Biogeosciences Discuss., 9, 14709–14750, 2012
www.biogeosciences-discuss.net/9/14709/2012/
doi:10.5194/bgd-9-14709-2012

Biogeosciences Discussions

Response of *Nodularia spumigena* to pCO_2 – Part 3: Turnover of phosphorus compounds

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Received: 17 September 2012 – Accepted: 11 October 2012 – Published: 22 October 2012

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Published by Copernicus Publications on behalf of the European Geosciences Union.

Abstract

Diazotrophic cyanobacteria often form extensive summer blooms in the Baltic Sea driving their environment into phosphate limitation. One of the main species is the heterocystous cyanobacterium *Nodularia spumigena*. *N. spumigena* exhibits accelerated uptake of phosphate through the release of the exoenzyme alkaline phosphatase that also serves as an indicator of the hydrolysis of dissolved organic phosphorus (DOP). The present study investigated the utilization of DOP and its compounds (e.g. ATP) by *N. spumigena* during growth under varying CO_2 concentrations, in order to estimate potential consequences of ocean acidification on the cell's supply with phosphorus. Cell growth, phosphorus pool fractions, and four DOP-compounds (ATP, DNA, RNA, and phospholipids) were determined in three set-ups with different CO_2 concentrations (341, 399, and 508 µatm) during a 15-day batch experiment. The results showed rapid depletion of dissolved inorganic phosphorus (DIP) in all pCO_2 treatments while DOP utilization increased with elevated pCO_2, in parallel with the growth stimulation of *N. spumigena*. During the growth phase, DOP uptake was enhanced by a factor of 1.32 at 399 µatm and of 2.25 at 508 µatm compared to the lowest pCO_2 concentration. Among the measured DOP compounds, none was found to accumulate preferentially during the incubation or in response to a specific pCO_2 treatment. However, at the beginning 61.9 ± 4.3 % of the DOP were not characterized but comprised the most highly utilized fraction. This is demonstrated by the decrement of this fraction to 27.4 ± 9.9 % of total DOP during the growth phase, especially in response to the medium and high pCO_2treatment. Our results indicate a stimulated growth of diazotrophic cyanobacteria at increasing CO_2 concentrations that is accompanied by increasing utilization of DOP as an alternative P source.

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1 Introduction

Cyanobacteria bloom events frequently occur in the Baltic Sea in summer (Kahru et al., 1994) and they are dominated by the filamentous diazotrophic cyanobacteria *Nodularia spumigena* and *Aphanizomenon* sp. (Sivonen et al., 1989; Finni, 2001; Vahtera et al., 2005). Calm conditions, a salinity of 7–8, temperatures $> 16^{\circ}$ C, and a N : P ratio < 8 promote the formation of extensive *Nodularia* blooms in the sea surface layer (Wasmund, 1997). Degerholm et al. (2006) suggested that *Nodularia* sp. is better adapted than *Aphanizomenon* sp. with respect to phosphorus (P) starvation and has a higher affinity for dissolved organic phosphorus (DOP) because of its lower substrate half-saturation constants (K_M) and the higher $V_{max} : K_M$ ratio of the enzyme alkaline phosphatase (AP). These findings were confirmed by Vahtera et al. (2007), who reported that under bloom conditions *Nodularia* is superior to *Aphanizomenon* in its ability to compete for phosphorus at low concentrations, more efficient in acquiring phosphate from organic sources, and better able to grow on intracellular phosphorus stores.

As a constituent of compounds mediating cellular energy transformation and metabolic processes, P is an essential macronutrient for all living organisms (Karl, 2000; Benitez-Nelson, 2000; Nausch and Nausch, 2011). The major forms of P in aquatic ecosystems are dissolved inorganic phosphorus (DIP) and dissolved organic phosphorus (DOP) (Orchard et al., 2010). Among the different forms of DIP, orthophosphate (PO_4^{3-}) is metabolically preferred by phytoplankton and bacteria, based on their direct uptake of this compound through the cell membrane (Løvdal et al., 2007). As P is the limiting nutrient besides nitrogen (N), its availability strongly influences primary production (Smith, 1984; Howarth, 1988; Ruttenberg and Dyhrman, 2005; Elser et al., 2007). After DIP depletion, phytoplankton are able to utilize DOP, as indicated by the increased activity of AP, responsible for hydrolyzing DOP (Ruttenberg and Dyhrman, 2005; Paytan and McLaughlin, 2007).

DOP, together with dissolved organic carbon and nitrogen (DOC and DON, respectively), comprise the dissolved organic matter (DOM) pool (Karl and Björkman, 2002).

