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Response of *Nodularia spumigena* to pCO_2 – Part 1: Growth, production and nitrogen cycling

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Abstract. Heterocystous cyanobacteria of the genus *Nodularia* form extensive blooms in the Baltic Sea and contribute substantially to the total annual primary production. Moreover, they dispense a large fraction of new nitrogen to the ecosystem when inorganic nitrogen concentration in summer is low. Thus, it is of ecological importance to know how *Nodularia* will react to future environmental changes, in particular to increasing carbon dioxide (CO_2) concentrations and what consequences there might arise for cycling of organic matter in the Baltic Sea. Here, we determined carbon (C) and dinitrogen (N_2) fixation rates, growth, elemental stoichiometry of particulate organic matter and nitrogen turnover in batch cultures of the heterocystous cyanobacterium *Nodularia spumigena* under low (median 315 µatm), mid (median 353 µatm), and high (median 548 µatm) CO_2 concentrations. Our results demonstrate an overall stimulating effect of rising pCO_2 on C and N_2 fixation, as well as on cell growth. An increase in pCO_2 during incubation days 0 to 9 resulted in an elevation in growth rate by 84 ± 38 % (low vs. high pCO_2) and 40 ± 25 % (mid vs. high pCO_2), as well as in N_2 fixation by 93 ± 35 % and 38 ± 1 %, respectively. C uptake rates showed high standard deviations within treatments and in between sampling days. Nevertheless, C fixation in the high pCO_2 treatment was elevated compared to the other two treatments by 97 % (high vs. low) and 44 % (high vs. mid) at day 0 and day 3, but this effect diminished afterwards. Additionally, elevation in carbon to nitrogen and nitrogen to phosphorus ratios of the particulate biomass formed (POC : POP and PON : POP) was observed at high pCO_2. Our findings suggest that rising pCO_2 stimulates the growth of heterocystous diazotrophic cyanobacteria, in a similar way as reported for the non-heterocystous diazotroph *Trichodesmium*. Implications for biogeochemical cycling and food web dynamics, as well as ecological and socio-economical aspects in the Baltic Sea are discussed.

1 Introduction

In summer, the heterocystous diazotrophic cyanobacteria of the genus *Nodularia* form extensive blooms in the open Baltic Sea with more than $200\,mg\,m^{-3}$ wet weight (Wasmund, 1997), along with cyanobacteria of the genus *Aphanizomenon*. These blooms are usually promoted by low nitrogen-to-phosphorus ratios in the surface waters (e.g. Niemistö et al., 1989; Nausch et al., 2008; Raateoja et al., 2011), exhibiting an average annually primary production rate of $\sim 21\,mol\,C\,m^{-2}\,yr^{-1}$ in the Baltic Proper (Wasmund et al., 2001b). The capacity of community N_2 fixation in the Baltic Sea is comparable to nitrogen inputs from the land and atmosphere (e.g. Larsson et al., 2001; Wasmund et al., 2001b, 2005b). Annual N_2 fixation rates during a moderate bloom in the Baltic Proper were averaged to 101–$263\,mmol\,N\,m^{-2}\,yr^{-1}$ (Wasmund et al., 2001a).

A significant fraction of the newly fixed nitrogen can be directly released by cyanobacteria, thereby dispensing 35 to 80 % of nitrogen into the surrounding environment (Wannicke et al., 2009; Ploug et al., 2011). Nitrogen fixed by

diazotrophs can be transferred to lower food web levels via dissolved organic matter (Ohlendieck et al., 2000) and to higher trophic levels by grazing directly on cyanobacteria or indirectly via the microbial loop (Engström-Ost et al., 2011). The extra load of nitrogen thus increases overall ecosystem productivity and meets 20 to 90 % of the nitrogen requirements for community primary production during summer blooms (Sörensson and Sahlsten, 1987; Larsson et al., 2001; Wasmund et al., 2005a). Aggregation and sedimentation of net primary production accounts for ∼ 30 up to 72 % of biomass loss from the upper mixed layer, which is a considerable proportion of the seasonal sinking flux (Lignell et al., 1993; Heiskanen and Leppänen, 1995). This process is supported by the formation of aggregates including *Nodularia* filaments (Engel et al., 2002).

To date, it is not well understood and barely investigated how future changes in climate caused by anthropogenic elevation of atmospheric CO_2 concentration will affect *Nodularia* performance and their potential to alter biogeochemical fluxes. At present, an atmospheric partial pressure of CO_2 (pCO_2) of 380 ppm prevails, elevated by 27 % compared to pre-industrial times of 280 ppm. This accounts for the highest levels since approximately half a million years (e.g. Lüthi et al., 2008). With atmospheric CO_2 dissolving in seawater, it is expected that current pCO_2 in the oceans will nearly double to 780 µatm by 2100, and lower the oceans' pH by about 0.35 units (IPCC, 2007), assuming that emissions will carry on at the present rate. This has severe implications for marine phytoplankton, as they appear to directly respond to increasing pCO_2 by altering their physiology (e.g. Riebesell et al., 2007), relative abundance (e.g. Tortell et al., 2002), and biogeography (e.g. Boyd and Doney, 2002). Additionally, unicellular marine cyanobacteria such as *Synechococcus* and *Prochlorococcus* can show species-specific responses to increasing pCO_2 (e.g. Fu et al., 2008). Several studies demonstrate that elevated pCO_2 supports C and N_2 fixation, as well as growth rates in the non-heterocyst diazotroph *Trichodesmium* (Hutchins et al., 2007; Levitan et al., 2007; Barcelos e Ramos et al., 2007). It has been hypothesized that these trends are facilitated by changes in activity of the carbon concentrating mechanism (CCM) and modified protein activity (Levitan et al., 2010a, b; Kranz et al., 2011) of the enzyme ribulose-1,5-bisphosphate carboxylase oxygenase (RUBISCO), resulting in a decrease of energy and nutrient demand of the cell at high pCO_2. The enzyme RUBISCO has a naturally low affinity to CO_2. Subsequently, energy saved can be relocated to other metabolic processes such as N_2 fixation. But experimental data so far are not able to prove this hypothesis on a gene expression level and no publication is available to verify this notion for heterocystous cyanobacteria.

In general, there is little knowledge on the response of heterocystous cyanobacteria to pCO_2. Czerny et al. (2009) directly addressed the effects of different pCO_2 conditions on growth and C fixation of the genus *Nodularia* and observed an overall detrimental effect of rising pCO_2 on the cells associated with a decrease in growth and production. They suggested that this pattern could be typical for heterocystous cyanobacteria compared to non-heterocystous cyanobacteria of the genus *Trichodesmium* and potentially relate to physiological and structural dissimilarities of both cyanobacteria groups. Even less information is available on the coupling of fluxes of carbon, nitrogen and phosphorus in relation to pCO_2 mediated by heterocystous cyanobacteria.

The purpose of this study was to examine the relationship between pCO_2 and diazotrophic growth of *Nodularia spumigena* and the related fluxes of carbon, nitrogen and phosphorus. Cultures of Baltic Sea *Nodularia spumigena* isolates were grown in a batch mode at three different pCO_2 levels (low, medium and high), which were supposed to simulating glacial (180 µatm), present day values (380 µatm) and values projected for the year 2100 (780 µatm), respectively. Here, we present data on growth and production parameters, as well as N_2 fixation and nitrogen turnover in response to increasing pCO_2. Carbon cycling and extracellular enzyme activities, as well as phosphorous cycling and utilization of dissolved organic phosphorous (DOP), will be presented in two companion publications (Endres et al., 2012; Unger et al., 2012).

2 Material and methods

2.1 Culture condition and design of the batch culture experiment

The experimental set-up we applied can be divided into three parts: firstly, the preparation of aged seawater, secondly, culturing of the parent culture and its acclimation to the pCO_2 treatments, and thirdly, inoculation with pre-acclimated cultures, amendment with DIP and the actual experimental run. The three individual steps taken are described in detail below and illustrated in Fig. 1.

The first part began with sampling of 1000 litre of seawater in the open Baltic Sea (54.22749° N, 12.1748° E, salinity of 9.1 psu), four months before the start of the experiment. Plankton growth was allowed for three months, resulting in aged sea water with low concentration in dissolved inorganic nutrients. After this growth phase, sterilisation was achieved by UV light treatment and 0.2 µm filtration under a clean bench. Concentrations of inorganic nutrients in this seawater (DIN and DIP) were below the detection limit.

