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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.

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The approach of the experiment presented by Wannicke et al. investigating the effect of future elevated CO2 concentrations on growth of Nodularia under phosphorus limited conditions can be seen as a reasonable continuation of the experiment presented by Czerny et al. (2009). In Czerny et al. (2009), cellular division and nitrogen fixation rates were found to decrease in response to increasing CO2. With carbon and phosphorus cellular uptake rates being unaffected, lowered division rates resulted in enhanced intracellular accumulation of these elements. 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 treat-

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ments 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. 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. 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 term or long term response. 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 (0.35μmol kg-1) was obviously depleted and therefore another 0.35μmol 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 (μ=0.319 /d-1 in Wannicke et al., 2009); $\mu\sim$ 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. 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

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180, 380, and 750 ppm are used as treatment levels by Wannicke et al., but measured values (Table 1) range between ~480 and ~590 μatm 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 IPPC (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". 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. 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. Presented data can hardly be compared to results of previous studies without appropriate reference to the physiological state of the cultures and the actual treatment CO2 levels.

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