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Measurable DOP components include deoxyribonucleic acid (DNA), ribonucleic acid (RNA) (e.g. Karl and Bailiff, 1989), adenosine-5'-triphosphate (ATP) (e.g. Björkman and Karl, 2001), and phospholipids (PL) (e.g. Suzumura and Ingall, 2001, 2004). DNA, in addition to its fundamental role in heredity for all self-replicating organisms (Karl and Bailiff, 1989), gained further attention when DeFlaun et al. (1987) examined the contribution of its dissolved form to general DOM dynamics. RNA is involved in protein synthesis, which is required for growth (Dortch et al., 1983). Both RNA and DNA are indicators of actively growing, metabolizing cells (Karl and Bailiff, 1989). As reported by Karl and Bailiff (1989), the dissolved DNA and RNA concentrations in marine coastal/estuarine and offshore regions range from 0.56 to 21 $\mu g\,l^{-1}$ and from 4.03 to 31.9 $\mu g\,l^{-1}$, respectively. ATP, one of the most P-rich organic molecules, mediates energy transfer in all living organisms. Dissolved ATP occurs in seawater in significant concentrations of 0.1–0.6 $\mu g\,l^{-1}$ (Azam and Hodson, 1977). Radiolabeled ATP is used to measure the hydrolysis of organic phosphorus compounds and the uptake of released P (Bentzen and Taylor, 1991; Casey et al., 2009). Phospholipids are ubiquitous in nature, serving as the structural and functional components of biological membranes (Suzumura and Ingall, 2001). They are classified according to their hydrophilic and hydrophobic portions, with dissolved forms providing a reservoir of organic P. So far, only a few studies have examined the distribution and abundance of phospholipids in marine environments, such that our understanding of their function in this respect remains limited (Suzumura, 2005). Parrish (1987) reported a wide range (4–88 $\mu g\,l^{-1}$) of dissolved phospholipid concentrations in coastal waters. In Pacific surface waters, concentrations of hydrophobic phospholipid-P between 6 and 16 $nmol\,l^{-1}$ were measured, thus constituting between 2 and 6 % of the DOP pool (Suzumura and Ingall, 2004).

The rising atmospheric CO_2 concentrations in the world's oceans have lowered pH and altered the carbonate chemistry of seawater faster than in the previous thousands of years (Siegenthaler et al., 2005; Hönisch et al., 2009). These changes are commonly referred to as ocean acidification (Doney et al., 2009). Since preindustrial times until today, the atmospheric CO_2 increased from 280 to 395 ppm (www.esrl.noaa.gov/gmd/

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ccgg/trends/). By the end of this century, the CO_2 concentration is expected to reach 800 ppm, assuming that anthropogenically induced CO_2 emissions continue to rise at the present rate (IPCC, business-as-usual emission scenario, 2007). At the same time, the average pH of ocean surface waters has fallen by approximately 0.1 units and is expected to decrease a further 0.3–0.4 pH units by 2100 (Orr et al., 2005). At present, the ecological implications of ocean acidification are largely unknown and are therefore the subject of numerous ongoing investigations.

So far, there has been little research directed toward improving our understanding of the effects of elevated pCO_2 on the marine P cycle as it has long been assumed that the P cycle was not directly affected by rising ocean pCO_2. However, indirect responses to the expected changes in C and N cycles are likely and, importantly, may serve as a relatively conservative indicator thereof (Hutchins et al., 2009). Published studies have preferentially concentrated on the cellular P quotas of different cyanobacterial and diatom species. For example, Burkhardt et al. (1999) analyzed the effect of low pH on the C : P ratios of six diatom and one dinoflagellate species, based on the premise that the increasing atmospheric pCO_2 does not affect global ocean Redfield ratios. Hutchins et al. (2007) and Fu et al. (2007) reported no effects of a similar pCO_2 increase on the cellular P quotas of the cyanobacteria *Trichodesmium erythtraeum*, *Synechococcus*, and *Prochlorococcus* whereas Czerny et al. (2009) noted a slightly increasing trend in cellular P quotas with elevated pCO_2 in their study of *Nodularia spumigena*. In a Norwegian fjord mesocosm experiment, AP activity (APA) was measured as a means to examine ^{33}P uptake rates and potential DOP utilization under three different pCO_2 concentrations (Tanaka et al., 2008); however, no statistically significant effects of pCO_2 on P biogeochemistry were determined.

This study is part of two other investigations based on the same experimental setup. Wannicke et al. (2012) and Endres et al. (2012) focused on N-cycling and exudation and extracellular enzyme activities, respectively. Here we investigated dissolved P pools, in particular their variation during the growth of the diazotrophic cyanobacterium *Nodularia spumigena* under conditions of pCO_2 elevation, and their contribution

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to *Nodularia* nutrition. To gain insight into the dynamics of DOP and P metabolism in general, we focused on the changes of DOP and its composition as well as on P transformation processes.

2 Materials and methods

2.1 Experimental setup and conditions

A 15-day batch culture experiment was conducted with the diazotrophic cyanobacterium *Nodularia spumigena* in April 2010. In preparation for the experiment, water from the Baltic Sea (54.22749° N, 12.1748° E) was collected and aged for 4 months to allow the removal of inorganic nutrients by phytoplankton and bacteria. Afterwards the seawater was filtered through 0.2 µm cellulose acetate (CA) filters to remove particulate material and then UV-sterilized for 5 days. Three weeks prior to the start of the experiment, parent cultures of *Nodularia spumigena* were grown in sterile Baltic Sea water in a walk-in cooling chamber (15 °C) under controlled light conditions (16 : 8 h light : dark cycle, 100 µmol photons m^{-2} s^{-1}). One week before the acclimation phase was started, the parent cultures were allowed to adapt to the experimental temperature of 23° C and to the doubled light supply of 200 µmol photons m^{-2} s^{-1}. In the meantime, the sterilized seawater was filtered again through 0.2 µm CA filters under a clean bench into 10 l Nalgene bottles (39 bottles in total). Over a period of 3 days, *Nodularia* maintained in this water was allowed to acclimate to three different CO_2 concentrations by aeration with premixed gases (Linde Gas) of 180 pCO_2 (ppm), 380 pCO_2 (ppm), and 780 pCO_2 (ppm), representing pre-industrial, present, and future pCO_2 conditions, respectively. The acclimated cyanobacteria were then inoculated into thirteen 10 l bottles per treatment. These batch cultures were aerated with the respective CO_2 gases, continuously for the first day and then once a day for 1 h (at 02:00 p.m.). The cultures were routinely mixed by gently shaking the bottles, avoiding aggregate formation and strong turbulence. During sampling, while the pCO_2-levels were clearly different from one another,

