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

**Anonymous Referee #1**

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The manuscript “Response of *Nodularia spumigena* to  $p\text{CO}_2$  – Part I: Growth, production and nitrogen cycling” by Wannicke et al. provides data on the ecophysiological response of the cyanobacteria *Nodularia spumigena* which has an important role in primary production in the Baltic Sea. The present study investigates the sensitivity of this phytoplankton to changes in  $p\text{CO}_2$ , with the aim to project the data to potential implications for future increasing atmospheric  $\text{CO}_2$  levels and climate change. This work is a valuable addition to a study by Czerny et al (2009) who in contrast found a detrimental effect of enhanced  $p\text{CO}_2$  to growth and  $\text{N}_2$  fixation for *Nodularia*. The authors refer to this study and partly debate the discrepancies in the discussion part (page 2498 line 4-20). Parts of this discrepancy can be related to some methodical differences such as light intensity (as discussed in the manuscript) but also differences in growth media and phosphorus concentrations. Also the shaking of the bottles might influence the results.

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While Czerny et al. used a semi continuous batch approach with continuous shaking of the cultures this study investigated the cellular response over the duration of a bloom a so-called batch culture approach (with shaking and bubbling once per day) including data points from the stationary phase. The shaking will influence the cellular boundary layer and thus affect nutrient availability which might explain parts of the differences. The authors suggest that the technique to control the carbonate chemistry might affect the results (acid/ base in Czerny et al or CO<sub>2</sub> 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 pCO<sub>2</sub> effects in this organism which the authors did not account for. The effect of light intensity in the culture is also mentioned to be responsible for the stimulatory effect on growth and N<sub>2</sub> fixation referring to a study by Kranz et al 2011. However, high light diminished the stimulatory effect of pCO<sub>2</sub> for *Trichodesmium* in Kranz et al and thus the differences between Czerny and Wannicke et al. cannot be explained by this. 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 pCO<sub>2</sub> at day 0 is above ambient pCO<sub>2</sub> concentrations. 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 pCO<sub>2</sub> 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 pCO<sub>2</sub> as estimated for the year 2100. The authors should thus refer to their obtained pCO<sub>2</sub> rather than to their target pCO<sub>2</sub>. As the CO<sub>2</sub> 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 pCO<sub>2</sub> from the “380” culture at date 03/29 compared to the “750” culture at date 04/01

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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 reach a CO<sub>2</sub> 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. 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. 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 PO<sub>4</sub><sup>-</sup> available, yet DOP decreased). The lower P per filament in the high pCO<sub>2</sub> cells as well as a lower DOP concentration at the end of the experiment in this culture suggest that high pCO<sub>2</sub> leads to a more efficient P usage as well as DOP uptake. The authors might elaborate on this. C and N<sub>2</sub> 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. It is puzzling that the high pCO<sub>2</sub> cells have more Heterocysts between day 0 and day 3 compared to ambient and low pCO<sub>2</sub> cultures, yet N<sub>2</sub>fixation does not reflect this morphological pattern. Please add a possible explanation for this.

Karin Wesslander, Anders Omstedt, Bernd Schneider. (2010) Inter-annual and seasonal variations in the air–sea CO<sub>2</sub> balance in the central Baltic Sea and the Kattegat, *Continental Shelf Research*, 30 (14), pp.

Hoppe, C., Langer, G. and Rost, B. (2011) *Emiliania huxleyi* shows identical responses to elevated pCO<sub>2</sub> in TA and DIC manipulations, *Journal of Experimental Marine Biology and Ecology*, 406 (1), pp. 54-62.

Kranz, S., Levitan, O., Richter, K., Prasil, O., Berman-Frank, I. and Rost, B. (2010) Combined effects of CO<sub>2</sub> and light on the N<sub>2</sub> fixing cyanobacterium *Trichodesmium*

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IMS101: Physiological responses , Plant Physiology, 154 , pp. 334-345 .

Czerny, J., Barcelos e Ramos, J., and Riebesell, U. (2009) Influence of elevated CO<sub>2</sub> concentrations on cell division and nitrogen fixation rates in the bloom-forming cyanobacterium *Nodularia spumigena*, *Biogeosciences*, 6, 10.5194/bg-6-1865-2009

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