, wouldn't all heterocystous cyanobacteria exhibit the same response?

We apologize for the confusion. Since the last version of this manuscript the results for the CO2 effect on Anabeana could be extended by further experiments and now allow a clearer statement. The section is now changed in the text to: "For Anabaena, a cyanobacterium often found together with Nodularia, a fertilising effect of elevated [CO2] could also not be found whereas Anabeana reacted with different shifts in elemental composition than Nodularia (Franz et al., in prep.)." For Anabeana this study

also finds a decrease in nitrogen fixation rates, and an increase in cellular C/N elemental ratios, while P/C ratios reacted different to the CO2 treatment than in the present study. The dataset will be available on the database PANGEA as soon as the paper is published.

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Interactive comment on Biogeosciences Discuss., 6, 4279, 2009.