The second part included the growth of three litre parent culture of the heterocystic cyanobacterium *N. spumigena*, which was isolated by L. Stal and coworkers (NIOO) from the Baltic Sea and maintained since 2000 at the Leibniz Institute for Baltic Sea Research in batch cultures in $f/2$ medium free of any combined inorganic N compounds. Three weeks prior to the start of the experiment, an axenic parent culture was transferred to sterile filtered aged

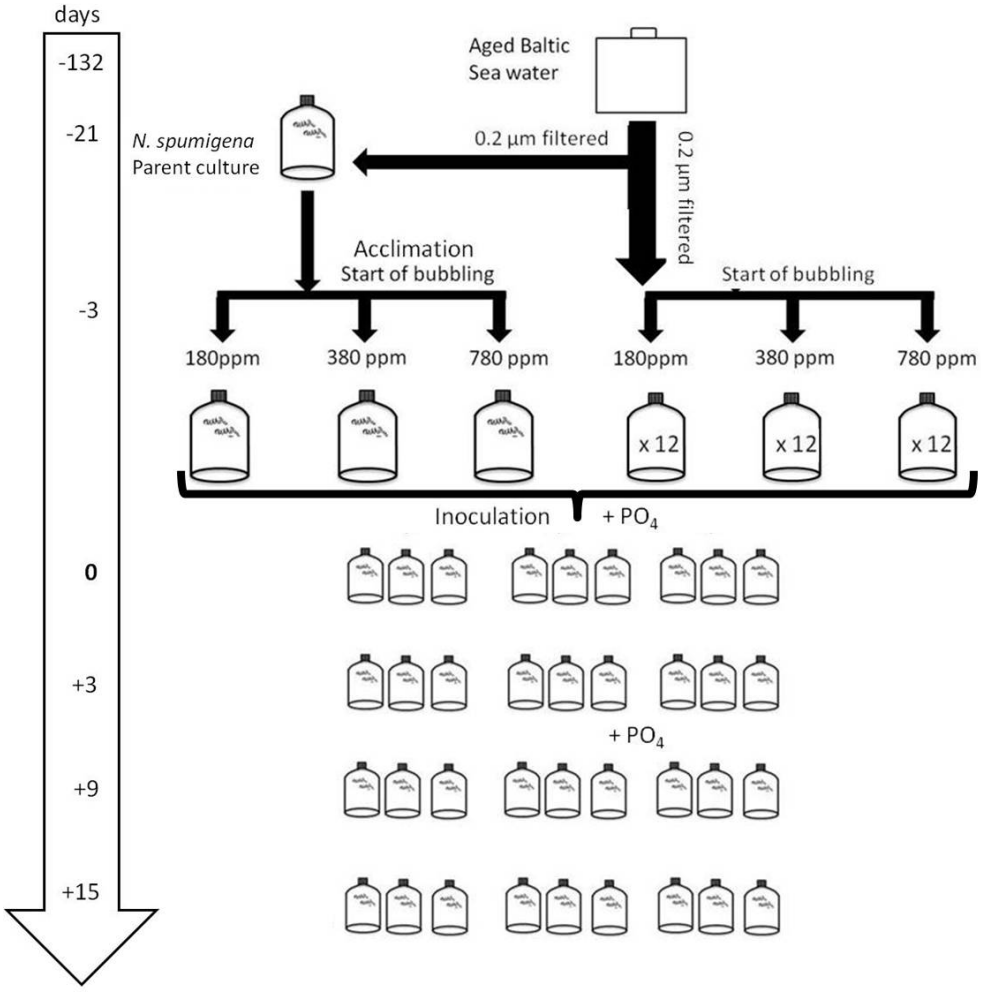

Fig. 1. Schematic overview of the experimental set-up and time flow of the single steps taken from preparation (−132 days) to the end of the experiment (+15 days). See text for detailed information.

Baltic Sea water amended with 0.3 µmol l^{-1} PO_4 every week to assure an exponential growth phase. The parent culture was cultured at 15 °C in a walk-in incubation chamber under 16 : 8 h light : dark cycle (cool, white fluorescent lighting, 100 µmol photons m^{-2} s^{-1}). 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. There, temperature was increased to 23 °C (representing typical summer temperatures at the Baltic Sea water surface) and light supply raised to 200 µmol photons m^{-2} s^{-1} (light: dark cycle of 16 : 8 h, cool, white fluorescent lighting). The chosen light intensity was rather low compared to light intensities experienced by cyanobacteria in summer time in the Baltic Sea (ca. 1000 µmol photons m^{-2} s^{-1}). Higher light intensities (> 200 µmol photons m^{-2} s^{-1}) were not achievable by using fluorescent light and would have led to an unwanted large increase in experimental temperature. Cultures were routinely mixed by manually shaking to prevent adhesion of cyanobacteria to the walls of the culture vessels.

Acclimation of the *N. spumigena* parent culture to the different pCO_2 level started three days before the beginning of the experiment. The parent culture was separated into three pre- cultures, one litre for each CO_2 treatment, and acclimated to the target pCO_2 by aeration with premixed gases (Linde gas). Three levels of pCO_2 were used, i.e. 180 µatm representing glacial conditions, 380 µatm representing present day and 780 µatm representing year 2100 conditions (Boer et al., 2000). During the course of the experiment (including the acclimation period; 18 days), aeration took place in the early afternoon (02:00 p.m.) for one hour per day in order to avoid continuously high turbulence that could potentially harm the integrity of the cyanobacteria filaments.

Aeration, however, was not sufficient to yield equilibration with the pre-mixed gases, i.e. the calculated pCO_2 (from CT and pH) values in the pre-cultures were 402 µatm, 422 µatm

and 548 µatm for the glacial, present-day and future pCO_2 treatments, respectively. For this reason, we re-defined the three pCO_2 levels based on the true pCO_2 determined during the experiment. These are the low pCO_2 treatment (median 315 µatm), medium pCO_2 treatments (median 398 µatm) and high pCO_2 treatment (median 548 µatm) pCO_2 treatment.

The third part started with the inoculation of each experimental bottle. After three days of pre-acclimation, chlorophyll *a* concentrations were determined in the three pre-cultures, yielding 28 µg chl *a* l^{-1} in the low pCO_2 treatment, 27 µg chl *a* l^{-1} in the mid and 38 µg chl *a* l^{-1} in the high pCO_2 scenario. In order to inoculate the same quantity, 0.8 µg chl *a* l^{-1}, we added 296 ml to replicate bottles of the low pCO_2 treatment, 308 ml to the mid pCO_2 treatment and 221 ml to replicate bottles of the high pCO_2 treatment. Heterotrophic bacteria cells counts at the start of the experiment were below the blank value of 1000 cells l^{-1}. Overall, bacterial biomass during the course of the experiments (18 d, 15 d + 3 days acclimation) never exceeded 1 % of cyanobacterial biomass.

After inoculation with *N. spumigena*, each of the 36 bottles was amended with phosphate to 0.35 µmol l^{-1} at time 0 and at day 3.

Four sampling time points were chosen for the three pCO_2 treatments (time 0, +3, +9 and +15 days, Fig. 1) with three replicate bottles harvested at each time point. Each bottle contained 10 litres of aged and sterile filtered seawater that had been aerated with premixed gases for three days in parallel with the pre-cultures. One replicate bottle of the low pCO_2 treatment at day 9 was omitted in the data compilation due to inaccurate inoculation with DIP. Samples were taken between 08:00 and 09:00 LT before daily aeration.

2.2 Analytical methods

2.2.1 Carbonate chemistry

The pH was measured with an electrode (Knick Mikroprozessor pH Meter 761 with Typ SE 100 glass electrode), calibrated directly before measurement with standard NBS buffer. Values of pH are given relative to the total scale.

Total carbon (C_T) was analysed directly after sampling using the colorimetric SOMMA system according to Johnson et al. (1993). The system was calibrated with carbon reference material provided by A. Dickson (University of California, San Diego) and yielded a precision of about ± 2 µmol kg^{-1}.

Total alkalinity and pCO_2 were calculated using CO2SYS (Lewis et al., 1998) parallel to C_T, pH, salinity and temperature.

2.2.2 Nutrient and chlorophyll *a* analysis

Dissolved inorganic nutrients (NH_4^+, NO_3^- and PO_4^{3-}) were determined colorimetrically from 60 ml filtered subsamples (combusted GF/F) using a spectrophotometer U 2000 (Hitachi-Europe GmbH, Krefeld, Germany) according to Grasshoff et al. (1983). The detection limits were 0.02 µmol l^{-1} for DIP, 0.05 µmol l^{-1} for ammonium and 0.05 µmol l^{-1} for NO_3. A subsample of 100 ml was filtered onto Whatman GF/F filters for chlorophyll *a* analysis, immediately after sampling. Filters were stored in liquid nitrogen or at −80 °C and were extracted with 96 % ethanol for at least 3 h. Chlorophyll *a* fluorescence was measured with a TURNER fluorometer (10-AU-005) at an excitation wavelength of 450 nm and an emission of 670 nm (HELCOM, 2005). Chlorophyll *a* concentrations were calculated according to the method of Jeffrey and Welschmeyer (1997).

2.2.3 *Nodularia* filament and bacteria cell counts

Subsamples of 50 ml were taken for phytoplankton analysis (preserved with acetic Lugol's (KI/I2) solution to 1 % fixation) and counted using an inverted microscope (Leica) (Utermöhl, 1958) at 100× magnification. Cell length and diameter were measured using a micrometer eyepiece and converted to biovolume assuming the geometrical approximation of a cylinder.