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the determined pCO_2-levels deviated from the target values (Fig. 1c). Thus, in the following we refer to them as low (341 ± 81 µatm), medium (399 ± 104 µatm), and high (508 ± 90 µatm) pCO_2 treatments. One bottle per treatment was used to obtain background information regarding nutrient status. In the remaining 36 bottles, the starting concentration of chlorophyll *a* (Chl *a*) of 0.8 µg l^{-1} was adjusted and DIP was added to a final concentration of 0.35 µmol l^{-1}. After day 3, an additional 0.35 µmol DIP l^{-1} was added because the phosphate in the medium was nearly depleted. The first set of samples was taken immediately after starting the experiment and then at days 3, 9, and 15 of the incubation. The sampling time for all three conditions was between 08:00 and 09:00 a.m. On each sampling day, three bottles per pCO_2 treatment were harvested. The experimental design is described in detail in Wannicke et al. (2012).

In parallel, the same experimental design and sampling mode were applied to investigate the transformation of DIP, using $[^{33}P]PO_4$. The difference was that the cultures were maintained in 500-ml-bottles (Schott). At the beginning of the experiment, 50 pM $[^{33}P]PO_4$ (6.6 MBq l^{-1}) (Hartmann Analytics, specific activity 110 TBq $mmol^{-1}$) were added to each bottle, with the radioactivity in the dissolved and the particulate fractions then measured at each sampling point. In addition, biomass parameters such as Chl *a*, particulate organic carbon (POC), and particulate organic phosphorus (POP) were measured to compare the growth of *Nodularia* in these bottles and in the larger ones.

2.2 Carbonate chemistry

The carbonate system was characterized by measuring pH and total dissolved inorganic carbon (C_T) on every sampling day. The pH was determined with an electrode (Knick Mikroprozessor pH Meter 761 with Typ SE 100 glass electrode) and calibrated with standard NBS buffers directly before each measurement. The pH values are expressed relative to the total scale. C_T was analyzed with the colorimetric SOMMA system according to Johnson et al. (1993) and calibrated with carbon reference material provided by A. Dickson (University of California, San Diego). The reported precision of

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this method is ±2 µmol kg^{-1}. Total alkalinity (A_T) and pCO_2 were calculated with the program CO2SYS (Lewis et al., 1998). C_T, pH, salinity, temperature, total phosphate, and total silicate were set as parameters for the calculations.

2.3 Sample analysis

2.3.1 Biomass and cell counts

The chlorophyll *a* (Chl *a*) concentration was determined by filtering 100-ml samples onto Whatman glass-fiber filters (GF/F), applying a vacuum of 200 mbar. The filters were stored in liquid nitrogen or at −80 °C until they were extracted with 96 % ethanol for at least 3 h. Chl *a* fluorescence was measured with a TURNER fluorometer (10-AU-005) at an excitation wavelength of 450 nm and an emission wavelength of 670 nm (HELCOM, 2005). Calculation of the Chl *a* concentrations was based on the method of Jeffrey and Welschmeyer (1997).

The abundance of *Nodularia spumigena* was determined by preserving 50-ml samples with acetic Lugol's (KI/I2) solution (1 % final concentration). The samples were counted at 100 × magnification using an inverted Leica microscope (Utermöhl, 1958).

Bacteria were analyzed using a flow cytometer (Facs Calibur, Becton Dickinson) according to Gasol and del Giorgio (2000). Samples of 4 ml were preserved with 100 µl of formaldehyde (1 % *v*/*v* final concentration), shock frozen in liquid nitrogen, and stored at −70 °C until further processing. For analysis a stock solution of SYBR GREEN (Molecular Probes) was prepared by mixing 1 µl of dye with 49 µl of dimethyl sulfoxide (DMSO, Sigma Aldrich). The samples were thawed and 300 µl were then mixed with 3 µl potassium citrate solution, 10 µl of the dye stock solution, and 10 µl fluoresbrite microspheres (Polysciences), followed by incubation in the dark for 30 min. The cells were counted at a medium flow rate. The values of interest were calculated using the software program "Cell Quest Pro".

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2.3.2 Inorganic nutrient analyses

Water samples (60 ml) of the batch cultures were filtered through combusted (450 °C, 4 h) Whatman GF/F filters and stored at −20 °C before the inorganic nutrient concentrations (DIP, nitrate/nitrite, silicate, and ammonium) were determined using the autoanalyzer system "Evolution III" (Rohde and Nehring, 1979) and standard colorimetric methods (Grasshoff et al., 1983) except for ammonium which was determined manually according to Grasshoff et al. (1983). The detection limit was 0.02 µmol l^{-1} for DIP, 0.05 µmol l^{-1} for nitrate/nitrite, and 0.1 µmol l^{-1} for silicate. Ammonium concentrations were below the detection limit of 0.05 µmol l^{-1} throughout the experiment. To estimate the development of the cultures immediately after sampling, DIP concentrations were determined manually according to Murphy and Riley (1962). For data analysis, DIP values from both measurements were pooled.

2.3.3 Organic matter analyses

To determine total and dissolved phosphorus (TP and DP, respectively), 40-ml samples were stored frozen at −20 °C either unfiltered or after filtration through 0.2 µm CA filters. The thawed samples were then oxidized with an alkaline peroxodisulfate solution (Grasshoff et al., 1983) in a microwave (MWS µPrep-A) to convert organic phosphorus into DIP. The procedure lasted 4 h in total including warming, incubating 1 h at 170 °C, and cooling. Further DIP analysis was done as described above but using a 10 cm-cuvette, which reduced the detection limit to 0.01 µmol l^{-1}. DOP was calculated as the difference between DP and DIP. POP was calculated as the difference between TP and DP and is referred to as *Nodularia*-P hereafter.

Both DOC and total dissolved nitrogen (TDN) were analyzed by collecting subsamples in combusted 20-ml glass ampoules (8 h, 500 °C), pre-filtered through combusted GF/F filters, followed by acidification with 80 µl of 85 % phosphoric acid, and stored at 0–2 °C until further processing. DOC and TDN concentrations were determined simultaneously in the filtrate by high-temperature catalytic oxidation with a Shimadzu

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TOC-VCSH analyzer equipped with a Shimadzu TNM-1 module. DOC and TDN concentrations were measured as quadruplicates and then averaged. The TDN values were corrected for nitrate/nitrite and ammonium, and subsequently defined as DON.

Particulate organic carbon (POC) and nitrogen (PON) were analyzed by filtering 200-ml samples onto GF/F filters which were subsequently stored frozen at −20 °C. Concentrations were measured by means of flash combustion in a Carlo Erba EA 1108 at 1020 °C and a Thermo Finnigan Delta S mass-spectrometer.

2.3.4 Dissolved ATP

Dissolved ATP (dATP) was determined according to Björkman and Karl (2001) but modified for Baltic Sea conditions. Samples of 200 ml each were pre-filtered through combusted (4 h, 450 °C) Whatman GF/F filters followed by filtration through 0.2 µm CA filters. A $Mg(OH)_2$ precipitate including the co-precipitated nucleotides was obtained by the addition of 1 M NaOH (0.5 % v/v). The precipitate settled overnight and was then centrifuged for 20 min at 1000 × g. The supernatant was aspirated and the precipitate was transferred into 50-ml Falcon tubes, centrifuged again (1.5 h, 1680 × g) to obtain the final pellet, and then resuspended with 5 M HCl, added dropwise. A final pH of 7.2 was reached by the addition of TRIS buffer (pH 7.4, 20 mM, Sigma-Aldrich, T7693). The final volume was recorded and standard concentrations were prepared as for the samples, thus yielding a blank with aged Baltic Sea water and six ATP concentrations (adenosine 5′-triphosphate disodium salt hydrate, Sigma-Aldrich, A2383) ranging from 1 to 20 nmol l^{-1}.

ATP concentrations were measured by the firefly bioluminescence assay using a Sirius Luminometer (Berthold Detection Systems). The 30-µl samples were each treated with 240 µl of firefly lantern extract mixture prepared according to Björkman and Karl (2001). The detection limit was 2.5 pmol ml^{-1} of the concentrated sample with a precision of < 5 % at 40 pmol l^{-1} dATP in the original water sample.

The fluorescence slope of the standard concentrations was used to calculate the dATP concentrations, with correction for the final sample volume. The P-content was

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calculated based on the fact that 1 mol ATP is equivalent to 3 mol P and is hereafter referred to as dATP-P.

2.3.5 Dissolved phospholipids

The phosphate concentration of dissolved phospholipids (dPL-P) was analyzed according to Suzumura and Ingall (2001, 2004), adopting the method to Baltic Sea conditions. For the extraction of dPL-P, 400-ml aliquots of GF/F (combusted, 4 h, 450 °C) and 0.2 µm CA filtered batch samples were stored frozen at −20 °C until further processing. The samples were thawed in a water bath at 30 °C and then extracted twice with 100 ml of chloroform (Merck 1.07024.2500). The chloroform phase was collected and concentrated to 5 ml in a rotary evaporator (Heidolph Hei-VAP Advantage). The concentrate was then transferred into microwave tubes (suitable for MWS µPrep-A) to completely evaporate the chloroform in a 60 °C water bath overnight. Twenty ml of Milli-Q water was added, after which processing in a microwave was the same as described for the analysis of TP and DP. Six standard concentrations ranging from 0 to 125 µg l^{-1} were prepared by adding the appropriate amounts of a 5 mg PG (L-α-phosphatidyl-DL-glycerol sodium salt, Sigma Aldrich, P8318) ml^{-1} stock solution to aged seawater. A reagent blank of chloroform was also measured. Based on the slope, the dPL-P concentration was determined. The detection limit was 0.8 nmol l^{-1}.

2.3.6 Dissolved DNA and RNA

Dissolved DNA and RNA were determined according to Karl and Bailiff (1989). For each sample a volume of 200 ml was filtered through combusted GF/F (4 h, 450 °C) and 0.2 µm CA filters. The same volume of ethylenediaminetetracetic acid (EDTA, 0.1 M, pH 9.3, Merck, 1.08454.1000) and 4 ml of cetyltrimethylammonium bromide (CTAB, Sigma-Aldrich, H5882) were added. The samples were gently mixed and stored frozen at −20 °C for at least 24 h. After defrosting the samples, the precipitate that had formed was collected onto combusted (450 °C, 4 h) GF/F filters (25 mm, Whatman), placed

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into annealed vials, and stored frozen at −80 °C until further analysis. Dissolved DNA and RNA (dDNA and dRNA) were detected according to Karl and Bailiff (1989) using the fluorescence-spectrophotometer F2000 (HITACHI) to determine dDNA and the dual-beam-UV/VIS-spectrophotometer U3010 (HITACHI) to determine dRNA. Coupled standards (DNA + RNA) with concentrations between 1–10 µg DNA l^{-1} (Sigma Aldrich, D3779) and 20–120 µg RNA l^{-1} (Sigma Aldrich, R1753) were prepared in aged seawater as described above. A reagent blank served as reference and aged seawater as the background control. Dissolved DNA and RNA concentrations were translated into P concentrations by multiplication by a factor of 2.06 nmol P for 1 µg dDNA and 2.55 nmol P for 1 µg dRNA, detected by DP determination in the microwave. Hereafter, these amounts are referred to as dDNA-P and dRNA-P. The detection limit was 10–20 ng for DNA and 250–500 ng for RNA.