Bacteria were counted using a flow cytometer (Facs Calibur, Becton Dickinson) and the protocol of Gasol and del Giorgio (2000). Four ml samples were preserved with 100 µl formaldehyde (1 % v/v final concentration), shock frozen in liquid nitrogen and stored at −70 °C until measurement. A stock solution of SYBR GREEN (Molecular Probes) was prepared by dilution of 1 µl dye with 49 µl DMSO. Three µl potassium citrate solution, 10 µl of the dye stock solution and 10 µl fluoresbrite microspheres (Polysciences) were added to 300 µl of the thawed sample and incubated for 30 min in darkness. Cell counting was done at a medium flow rate and calculations were performed using the software program "Cell Quest Pro". Mean abundance of heterotrophic bacteria was $4.45 \pm 2.28 \times 10^5$ cells l^{-1} (low pCO_2), $2.38 \pm 2.09 \times 10^5$ cells l^{-1} (mid pCO_2) and $4.80 \pm 2.82 \times 10^5$ cells l^{-1} (high pCO_2).

2.2.4 Dissolved organic matter (DON, DOC, DOP)

For analysis of dissolved organic carbon (DOC) and total dissolved nitrogen (TDN), subsamples were filtered through pre-combusted GF/F filters, collected in 20 ml pre-combusted (8 h, 500 °C) glass ampoules, acidified with 80 µl of 85 % phosphoric acid and stored at 2–5 °C in a refrigerator. TDN and DOC concentrations were determined simultaneously by high temperature catalytic oxidation with a Shimadzu TOC-VCSH analyser. In the auto sampler, 18 ml of sample volume plus 9 ml of ultrapure (Type 1) water (in pre-combusted vials) were acidified with 50 µl HCl (1 M) and sparged with oxygen (150 ml min^{-1}) for 6 min to remove all inorganic C. 100 µl sample volume was injected directly on the catalyst (heated to 720 °C). Detection of the generated CO_2 was performed with an infrared detector. Final DOC

concentrations were average values of quadruplicate measurements. If the coefficient of variation exceeded 0.1 %, up to 4 additional analyses were performed and outliers were eliminated. Total N was quantified by a chemiluminescence detector (gas flow oxygen: 0.6 l min^{-1}). After every 8th sample, one standard for quality control and one blank was measured. Values of TDN were corrected for nitrate, and ammonium, and thereafter referred to as DON.

Subsamples (40 ml) for the determination of total (TP) and dissolved phosphorus (DP) were stored at −20 °C until processing either unfiltered (for TP) or filtered through pre-combusted (450 °C, 4 h) Whatman GF/F filters (for DP). Thawed samples were oxidized with an alkaline peroxodisulfate solution (Grasshoff et al., 1983) in a microwave (MarsXpress, CEM) to convert organic phosphorus into DIP. The subsequent DIP determination was done using a 10 cm-cuvette reducing the detection limit to 0.01 µmol l^{-1}. Dissolved organic phosphorus (DOP) was calculated as the difference between dissolved phosphorus (DP) and dissolved inorganic phosphorous (DIP), detected as described above.

2.2.5 Particulate organic matter analysis (PON, POC, POP)

Stable N and C isotope ratios (δ^{15}N-PON, δ^{13}C-POC), as well as PON and POC concentration were measured by means of flash combustion in a Carlo Erba EA 1108 at 1020 °C and a Thermo Finnigan Delta S mass-spectrometer. Filters containing particle samples were trimmed, sectioned and then loaded into tin capsules and pelletised for isotopic analysis. Particulate organic phosphorus (POP) was calculated as the difference between total and dissolved phosphorus.

2.2.6 Isotopic analysis and rates measurements (primary production, N_2 fixation)

The stable N and C isotope ratios measured for each sample were corrected for values obtained from standards with defined N and C isotopic compositions (International Atomic Energy Agency IAEA: IAEA-N1, IAEA-N2, NBS 22 and IAEA-CH-6) by means of mass balance. Values are reported relative to atmospheric N_2 (δ^{15}N) and VPDB (δ^{13}C-Vienna Peedee belemnite). The analytical precision for both stable isotope ratios was ± 0.2 ‰. Calibration material for C and N analysis was acetanilide (Merck). N_2 fixation activity was measured using the ^{15}N-N_2 assay, C fixation using the ^{13}C-$NaHCO_3$ assay. Tracer incubations were terminated by gentle vacuum filtration (< 200 mbar) through pre-combusted GF/F filters. These filters were dried at 60 °C and stored for isotopic analysis. Rates were calculated using the approach of Montoya et al. (1996). Incubation time for rate measurements was 6 h, guaranteeing a sufficient dissolution of the ^{15}N gas in the incubation bottle (method consideration, Mohr et al., 2010).

2.3 Statistical analysis

Statistical analyses were done using the software SPSS 9.0 and Sigma Plot 10. The effect of the pCO_2 treatment on biological and chemical variables was tested by analysis of variance of data (ANOVA, t-test). Dependencies of growth and production parameters from other environmental parameters were tested using Pearson's correlation "stepwise" multiple regression analysis. Prior to ANOVA and correlation analysis, data were tested for normality and homogeneity of variances using Wilk-Shapiro and Levene's tests. Linear regression analysis was applied to calculate growth rates from changes in natural logarithm transformed filament/cell numbers, PON, POC, as well as chlorophyll *a* values.

3 Results

3.1 Carbonate chemistry

Throughout the study, the pCO_2 treatments were different with respect to pH and total carbon (C_T), as well as calculated total alkalinity (A_T) and pCO_2 (Table 1). The pCO_2 treatments differed significantly in pH and C_T between mid and high pCO_2 treatment, as well as between the low and high pCO_2 treatment ($p \leq 0.001$, $n = 12$, Supplement Table S2). Ranges of calculated values for the single treatments were for the low pCO_2 treatment 248.5–498.6 µatm with a median of 315.7 µatm (mean value 340 ± 80 µatm), for the mid pCO_2 treatment 286.5–571.1 µatm with a median of 353.3 µatm (mean value 398 ± 104 µatm) and for the high pCO_2 treatment 395.2–630.4 µatm with a median of 548.8 µatm (mean value 508 ± 89 µatm). The large deviation in pCO_2 within the treatments occurred partly because CO_2 was consumed during photosynthesis. The calculated pCO_2 was significantly different between all three pCO_2 set-ups ($p \leq 0.001$, $n = 12$, Supplement Table S2).

3.2 Inorganic nutrients

There were no significant differences in concentrations of inorganic nutrients between pCO_2 treatments (Supplement Table S2). Dissolved inorganic phosphate was depleted in all treatments after three days of incubation (Table 2). DIP amended on day 3 was again below the detection limit at day 9. Throughout the experiment, mean concentration of dissolved inorganic nitrogen ($DIN = NO_3^- + NO_2$) was 0.26 ± 0.1 µmol l^{-1} in the low pCO_2 treatment, 0.13 ± 0.1 µmol l^{-1} in the mid pCO_2 treatment and 0.1 ± 0.1 µmol l^{-1} in the high pCO_2 treatment (Table 2), whereas ammonium was not detectable. Due to the uptake of nutrients during cell growth, an inverse relationship was observed between DIP and abundance, chlorophyll *a*, PON and POP ($R^2 = -0.567$, −0.686 and −0.599, 0.359, respectively, $p \leq 0.05$ and $p \leq 0.01$, $n = 12$, Supplement Table S1).

Table 1. Carbonate system variables for the four sampling time points. pH and total carbon (C_T) were measured, total alkalinity (A_T) and pCO_2 in seawater were calculated from pH and C_T using CO2SYS (Lewis et al., 1998). Values are means and standard deviations of three replicates (except one replicate bottle of the 180 ppm treatment at day 9). Samples were taken between 08:00 and 09:00 LT, after daily aeration.

Date	pCO_2 Target [µatm]	Incubation Time [d]	pH Total scale	C_T [µmol kg^{-1}]	A_T [µmol kg^{-1}]	pCO_2 [µatm]
29 March 2010	180	0	8.02 ± 0.02	1651.9 ± 8.9	1724.2 ± 6.4	472.9 ± 29.9
	380	0	7.95 ± 0.01	1656.8 ± 3.6	1713.4 ± 2.8	561.0 ± 8.7
	780	0	7.93 ± 0.03	1676.7 ± 2.7	1730.3 ± 2.6	590.7 ± 35.3
1 April 2010	180	3	8.16 ± 0.03	1607.4 ± 10.2	1709.7 ± 3.2	330.1 ± 26.3
	380	3	8.09 ± 0.03	1617.5 ± 11.4	1703.5 ± 3.5	388.8 ± 36.0
	780	3	7.94 ± 0.02	1667.9 ± 1.9	1720.5 ± 5.1	576.8 ± 23.3
7 April 2010	180	9	8.22 ± 0.04	1570.4 ± 14.6	1690.8 ± 5.0	276.7 ± 27.1
	380	9	8.18 ± 0.03	1581.1 ± 2.8	1690.9 ± 5.9	305.3 ± 20.3
	780	9	8.07 ± 0.02	1620.7 ± 5.3	1702.3 ± 3.8	412.2 ± 16.9
13 April 2010	180	15	8.20 ± 0.04	1568.0 ± 11.4	1683.3 ± 22.6	291.8 ± 29.3
	380	15	8.14 ± 0.01	1595.9 ± 4.9	1696.7 ± 2.0	339.6 ± 12.5
	780	15	8.03 ± 0.06	1626.9 ± 21.1	1702.2 ± 6.7	452.9 ± 82.2

Table 2. Abiotic and biotic variables for the four sampling time points. Values are means and standard deviations of three replicates (except one replicate bottle of the 180 ppm treatment at day 9).