The concentrations of the measured DOP fractions (dATP, dPL-P, dDNA-P and dRNA-P) were totaled and the amount subtracted from the total DOP concentration. The difference is defined as the uncharacterized DOP.

2.4 [^{33}P]PO_4 uptake and transformation

Total [^{33}P]-activity was measured in 1-ml volumes of each of the nine sub-samplings by liquid scintillation counting (Tri-Carb 2800TR, Perkin Elmer).

[^{33}P]PO_4 incorporated in *Nodularia* filaments was determined by filtering a 5-ml subsample onto 0.2 µm polycarbonate (PC) filters pre-soaked with a 20 mM cold PO_4 solution. The filters were rinsed with 5 × 1 ml of particle-free aged seawater. Dissolved inorganic and organic [^{33}P]-phosphorus were distinguished according to the method described by Ammerman (1993) for the uptake of dissolved ATP. To detect the total dissolved activity, 1 ml of the filtrate (filtrate 1) was transferred into scintillation vials for counting. Activated charcoal (20 mg) and 1 ml 0.03 N H_2SO_4 were then added to the remaining 4-ml filtrate; the mixture was shaken for 15 min and then filtered through 0.45 µm filters. One ml of this filtrate (filtrate 2) was counted again. Filtrate 1 contained inorganic and organic bound [^{33}P]PO_4; in filtrate 2, organic phosphorus was removed

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on activated charcoal, leaving only inorganic [^{33}P]PO_4. Organic, bound [^{33}P]PO_4 was calculated as the difference between filtrates 1 and 2. The procedure was repeated two times.

In preliminary tests with sterile Milli-Q and aged seawater, the dilution of [^{33}P] by the addition of 1 ml 0.03 N H_2SO_4 and non-specific binding to charcoal were checked, indicating that 25 % of the variation can be explained by these effects. Therefore, the values of filtrate 2 were corrected by this amount.

2.5 Data and statistical analyses

The data and illustrations shown represent the average values of the three parallel incubations, except one outlier (sample: low-II at day 9) in which double the amount of PO_4 was inadvertently added. Statistical significance was tested by an unpaired *t*-test, with a significance level of $p < 0.05$. Prior to the t-test, the data were tested for normality by the Shapiro–Wilk test. If the normality test failed, a Mann–Whitney rank sum test was used. Correlation analyses were performed using Spearman's rank test, assuming a significant correlation as a correlation coefficient $|R| > 0.6$, and $p < 0.001$. The operations were performed with "Sigma Plot 11" (Systat Software Inc.).

3 Results

3.1 Carbonate system

Average pH values for the low, medium and high pCO_2 treatments were 8.15 ± 0.08, 8.09 ± 0.09, and 7.99 ± 0.07, respectively. The high pCO_2 treatment differed significantly from the low ($p < 0.001$, $n = 12$) and medium pCO_2 treatments ($p = 0.009$, $n = 12$). The means of the corresponding C_T values were 1598 ± 35, 1613 ± 30, and 1648 ± 27 µmol kg^{-1}, respectively. The differences in C_T between the low and high as well as the medium and high pCO_2 treatments were significant ($p > 0.001$ and $p = 0.006$,

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n = 12). The calculated values for pCO_2 and A_T (total alkalinity) resulted in an average of 341 ± 81 µatm pCO_2 and 1701 ± 19 µmol kg^{-1} A_T in the low pCO_2 treatment, 399 ± 104 µatm pCO_2 and 1701 ± 9 µmol kg^{-1} A_T in the medium pCO_2 treatment, and 508 ± 90 µatm pCO_2 and 1714±13 µmol kg^{-1} A_T in the high pCO_2 treatment. The significance of the differences in pCO_2 between the low and high as well as the medium and high pCO_2 treatments ($p < 0.001$ and $p = 0.009$, n = 12) was verified by a t-test (Fig. 1).

3.2 *Nodularia* growth and bacterial occurrence

A detailed description of *Nodularia* abundance, filament length, and number of heterocysts in response to changing pCO_2 is given in Wannicke et al. (2012). Briefly summarized, the abundance of *Nodularia spumigena* increased by a factor of 2.5, 3.4, and 8.5 in the low, medium, and high pCO_2 treatment, respectively, until day 9. Afterwards, cyanobacterial growth under low and medium pCO_2 proceeded at a lower rate. At high pCO_2, the abundance declined slightly. A similar trend was observed for chlorophyll *a* (Chl *a*). Additionally, the Chl *a* concentration increased by a factor of 6.1 at low pCO_2, 5.9 at medium pCO_2, and 10.2 at high pCO_2 until day 9 and then dropped, regardless of the CO_2 concentration. Accordingly, the period between day 0 and day 9 was considered to be the growth phase (Fig. 2). During the total time of the experiment, *Nodularia* abundance positively correlated with Chl *a*, *Nodularia*-P, POC, and PON ($|R|$ = 0.741, 0.86, 0.841, and 0.888, $p < 0.001$, n = 36).