Date	pCO_2 treatment	PO_4^{3-} [µM]	DIN [µmol l^{-1}]	Chlorophyll *a* [µg l^{-1}]	Bacterial abundance [10^5 l^{-1}]	DOC [µmol l^{-1}]	DON [µmol l^{-1}]	DOP [µmol l^{-1}]
29 March 2010	low	0.29 ± 0.02	0.22 ± 0.31	0.74 ± 0.08	4.31 ± 2.54	306.5 ± 18.6	16.02 ± 0.07	0.34 ± 0.04
	medium	0.32 ± 0.08	0.28 ± 0.16	0.87 ± 0.09	5.61 ± 2.22	295.6 ± 23.9	18.02 ± 1.17	0.33 ± 0.06
	high	0.34 ± 0.02	0.22 ± 0.02	0.71 ± 0.04	3.14 ± 0.22	330.1 ± 76.3	17.67 ± 1.74	0.38 ± 0.06
1 April 2010	low	0.05 ± 0.02	0.41 ± 0.35	3.04 ± 0.12	2.68 ± 0.82	270.6 ± 8.7	15.61 ± 0.01	0.27 ± 0.02
	medium	0.03 ± 0.01	0.24 ± 0.03	4.04 ± 0.79	4.95 ± 1.68	297.8 ± 8.5	15.35 ± 0.14	0.24 ± 0.02
	high	0.04 ± 0	0.22 ± 0.02	3.46 ± 0.07	4.33 ± 1.65	296.8 ± 1.6	16.18 ± 1.56	0.28 ± 0.07
7 April 2010	low	0.01 ± 0.01	0.28 ± 0.27	3.38 ± 2.27	5.14 ± 1.56	308.7 ± 19.6	15.44 ± 1.79	0.27 ± 0.08
	medium	0.02 ± 0.02	0.20 ± 0.12	5.15 ± 0.23	5.41 ± 2.39	319.9 ± 17.2	15.91 ± 0.82	0.25 ± 0.09
	high	0.01 ± 0	0.14 ± 0.15	7.27 ± 0.42	5.34 ± 3.85	322.6 ± 6.1	15.81 ± 0.44	0.23 ± 0.04
13 April 2010	low	0.03 ± 0.01	0.20 ± 0.04	1.49 ± 0.28	6.62 ± 3.22	318.7 ± 26.8	14.65 ± 0.74	0.24 ± 0.07
	medium	0.02 ± 0.01	0.21 ± 0.15	3.05 ± 0.45	2.20 ± 0.33	323.7 ± 16.4	15.13 ± 0.49	0.19 ± 0.02
	high	0.02 ± 0.01	0.10 ± 0	4.41 ± 1.09	6.10 ± 5.24	306.0 ± 15.8	16.79 ± 0.41	0.21 ± 0.04

3.3 Dissolved organic matter (DOM)

DOM concentration and stoichiometry did not differ significantly between the treatments.

DOC concentrations were 303 ± 26 µmol l^{-1} in the low pCO_2 treatment, 309 ± 21 µmol l^{-1} in the mid and 313 ± 36 µmol l^{-1} in the high pCO_2 treatment. During the first 3 days of the experiment, DOC concentration decreased in the low and high pCO_2 treatment by 35 and 33 µmol l^{-1}, respectively, while it increased in the mid pCO_2 treatment by 2 µmol l^{-1}. From thereon, until the end of the experiment at day 15, concentrations of DOC increased by 24 µmol l^{-1} in the low pCO_2 treatment, by 13 µmol l^{-1} in the mid pCO_2 treatment and by 5 µmol l^{-1} in the high pCO_2 treatment.

DON concentrations were 15 ± 1.0 µmol l^{-1} in the low, 16 ± 1.3 µmol l^{-1} in the mid and 17 ± 1.2 µmol l^{-1} in the high pCO_2 treatment. They were reduced in the low compared to high pCO_2 level, indicating a higher accumulation of DON at high pCO_2. From day 3 onwards, DON concentration decreased by 0.3 µmol l^{-1}, 0.11 µmol l^{-1} and 0.9 µmol l^{-1}, respectively. Nevertheless, it has to be kept in mind that calculated differences in concentration were of the same magnitude as standard deviation of the single measurements and have to be considered carefully. DON showed a significantly negative correlation with PON, POP and pH ($R^2 = -0.351$, -0.574 and -0.619, $p < 0.05$ and $p \leq 0.01$, $n = 12$, Supplement Table S1) and positive ones with C

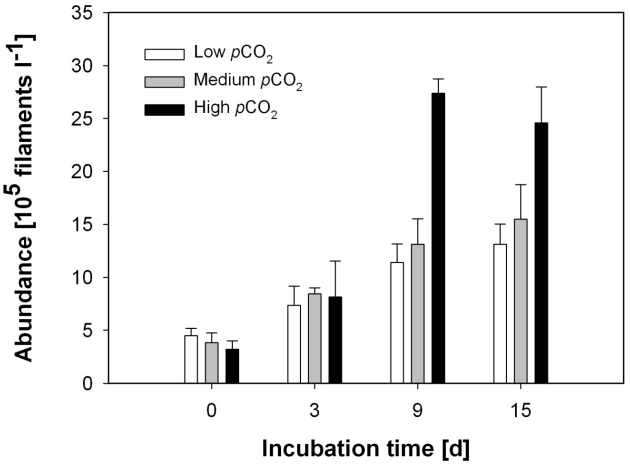

Fig. 2. Changes in abundance with time for the three pCO_2 treatments labelled with the partial pressure of premixed gas with which the cultures were aerated (low pCO_2 – white bars; medium pCO_2 – grey bars; high pCO_2 – black bars). Bars represent mean values of three replicates with respective standard deviation.

fixation, PO_4^{3-}, pCO_2 and C_T ($R^2 = 0.557$, 0.599 and 0.622, $p \leq 0.01$, $n = 12$, Supplement Table S1).

DOP concentrations were $0.3 \pm 0.1\,\mu mol\,l^{-1}$ in the low, $0.27 \pm 0.1\,\mu mol\,l^{-1}$ in the mid and $0.27 \pm 0.08\,\mu mol\,l^{-1}$ in the high pCO_2 treatment.

Mean values for DOC : DON ratios were 20 ± 3 (low pCO_2), 19 ± 2 (mid pCO_2) and 19 ± 2 (high pCO_2). Mean DOC:DOP ratios were 1094 ± 383 (low pCO_2), 1249 ± 421 (mid pCO_2) and 1243 ± 378 (high pCO_2). Ratios of DON : DOP were 57 ± 14 (low pCO_2), 64 ± 17 (mid pCO_2) and 66 ± 19 (high pCO_2).

3.4 Responses of *Nodularia*

3.4.1 *Nodularia* abundance

There was a steady increase in the abundance of *Nodularia* filaments in all pCO_2 treatments until day 9 (Fig. 2). Afterwards, abundance remained in a stationary phase. *Nodularia* abundance correlated significantly positive with chlorophyll *a*, POC, PON and POP ($R^2 = 074$, 0.83, 0.88 and 0.88, $p < 0.01$, $n = 12$).

Mean filament length in the high pCO_2 treatment increased from $74 \pm 28\,\mu m$ (day 0) to $88 \pm 38\,\mu m$ (day 9), but this trend was not statistically significant. Filament length in the low pCO_2 treatment and mid pCO_2 treatments increased from $67 \pm 24\,\mu m$ to $117 \pm 53\,\mu m$, and from $71 \pm 29\,\mu m$ to $100 \pm 50\,\mu m$, respectively. Differences in filament length between treatments were not statistically significant, but filaments were slightly shorter at the highest pCO_2 by ~23 %. On day 3, there was a significant higher number of heterocysts per filament in the high pCO_2 treatment compared to the mid pCO_2 ($p = 0.005$, $n = 50$, data not shown) and to the low pCO_2 treatment ($p = 0.0001$, $n = 50$, data not shown). From thereon, heterocyst number per filament decreased significantly in all treatments, while differences between the treatments were no longer statistically significant.

3.4.2 Chlorophyll *a*

Chlorophyll *a* increased over time in all pCO_2 treatments (Table 2). Highest mean chlorophyll *a* values occurred in the high pCO_2 treatment ($3.96 \pm 0.42\,\mu g\,l^{-1}$), medium values at the mid pCO_2 treatment ($3.28 \pm 0.72\,\mu g\,l^{-1}$), and lowest values at low pCO_2 levels ($2.28 \pm 0.79\,\mu g\,l^{-1}$). Nevertheless, only differences between the high pCO_2 treatment and the low pCO_2 treatment were statistically significant according to the t-test ($p = 0.009$, $n = 12$).

3.4.3 Concentration and stoichiometry of particulate organic matter (POM)

Concentrations of POC, PON and POP increased in all pCO_2 treatments, but most pronounced at the high pCO_2 level (Fig. 3). POC and PON concentration differed significantly between pCO_2 treatments, with highest concentrations being observed at high pCO_2 (Table 2). Normalized to filament abundance, however, POC, PON, and POP were lowest in the high pCO_2 treatment (Fig. 3). Thereby, differences in POC content per filament were statistically significant for the mid vs. the high pCO_2 level ($p = 0.05$, $n = 12$, data not shown), but not for the other combinations of pCO_2 levels. PON and POP per filament differed significantly between the low and high pCO_2 ($p = 0.05$ and $p = 0.01$, $n = 12$, data not shown) and between mid and high pCO_2 ($p = 0.05$ and $p = 0.01$, respectively, $n = 12$, data not shown).

Box-plots of POM elemental composition demonstrate an elevation in all treatments relative to Redfield ratios for POC : POP and PON : POP, but near Redfield stoichiometry for POC : PON (Fig. 4).

Elemental ratios decreased with pCO_2 ($R^2 = -0.552$, -0.653 and -0.634, respectively, $p \leq 0.01$, $n = 12$, Supplement Table S1), as well as with biomass specific C fixation ($R^2 = -0.708$, -0.732 and -0.711, respectively, $p \leq 0.01$, $n = 12$, Supplement Table S1) and DIP ($R^2 = -0.633$, -0.653 and -0.634, respectively, $p < 0.01$, $n = 12$, Supplement Table S1). This might imply a more balanced incorporation of C, N and P at higher pCO_2 if we assume incorporation of nutrients according to Redfield ratios.

POC : PON, POC : POP and PON : POP increased with abundance of *Nodularia* filaments ($R^2 = 0.751$, 0.795 and 0.789, respectively, $p \leq 0.01$, $n = 12$, Supplement Table S1), chlorophyll *a* ($R^2 = 0.823$, 0.883 and 0.829, respectively, $p < 0.01$, $n = 12$, Supplement Table S1) and pH ($R^2 = 0.529$, 0.556 and 0.529, respectively, $p \leq 0.01$, $n = 12$, Supplement Table S1).

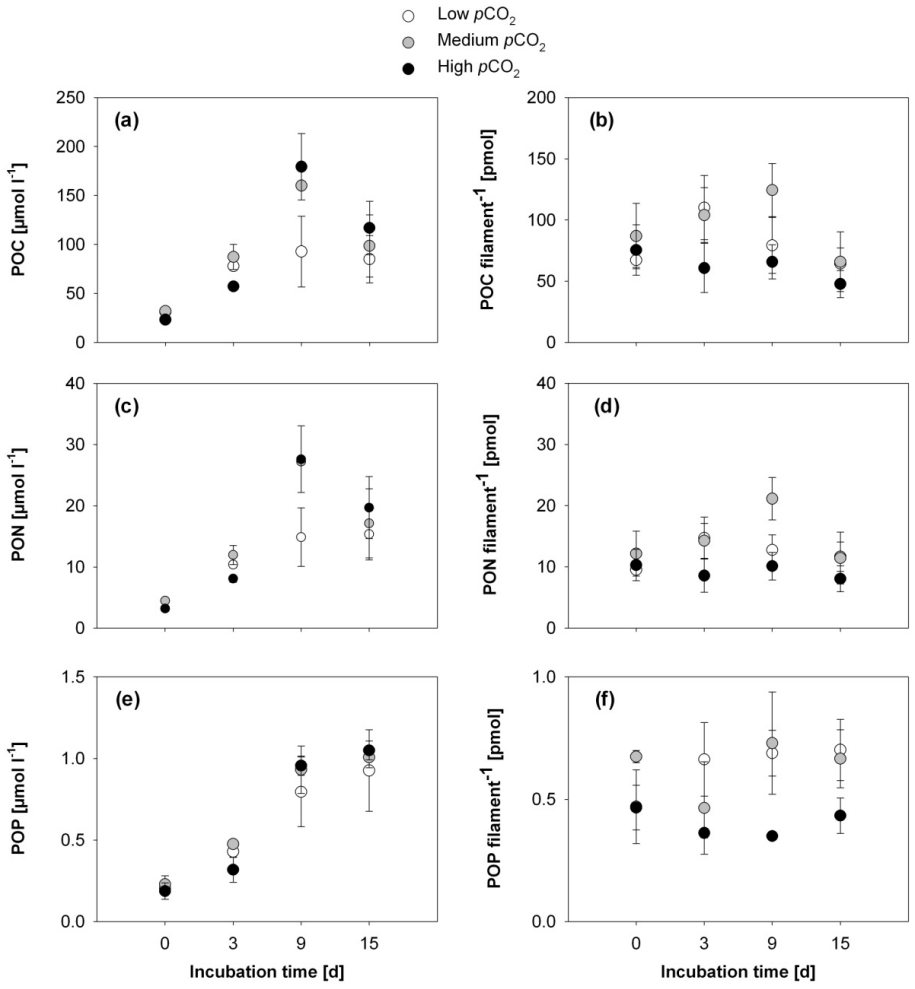

Fig. 3. Time depended variation in particulate organic carbon (POC), particulate organic nitrogen (PON), particulate organic phosphorous (POP) per volume **(a, c, e)** and per filament **(b, d, f)** for the three pCO_2 treatments (low pCO_2, white circles; medium pCO_2, grey circles; high pCO_2, black circles). Values are means and standard deviations of three replicates.

3.4.4 Growth rates

Growth rates calculated for the exponential growth phase (days 0–9) from changes in POC and PON were lower than growth rates derived from abundance and chlorophyll *a* in the low pCO_2 treatment, while they were equal in the mid and high pCO_2 treatment (Fig. 5). Compiled growth rates based on all parameters were significantly different between the pCO_2 treatments ($p < 0.05$ and $p = 0.001$, $n = 12$, Supplement Table S2), with the highest growth rate at high pCO_2 ($0.212 \pm 0.018\,d^{-1}$)

3.4.5 C and N_2 fixation

Biomass specific C and N_2 fixation rates decreased with incubation time in all pCO_2 treatments (Fig. 6). Mean values of C fixation averaged over the sampling period of 15 days were 21 ± 15 nmol C µmol POC^{-1} h^{-1} at low pCO_2, 30 ± 19 nmol C µmol POC^{-1} h^{-1} at mid pCO_2 and 35 ± 31 nmol C µmol POC^{-1} h^{-1} at high pCO_2. Mean values of N_2 fixation, excluding day 3, were 0.32 ± 0.18 nmol N µmol POC^{-1} h^{-1} at low pCO_2, 0.36 ± 0.21 nmol N µmol POC^{-1} h^{-1} at mid pCO_2 and 0.48 ± 0.20 nmol N µmol POC^{-1} h^{-1} at high pCO_2. Mean values of N_2 fixation of day 3 were 2.56 ± 0.44 nmol N µmol POC^{-1} h^{-1} at low pCO_2, 3.89 ± 0.12 nmol N µmol POC^{-1} h^{-1} at mid pCO_2 and 5.36 ± 0.32 nmol N µmol POC^{-1} h^{-1} at high pCO_2. C uptake rates showed high standard deviations within treatments and in between sampling days. Nevertheless, C fixation in the high pCO_2 treatment was elevated compared to the other two treatments by 97 % (high vs. low) and 44 % (high vs. mid) at day 0 and day 3, but this effect diminished afterwards.