A comparison of the growth in the small and the large bottles showed no differences. Significant correlations between both experimental set-ups were thus determined ($p < 0.001$, n = 36) for Chl *a* ($|R|$ = 0.76), POC ($|R|$ = 0.817) and *Nodularia*-P ($|R|$ = 0.798).

Heterotrophic bacterial abundance in the low, medium, and high treatments was $4.69\times10^5\pm1.64$, $4.54\times10^5\pm1.59$, and $4.73\times10^5\pm1.28$ cells l^{-1}, respectively. There was no significant increase in abundance of heterotrophic bacteria. Moreover, heterotrophic bacterial abundance did not correlate with any other parameters. Thus, the influence of heterotrophic bacteria could be neglected.

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3.3 Phosphorus pool

The initial TP concentration (day 0) was 0.83 ± 0.3, 0.91 ± 0.08, and 0.87 ± 0.08 µmol l^{-1} in the low, medium, and high pCO_2 treatment, respectively. An increase of around 0.36 µmol l^{-1} at day 9 was due to the additional PO_4 supply after sampling at day 3 (Table 1). The initial concentrations of the DP pool under low, medium, and high pCO_2 were, respectively, 0.63 ± 0.04, 0.65 ± 0.02, and 0.72 ± 0.04 µmol l^{-1}. During the 15-day incubation, the DP concentration declined by 0.35, 0.44, and 0.50 µmol l^{-1}. Both the initial amount of DIP and the additional amount of DIP after sampling at day 3 were taken up completely by *Nodularia*. From day 3 onwards, DIP concentrations were below the detection limit (Table 1). Differences in DIP uptake between the respective pCO_2 treatments were not significant. DIP correlated negatively with *Nodularia*-P, POC, PON, Chl *a*, and *Nodularia* abundance ($|R| = -0.843$, -0.839, -0.854, -0.822, and -0.834, $p < 0.001$, $n = 35$). *Nodularia*-P increased in all treatments over the course of the experiment. The increment during the growth phase accounted for 0.59, 0.70, and 0.77 µmol *Nodularia*-P l^{-1} for the low, medium and high treatments. The amounts obtained in response to medium and high pCO_2 were 1.19- and 1.30-fold higher than those measured under low pCO_2 (Fig. 3a), but differences were not significant. At day 15, there was a slight increase in the *Nodularia*-P concentration in all pCO_2 treatments. *Nodularia*-P correlated negatively with DOP ($|R| = -0.844$, $p < 0.001$, $n = 36$) and positively with APA ($|R| = 0.824$, $p < 0.001$, $n = 36$).

During the first 3 days, DOP concentrations increased slightly in the low and medium pCO_2 treatments by about 0.05 and 0.02 µmol l^{-1}, respectively, suggesting that DOP was produced by *Nodularia* at the beginning of the experiment. Under high pCO_2, the DOP concentration decreased already from day 0 until day 3 by 0.10 µmol l^{-1}. From this point on, the DOP concentration continued to decrease in the high pCO_2 treatment and also in the two other pCO_2 treatments. During the growth phase, DOP utilization varied as a function of pCO_2, with concentrations declined by 0.14, 0.09, and 0.06 µmol l^{-1} at high, medium, and low pCO_2, respectively (Fig. 4a). However, DOP uptake differed

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significantly only between the high and low pCO_2 treatments ($p = 0.04$, $n = 12$). The decrement in DIP plus DOP by 0.64, 0.69, and 0.77 µmol l^{-1} under low, medium, and high pCO_2, respectively, was reflected in the increase of *Nodularia*-P by nearly the same amount (Fig. 3a). The parallel decline of DIP and DOP indicated the utilization of both pools, as confirmed by the positive correlation between both ($|R| = 0.675$; $p < 0.001$, $n = 35$).

3.4 DOP components

3.4.1 Dissolved ATP

On sampling days 0 and day 3, dATP-P concentrations remained constant, at 2.5 ± 0.4 nmol l^{-1}, in all treatments and accounted for 0.7 ± 0.2 % of total DOP. On day 9 dATP-P concentrations escalated by 5.61, 5.23, and 5.60 nmol l^{-1} at low, medium, and high pCO_2, thus comprising 3.1, 3.4, and 3.3 %, respectively, of the total amount of DOP. However, at day 15 the dATP-P concentration was reduced by 3.2 (low pCO_2), 2.8 (medium pCO_2), and 3.0 (high pCO_2) nmol l^{-1} (Fig. 4e), without significant differences between treatments. The dATP-P concentration correlated positively with *Nodularia*-P, POC, PON, and *Nodularia* abundance ($|R| = 0.673$, 0.768, 0.816, 0.727, $p < 0.001$, $n = 36$) and negatively with DIP ($|R| = -0.736$, $p < 0.001$, $n = 35$).