Statistically significant differences between pCO_2 treatments according to ANOVA and Tukey's post hoc test were observed for biomass specific C and N_2 fixation

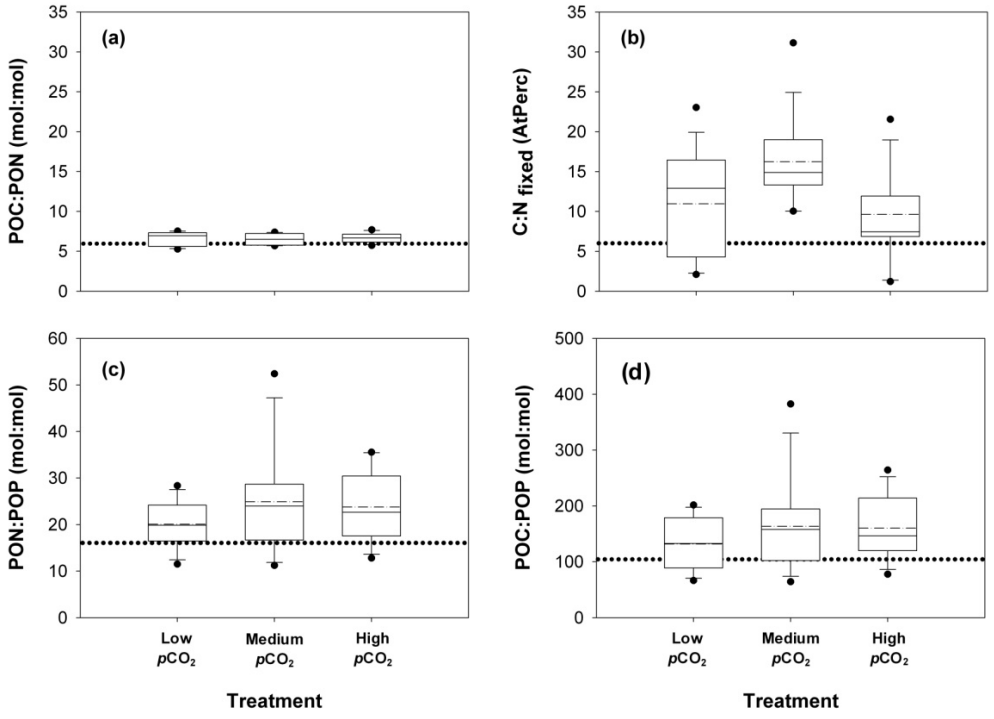

Fig. 4. Box plot ($n = 12$) of particulate organic matter stoichiometry and stoichiometry of carbon to nitrogen fixed for the three pCO_2 treatments (low pCO_2, medium pCO_2, high pCO_2). **(a)** Molar ratio of particulate organic carbon to particulate organic nitrogen (POC : PON), **(b)** atom percent ratio of carbon and nitrogen fixed, **(c)** molar ratio of particulate organic carbon to particulate organic phosphorous (POC : POP), **(d)** molar ratio of particulate organic nitrogen to particulate organic phosphorous (PON : POP). Dotted lines represent Redfield stoichiometry. Dashed dotted line represents mean values.

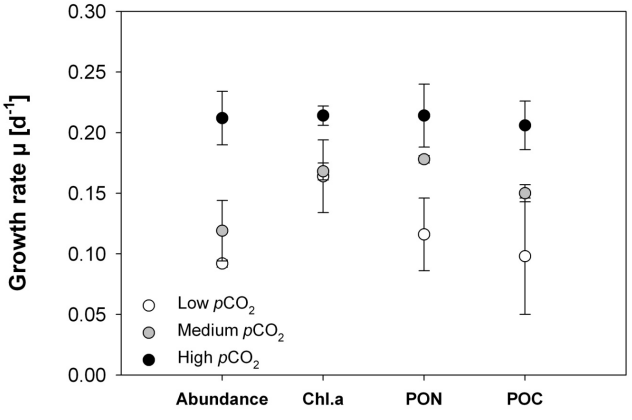

Fig. 5. Calculated growth rates (μ) per day based on changes in abundance, chlorophyll *a* (Chl *a*), particulate organic nitrogen (PON) and particulate organic carbon (POC) for the three pCO_2 treatments (low pCO_2, open circles; medium pCO_2, grey circles; high pCO_2, black circles). Symbols represent means of 12 values with standard deviations.

rates ($p \leq 0.05$ and $p \leq 0.001$, Supplement Table S2). C and N_2 fixation increased significantly with pCO_2 ($R^2 = 0.747$ and 0.362, $p \leq 0.01$ and $p \leq 0.05$, $n = 12$, Supplement Table S1). Ratios of $C_{fixed} : N_{fixed}$ were higher than the Redfield ratio and yielded maximum values at mid pCO_2 (C : N = 16:1) and lowest values at high pCO_2 (C : N = 9.6) (Fig. 4).

4 Discussion

4.1 Growth and production under different pCO_2 conditions

In this study we assessed the response of *Nodularia spumigena* to changes in pCO_2. Growth rates in terms of biomass increase calculated for days 0 to 9 were highest in the high pCO_2 scenario (508 ± 89 µatm). They were statistically significant elevated by 40 ± 25 % relative to mid pCO_2 (398 ± 104 µatm) and by even 84 ± 38 % relative to low pCO_2 (340 ± 80 µatm) ($p = 0.001$, $n = 12$). In the same time period (days 0–9), N_2 fixation seemed to be more stimulated by high pCO_2 than C uptake (Fig. 6). Biomass specific C fixation at high pCO_2 increased by 9 ± 44 % compared to mid pCO_2 and by 60 ± 60 % relative to low pCO_2. C uptake rates showed high standard deviations within treatments and in between sampling days. Nevertheless, C fixation in the high pCO_2 treatment was elevated compared to the other two treatments by 97 % (high vs. low) and 44 % (high vs. mid) at day 0 and day 3, but this effect diminished afterwards.

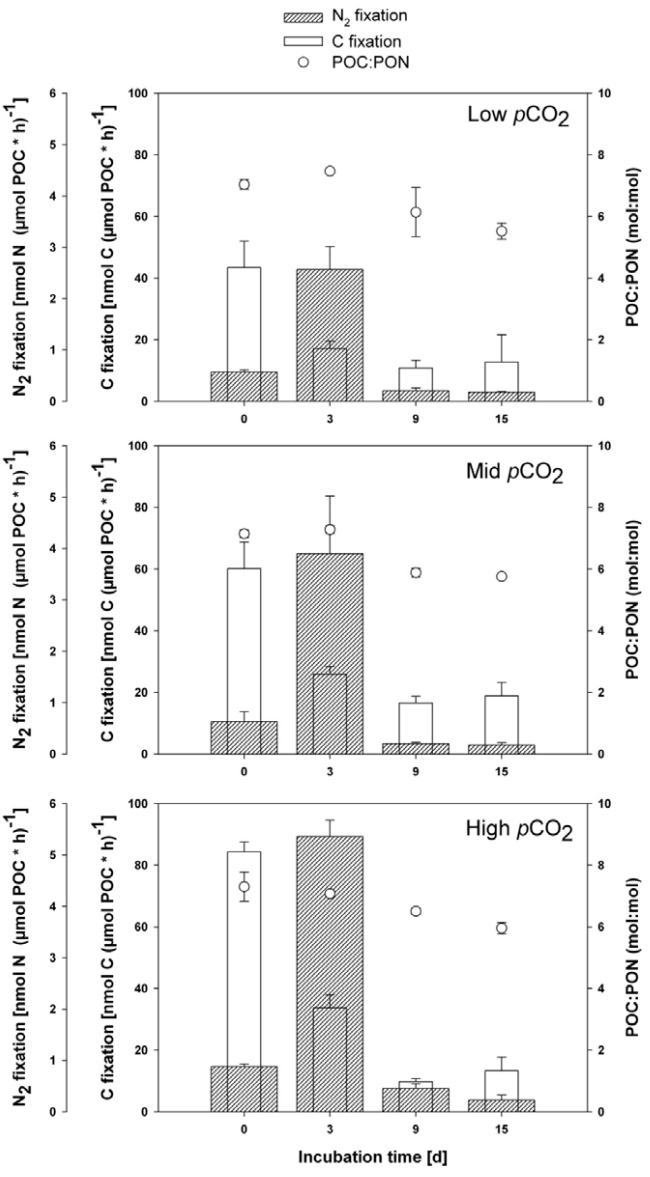

Fig. 6. Changes in biomass specific C (non filled bar charts), N_2 fixation (filled bar charts) and corresponding molar POC : PON ratios (scatter plots) versus incubation time for the three pCO_2 treatments low pCO_2, medium pCO_2, high pCO_2. Bars and scatter represent mean values of three replicates with respective standard deviation.

Elevation in N_2 fixation at the highest pCO_2 was accompanied by a higher number of heterocysts per filament. This went along with a shortening of filaments towards the end of the experiment (not statistically significant), because filaments tend to become more instable, fragile and break more easily. Nevertheless, heterocyst frequency declined over the course of the incubation in all treatments. Heterocyst frequency in *Nodularia* (Lindahl et al., 1980) and *Aphanizomenon* (Riddolls, 1985) has been shown to correlate with N_2 fixation rate, suggesting that it could be used as an indicator for N_2 fixation capacity.

If this tendency in morphology is repeatedly observed in future studies, it might have implications for grazing on filamentous cyanobacteria by zooplankton. Shorter filaments might not provide sufficient morphological grazing resistance, apart from chemical resistance. Moreover, Chan et al. (2004) demonstrated that in the presence of grazers, heterocysts showed a decline in N_2 fixation rates by 40 %. By reducing filament length, zooplankton grazing may act to preempt cyanobacteria blooms by suppressing N_2 fixation and cyanobacterial growth.

The simulative effect of high pCO_2 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 pCO_2 treatment along with a higher P concentration per filament, indicating a more efficient P usage at high pCO_2. Further investigation of the different DOP components and P turnover will be discussed in two companion manuscripts (Endres et al., 2012; Unger et al., 2012).

In our study growth rates increased with increasing pCO_2, despite DIP limitation, indicating a stimulating effect of DIC availability. This suggests a co-limitation by C and P in our experimental set-up at low and mid pCO_2 conditions, which might be applied to the Baltic Sea in summer, as well. A deficiency in DIP seems to be partly counter-balanced by excess C, which is opposing the concept of Liebig's law of only one limiting nutrient, which has already been noted by, e.g. Arrigo (2005) and Hutchins et al. (2007).

The first and only study available so far reporting the response of *Nodularia* growth and primary production to changing pCO_2 conditions was published by Czerny et al. (2009), who hypothesized a detrimental effect of high pCO_2 on *Nodularia* growth.

Both studies investigating *Nodularia* performance, Czerny et al. (2009) and ours used culture conditions that favoured the formation of single filaments without visible formation of larger aggregates. Czerny et al. continuously rotated their incubation bottles using a plankton wheel, representing a closed DIC manipulation set-up. We used slight agitation by manually rotating the bottles once a day and aeration at a low flow-rate, representing an open DIC manipulation. The different methods used might partly explain the observed opposing trends. Czerny et al. (2009) adjusted the pH by acid/base manipulation, which changes total alkalinity (TA) at constant dissolved inorganic carbon (DIC). Concentrations of DIC, HCO_3^-, and CO_2^{3-} in their study might have been lower than their actual target values, because seawater pH controls the relative proportion of the carbonate species and induces a lower percentage increase in HCO_3^- compared to a reduction of pH achieved by, e.g. aeration or by co-adding carbonate ions along with acid (e.g. $NaHCO_3$, e.g. Gattuso and Lavigne, 2009). This fact might have dampened a possible positive pCO_2 effect.

Additionally, it has been shown that light intensity strongly influences the magnitude of stimulation of growth and production by pCO_2 (e.g. Kranz et al., 2010), with significantly elevated rates at high pCO_2 and light conditions. Light intensity in our experiment was higher by a factor of 2.4 compared to those given by Czerny et al. (2009) (200 vs. 85 µmol photons m^{-2} s^{-1}, respectively). Experimental temperature differed by a factor of 1.3 with higher incubation temperatures in our study. Furthermore, DIP concentrations were non-limiting in the Czerny et al. (2009) study with 5 µmol l^{-1}, while reaching limiting conditions in our study after 3 days with DIP values near the detection limit. Czerny and co-workers hypothesized that the negative effect of high pCO_2 on N_2 fixation and growth occurred, because translocation of amino acids from heterocysts to vegetative cyanobacteria cells was restrained by a reduction in extracellular pH. However, intracellular amino acid translocation is not necessarily directly dependent on the external pH, because ionic exchange between adjacent cells takes place through the microplasmodesmata. These are intercellular channels linking cytoplasms of cells where intracellular pH is kept constant (Mullineaux, 2008; Flores and Herrero, 2010). Therefore, it is unlikely that amino acids will pass the outer and inner layers of the heterocyst envelope, but they will diffuse within a continuous periplasm and are re-imported into the cytoplasm of vegetative cells (Flores et al., 2006). Furthermore, Nicolaisen et al. (2009) showed that the outer membrane in heterocystous cyanobacteria is an efficient permeability barrier for glutamate and retains this metabolite within the filament. Nevertheless, a lower extracellular pH might hypothetically explain the reduction in N_2 fixation in the Czerny study by restraining the transport of nitrogenous metabolites, but it cannot explain the pronounced decrease in growth rate detected in parallel to a relative small reduction in N_2 fixation rate.

Supporting evidence towards a stimulation of growth and production at high pCO_2 has been shown previously for non-heterocystous cyanobacteria of the genus *Trichodesmium* both by adjustment of pCO_2 by aeration with pre-mixed gases (Hutchins et al., 2007; Kranz et al., 2009) or acid/base accomplished with DIC addition (Barcelos e Ramos et al., 2007). In these experiments, growth rates increased from present day to future pCO_2 levels by 34 to 38 % and decreased by 30 to 50 % when comparing the glacial vs. the present day pCO_2 levels (Levitan et al., 2007; Barcelos e Ramos et al., 2007). Furthermore, Hutchins et al. (2007) detected no growth in *Trichodesmium* cultures at pCO_2 conditions below 150 ppm.

Several researchers observed an elevation of N_2 fixation rates by approximately 35 to 40 % (with a maximum of even 400 %) over the respective pCO_2 range (Barcelos e Ramos et al., 2007; Levitan et al., 2007; Hutchins et al., 2007; Kranz et al., 2009). So far, all experiments were done with laboratory cultures, while field measurements are still scarce. Until today, there is only one publication, Hutchins et al. (2009), which reports a stimulation of cyanobacterial N_2 fixation rates by pCO_2 during three experimental runs (6, 21 and 41 %) in a field population of the Gulf of Mexico.

The underlying molecular and cell-physiological mechanisms of the beneficial effect of a high pCO_2 environment, however, is still speculative. Levitan et al. (2010a, b) and Kranz et al. (2009, 2010) assume energy savings achieved by down-regulating carbon concentration mechanisms (CCM). The acquisition of carbon in cyanobacteria involves the use of CCM to compensate for a low pCO_2 in aqueous environments, which are typically lower than the half saturation constant of RUBISCO, the major enzyme involved in C fixation. These CCMs often include bicarbonate transporter that allow access to the larger DIC reservoir (Tortell and Morel, 2002). *Trichodesmium*, as well as *Nodularia*, both belong to the β-group of cyanobacteria, classified based on the structural differences in RUBISCO (Badger et al., 2002). Both cyanobacteria share CCM components and possess a surplus of one DIC and CO_2 uptake system compared to α-cyanobacteria (BCT1, NADH-I_3). The operation of CCMs is energetically expensive and, because cell membranes are freely permeable for CO_2, additional metabolic costs are incurred in limiting the efflux of CO_2 from the cell. It has been proposed that CCM regulation might occur by changing the gene expression level, but studies by Levitan et al. (2010a, b) and Kranz et al. (2009, 2010) do not support this hypothesis in long-term studies. On the other hand, the discrepancy between CCM gene expression, CCM activity and stimulation of growth and production at high pCO_2, may be due to a modulation of the CCM activity at the translational and post-translational level or alteration of the transporter activity (Levitan et al., 2010b; Kranz et al., 2009, 2010). Within this line of arguments, Kranz et al. (2009) demonstrate an increase in activity of a special CCM transporter component at high pCO_2, the NDH-I_4 transporter, a low affinity transporter avoiding efflux of CO_2 from the cytosol by converting CO_2 to HCO_3^-. This elevated activity might lead to enhanced ATP production yielding in an energetic surplus available to fuel N_2 fixation. Regardless of the underlying molecular and cell physiological processes, C and N_2 fixation mechanisms compete for photo-generated reductants and any reduction in energy demand of the C fixation apparatus can be allocated to other metabolic processes including N_2 fixation and would explain the effect of CO_2 availability on potential C as well as N_2 fixation. A high plasticity of CCM regulation in *Trichodesmium* under different pCO_2, but also under variable light and temperature conditions and, moreover, in relation to the current N supply (Giordano et al., 2005), has emerged. This may modulate N and corresponding C demands to keep the respective C : N ratio at a constant level.

Overall, our results suggest a stimulating effect of high pCO_2 on *Nodularia*, which may be broadly applicable to non-heterocystous and heterocystous diazotrophic cyanobacteria of group β, unless no other growth factor becomes limiting.

4.2 Nitrogen and carbon turnover and elemental stoichiometry under different pCO_2 conditions

Total nitrogen (TN) within our experimental system increased over 9 days by $\sim 10\,\mu mol\,l^{-1}$ at low pCO_2, by $\sim 20\,\mu mol\,l^{-1}$ at mid pCO_2 and by $\sim 25\,\mu mol\,l^{-1}$ at high pCO_2. On a daily basis, N_2 fixation provided sufficient N to explain the build up of PON and POC. Nevertheless, a discrepancy occurred, because N_2 fixation per day was higher by 0.3 to 1.2 $\mu mol\,l^{-1}\,d^{-1}$ than the build up of PON and thus lead to a surplus of N_{fixed} compared to the build up of PON. Still, one has to keep in mind that N_2 fixation was measured for a period of 6 h at the sampling day, while accumulation of PON was determined for a longer period of 3 and 6 days, which might introduce errors and makes it inadequate for comparison.