3.4.2 Dissolved phospholipids

The initial concentrations of dPL-P were 6.7, 10.5, and 15.2 nmol l^{-1} at low, medium, and high pCO_2, accounting for 2, 3.2, and 4 % of total DOP, respectively. The concentrations of dPL-P in the low and medium treatments increased over the first 3 days by a mean of 10.5 and 17.8 nmol l^{-1}, respectively, without significant differences between treatments. From day 3 onwards, dPL-P concentrations decreased again, until they leveled out around the initial concentrations (10.1 and 14.5 nmol l^{-1}, respectively). In contrast, dPL-P concentrations in the high pCO_2 treatment were constant at around

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15.9 ± 0.6 nmol l^{-1} until day 9, with a slight increase by 2.9 nmol l^{-1} at day 15 (Fig. 4d). By this time, the proportion contributed by dPL-P to total DOP was higher in all three treatments (4.2, 7.6, and 9.3 %, for low, medium, and high, respectively), mainly due to the decrease of total DOP. However, neither a significant difference between the treatments nor a correlation to any other parameter was noted.

3.4.3 Dissolved DNA

Overall, the concentration of dDNA-P was very low and represented only a very small proportion of total DOP (0.07 ± 0.01 %). The concentration on day 0 was 0.28, 0.27, and 0.31 nmol l^{-1} at low, medium, and high pCO_2, respectively. At day 9, the dDNA-P concentration was reduced by half, to 0.14, 0.14, and 0.16 nmol l^{-1}, respectively, and remained constant afterwards (Fig. 4f). Dissolved DNA-P correlated positively with DOP ($|R| = 0.727$, $p < 0.001$, $n = 36$) and negatively with POP, POC, and PON ($|R| = -0.836, -0.637, -0.688$, $p < 0.001$, $n = 36$).

3.4.4 Dissolved RNA

Starting concentrations of dRNA at low, medium, and high pCO_2 were 108, 106, and 145 nmol l^{-1}, constituting 32, 32, and 38 % of total DOP, respectively. In the low and medium pCO_2 treatments, the concentration increased until day 3, by 46 and 55 nmol l^{-1}, respectively, and then fluctuated around 150.2 ± 3.4 nmol l^{-1} (low) and 150.5 ± 14.3 nmol l^{-1} (medium). In contrast, the dRNA concentration of the high pCO_2 treatment first slightly decreased, by 14.5 nmol l^{-1}, before increasing by 35 nmol l^{-1} at day 9, remaining constant thereafter (Fig. 4c). Thus, the dRNA-P concentrations increased over the course of the 15-day experiment, with the largest increment at low pCO_2 (by 41.7 nmol l^{-1}). At medium and high pCO_2 the increases were minor (28.1 and 12.8 nmol l^{-1}) and differences between both were not significant. Due to the decrease of DOP, the proportions of total DOP contributed by dRNA-P increased with

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time, finally reaching 63 % (low), 71 % (medium), and 77 % (high) of total DOP. Therefore, dRNA-P accounted for a major fraction of DOP.

3.4.5 Uncharacterized DOP

At the beginning of the experiment, uncharacterized DOP accounted for the majority of the total DOP, amounting to 65.1, 63.7, and 57.0 % at low, medium, and high pCO_2, respectively. During the growth phase of *Nodularia*, concentrations of uncharacterized DOP declined by 114.7, 150.3, and 171.2 nmol l^{-1}, respectively. This implied a decrease, albeit not significant, by a factor of 1.31 at medium pCO_2 and 1.49 at high pCO_2 compared to the low pCO_2 treatment. Until day 15, the decrement proceeded, progressively reducing the proportions of uncharacterized DOP to 30.8 % (low), 18.6 % (medium), and 11.7 % (high) of total DOP (Fig. 4b). The uncharacterized DOP fraction correlated positively with dDNA-P ($|R| = 0.738$, $p < 0.001$, $n = 36$) and negatively with *Nodularia*-P, *Nodularia* abundance, and APA ($|R| = -0.82, -0.682, -0.681$; $p < 0.001$, $n = 36$). This correlation analysis supports the finding that uncharacterized DOP was the largest fraction of DOP and thus served as the main source of *Nodularia*-P after DIP depletion.

3.5 $[^{33}P]PO_4$ uptake and transformation

The distribution of $[^{33}P]$ in the three fractions, *Nodularia*-P, DOP, and DIP, was similar in all CO_2 treatments during the incubation, despite a few deviations. $[^{33}P]PO_4$ was incorporated into biomass during the growth phase (Fig. 5), with 15.9–26.3 % of the added $[^{33}P]PO_4$ occurring in *Nodularia* in all treatments after 3.5 h. Thereafter, $[^{33}P]$ fixation seemed to be faster in the medium and high pCO_2 treatments, based on a mean at day 3 of 74 % and 58 %, respectively, detected in the biomass compared to 28 % in the low pCO_2 treatment. At day 9, nearly the whole $[^{33}P]PO_4$ (94.7–97.8 %) was fixed into biomass independent from the pCO_2 treatment. The stagnation or decline of the cyanobacteria population at day 15 (Fig. 2) was accompanied by a strong decrease in

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cellular P in the medium pCO_2 treatment and a weak decrease in the high pCO_2 treatment. In the low treatment, the proportion of [^{33}P]PO_4 in *Nodularia* remained as high as at the previous sampling time. Thus, P-turnover was faster under medium and high than under low pCO_2 conditions. [^{33}P] was released from *Nodularia* predominantly as DIP, with only a small proportion occurring as DOP (Fig. 5a, c, d). The 81 % decrease in biomass detected in the medium treatment was combined with a release of 78 % as DIP and 3 % as DOP. The phosphorus decrease of 6 % in the high treatment consisted of 5 % as DIP and to 1 % as DOP.

The transformation of [^{33}P]PO_4 into DOP by *Nodularia* was generally low (1.4–7.7 %), with most already released after 3.5 h. The calculation of DIP transformed into DOP based on the DIP additions indicated that the DIP conversion involved nanomolar concentration ranges (Table 5), in agreement with the decline of DOP (Fig. 3).