Differences in DON between the mid and low pCO_2 scenarios, as well as mid and high pCO_2 levels, were not statistically significant according to ANOVA, but overall a statistically significant positive correlation of DON concentration and pCO_2 was found. This presumes a tendency to elevated exudation of DON at high pCO_2, but caution has to be taken because of the different sampling intervals (3, 6 and 6 days). Moreover, the differences between replicate DOM were in the same concentration range as differences in concentration from one sampling day to the next. Thus, a significant accumulation of DIN due to exudation was not detectable. Again, our sampling interval and method chosen (sampling after 3, 9 and 15 days, exudation calculated from differences in concentration) made it difficult to directly determine exudation, which might indicate a faster cycling of N compounds than could be detected by our experimental approach. There was a constant although low number of heterotrophic bacteria in our incubation bottle, but our measurements revealed that active growth of bacteria did not occur. Therefore, this bacterial contamination might have resulted from a background of non-viable, but SYBR green stainable bacteria since also extracellular nucleic acids and dead, DNA containing, cells will be stained by the dye. In conclusion, uptake of DON and DIN by heterotrophic bacteria should have been negligible. Apart of such potential methodological constrains, some studies have shown that N_2 fixation and subsequent release of DON are possible mechanisms to dissipate excess light energy on a short term scale (Lomas et al., 2000; Wannicke et al., 2009), while no previous report on the effect of pCO_2 on DON release exists.

There are several studies showing indirectly via TEP and exudate formation (Engel et al., 2002) or directly (Kim et al., 2011) that DOC production is sensitive to changes in pCO_2. This lack of significant tendencies presumably results from a rapid response of the microbial food web superimposing short term trends of autotrophic processes which might have been significant. On the other hand, Borchard and Engel (2012) recently demonstrated a stimulating effect of greenhouse conditions (high pCO_2 and high temperature) on exudation processes in a laboratory study using *Emiliania huxleyi*. Ratios of newly fixed C : N were above the Redfield ratio and significantly higher than molar C : N ratios of cyanobacteria growing at low and mid pCO_2 ($p = 0.03$ and < 0.001, respectively, $n = 12$, data not shown), whereas ratios of newly fixed C : N and molar C : N did not change at high pCO_2 ($p = 0.08$, $n = 12$, data not shown).

At high pCO_2, a higher N_2 fixation rate along with a higher C fixation rate and a more balanced growth in terms of POC to PON to POP suggests synchronic ammonium incorporation into the carbon skeletons (2-oxoglutarat) through the GS–GOGAT (glutamine synthetase–glutamine oxoglutarate aminotransferase) cycle synthesizing glutamate.

POC : POP, as well as PON : POP in this study, were elevated relative to the Redfield ratios in all treatments and deviated significantly between low vs. mid and low vs. high pCO_2 treatments. The positive correlation between POC : POP and PON : POP ratios and biomass (chlorophyll *a* and abundance) presumes a higher C accumulation relative to N and P and of N relative to P.

In terms of trend and magnitude, our measured elemental ratios are comparable with those given by Hutchins et al. (2007) and Barcelos e Ramos et al. (2007), indicating constant C : N ratios, but an increase in N : P and C : P ratios at high pCO_2. This opposes the trend observed by Levitan et al. (2007), Czerny et al. (2009) and Kranz et al. (2009), who found an increase in C and N quota as well as the ratio at elevated pCO_2.

In general, to date there is no consensus on whether phytoplankton elemental ratios are likely to be altered in a systematic manner in a future acidified ocean.

Elemental ratios of most of eukaryotic phytoplankton investigated so far either remained near Redfield values, or were increased in some species (Hutchins et al., 2009; Liu et al., 2010 and references therein).

Similarly, natural populations display no clear trend in POM stoichiometry either with increased C : N ratios in some studies (Riebesell et al., 2007; Engel et al., 2005) and a decrease in N : P in others (Tortell et al., 2002; Bellerby et al., 2008).

4.3 Biogeochemical and ecological implications

Seasonally, cyanobacteria in the Baltic Sea exhibit pCO_2 fluctuations with minimum values close to or below glacial pCO_2 values ($< 180\,\mu atm$). In the Gulf of Finland, pCO_2 drops from winter time until May from atmospheric equilibrium values of $\sim 350\,\mu atm$ to $\sim 150\,\mu atm$ due to warming of water and increased sequestration by photosynthetic activity (Schneider et al., 2006). This corresponds to a decline in pCO_2 of 60 %. In July, pCO_2 concentrations rise slightly up to $\sim 200\,\mu atm$ and level off again to a minimum of 100 µatm with the onset of the cyanobacteria bloom.

Thus, the natural cyanobacteria community of the Baltic Sea seems to be periodically exposed to glacial like pCO_2

conditions. If we apply rate measurements obtained in our study at low pCO_2, growth rates, N_2 and C fixation at mid pCO_2 would be lower by up to 34 ± 29 %, 30 ± 29 % and 44 ± 8 %, respectively.

Nevertheless, this C limitation is balanced periodically by upwelling and turbulent mixing of CO_2 and nutrient rich intermediate winter water (Gidhagen, 1987) with pCO_2 up to 800 µatm (Schneider et al., 2006; Beldowski et al., 2010; Schneider, 2011).

Our results suggest that, as long as growth and production of cyanobacteria in the Baltic Sea are not limited by other factors, e.g. nutrients and light, maximum growth rates of *Nodularia* could potentially rise by 84 ± 38 % due to the predicted increase in pCO_2 throughout the next 100 yr.

N_2 fixation by *Nodularia* might be elevated by 67 ± 50 % in the next 100 yr, if we extrapolate rates we determined in our study.

N_2 fixation by *Nodularia* might be elevated by 67 ± 50 % in the next 100 yr, if we extrapolate rates we determined in our study (Levitan et al., 2007; Barcelos e Ramos et al., 2007). Current estimates of N_2 fixation by cyanobacteria are about 136 mmol N m^{-2} yr^{-1} for the Baltic Proper (Wasmund et al., 2001a, 2005b). If we assume that our experimental results can also be extrapolated to the field, this rate could rise to 227 ± 68 mmol N m^{-2} yr^{-1}, caused by the expected increases in pCO_2 alone. Nevertheless, this projected increase remains within the natural variability of rate measurements. Subsequently, pCO_2 induced increase in N_2 fixation would elevate the amount of bioavailable nitrogen by the release of dissolved nitrogenous compounds (DIN and DON) corresponding to a release rate of 113 ± 34 mmol N m^{-2} yr^{-1} or 182 ± 54 mmol N m^{-2} yr^{-1}, if we assume that 50 % or 80 % of total nitrogen fixed by cyanobacteria is exudated (Glibert and Bronk, 1994; Ohlendieck, 2000). However, to date it is not known whether DON exudation will be affected by an increase in pCO_2 itself. In comparison, atmospheric N input to the Baltic Sea accounts for ~ 80 mmol N m^{-2} yr^{-1} (Larsson et al., 2001; Rolff et al., 2008), while 45 % of the total N input derives from N_2 fixation. Riverine N load is higher and adds up to 76×10^3 mmol N m^{-2} yr^{-1} (HELCOM, 2005).

Since diazotrophic cyanobacteria can exploit elemental N_2, as well as organic N sources, they do not solely rely on dissolved inorganic nitrogen sources like nitrate and ammonia. Moreover, they still can exploit inorganic phosphorous, as well as organic phosphorous, although dissolved inorganic nitrogen is already limiting. As a result, they drive the ecosystem towards P instead of N limitation. Recently, this phenomenon has been described for the Atlantic Ocean by Moore et al. (2009) and Fernández et al. (2010). Our results suggest that this phenomenon will be amplified in the future ocean when rate and extent of mass occurrences of diazotrophs develop, in particular when temperature increases at the same time. Cyanobacteria mass developments not only impact N and P cycling in the phototrophic zone, but also reduce oxygen concentrations in the deeper water layers and on the sediment when their biomass settles out. This will increase oxygen consumption and hence expand hypoxia in the Baltic Sea, which are known to release large quantities of inorganic P (Mohr et al., 2010).

In addition to this, we have detected an increase in C : P and N : P ratios at high pCO_2. Extrapolating our results to a potential Baltic Sea in 2100 suggests that the nutritional value of organic matter produced in the euphotic zone will decrease in the future ocean. This could impact the efficiency of bacterial degradation on the one hand, and zooplankton production on the other hand, affecting the remineralisation potential in deep water layers. Overall, the environmental significance of diazotrophic blooms in the Baltic Sea goes far beyond the detrimental effects of changes in stoichiometry and quantity of degradable biomass to the point of recreational issues. Eutrophication might play a substantial role in the expansion of cyanobacterial blooms (e.g. O'Neil et al., 2012). The future N input into the Baltic Sea, caused by a pCO_2 induced stimulation of cyanobacteria, might counteract the nitrogen load reductions aimed to mitigate eutrophication (e.g. Vahtera et al., 2007; Voss et al., 2011) and in the worst case impair the socio-economic value of the Baltic Sea.

Supplementary material related to this article is available online at: http://www.biogeosciences.net/9/2973/2012/bg-9-2973-2012-supplement.pdf.

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