4 Discussion

This joint study investigated *Nodularia spumigena* growth as well as carbon, nitrogen, and phosphorus transformation under different pCO_2 conditions. Part I focused on growth, production and nitrogen cycling (Wannicke et al., 2012). Part II dealt with exudation and extracellular enzyme activities (Endres et al., 2012). Here (part III), we discuss the turnover of the dissolved P pool, including DOP composition, to illustrate the P-based nutrition of *Nodularia* and its P transformation processes.

4.1 CO_2 effects on phosphorus nutrition of *Nodularia spumigena*

During the first 9 days of the experiment, *Nodularia* growth was significantly enhanced with increasing pCO_2, as evidenced by the increases in Chl *a*, POC, PON, and filament abundances. Carbon and nitrogen fixation rates were stimulated as well (Wannicke et al., 2012). The response of *N. spumigena* to pCO_2 elevation was similar to that reported for the oceanic filamentous cyanobacterium *Trichodesmium* (Barcelos e

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Ramos et al., 2007; Hutchins et al., 2007; Levitan et al., 2007). When DIP reached the detection limit, *Nodularia* obtained P from DOP. While the DOP concentration decreased in all treatments, the decline was strongest at medium and especially at high pCO_2. Dissolved phosphorus (DIP and DOP) uptake was reflected in the P content of *Nodularia* (Fig. 3a, 5). In the [^{33}P]-experiments, during the growth phase of *Nodularia* nearly all of the DIP (95–98 %) was fixed in biomass. The reason might be different for the higher amount of [^{33}P]PO_4 retention in the low and high treatments compared to the medium treatment, such as differences in the growth rate. In the low pCO_2 treatment, growth and [^{33}P]PO_4 incorporation were slower than in the high pCO_2 treatment such that senescence, in which P is released, might not have been reached within the time limits of the experiment. In the high treatment, the greater P demand of *Nodularia* could have caused the persistence of P in the cells. While this hypothesis has to be supported by further experiments, a similar increase in the P demand of *Nodularia* will be likely under the conditions predicted for the Baltic Sea. Even the slight elevation in pCO_2 in our experiment, from 341 to 391 µatm, was shown to have a stimulating effect. Accordingly, small variations in the present pCO_2 (spatially and temporally) can be expected to influence *Nodularia* growth, nitrogen fixation, and P demand. Currently, the pCO_2 in the central Baltic Sea ranges between 120 and 250 µatm during the summer season. Thus, elevation of atmospheric pCO_2 might result in a lower pCO_2 level in seawater as assumed in our experiments.

4.2 DOP as phosphorus source

The ability of phytoplankton to utilize DOP as an alternative P source was frequently demonstrated in earlier studies (e.g. Currie and Kalff, 1984; Cotner and Wetzel, 1992; Dyhrman et al., 2006). In fact, it is generally accepted that DOP is the main P source when DIP is exhausted (Nausch and Nausch, 2004). Accordingly, a high alkaline phosphatase activity (APA) is indicative of DOP utilization (Cembella et al., 1984; Nausch,

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1998; Hoppe, 2003) and is often used as an indicator of P stress, especially in association with cyanobacteria (Paasche and Erga, 1988; Wu et al., 2012).

In this joint experiment, DIP depletion and the decrease in DOP occurred in parallel with enhanced APA, as previously reported by Endres et al. (2012). We not only confirmed the negative correlation between APA and DIP and DOP ($|R| = -0.852$, $n = 35$, and -0.635, $n = 36$, $p < 0.001$) but also found similar degrees of change in APA and DOP (by 2.4- and 1.5-fold) in the high and medium pCO_2 treatments, respectively, versus the low pCO_2 treatment. This is an additional indication for the enhanced P-demand of *Nodularia* with increasing pCO_2.

AP preferentially hydrolyzes phosphomonoester bonds, cleaving orthophosphate from the organic moiety and making it available for cellular assimilation (Sebastián et al., 2004). ATP, DNA, RNA and PL, as the most P-rich organic compounds, are cycled differently due to their varying reactivity (Kolowith et al., 2001). ATP, a phosphoanhydride, is hydrolyzable by AP (Hernández et al., 1996; Hansen and Heath, 2005). Phosphate is cleaved from DNA and RNA by AP at the 5′ terminal end (Hino, 1989) rather than from within the DNA or RNA strands. Phospholipids react with AP only if they contain a phosphate monoester group at the C_3 position of glycerol (Blank and Snyder, 1970). In contrast to these DOP compounds, phosphomonoesters (e.g. sugar phosphates) are easily cleaved by AP and thereby are able to significantly contribute to the bioavailable DOP pool and, therefore, to P-nutrition. Labry et al. (2005) reported that in the Gironde plume phosphomonoester concentrations make up between 11 and 65 % of the total DOP pool. In our study, an uncharacterized DOP proportion accounted for 20.4 to 61.9 % of total DOP, and may be attributed to phosphomonoesters that were not determined during this study. The importance of this pool of uncharacterized DOP derives from the fact that it accounted for the bulk of P-nutrition in *Nodularia*, a finding supported by the negative correlation with *Nodularia*-P and APA ($|R| = -0.82$ and -0.681, $p < 0.001$, $n = 36$) as well as the positive correlation with DOP ($|R| = 0.932$, $p < 0.001$, $n = 36$).

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4.3 Composition of DOP in the presence of *Nodularia spumigena*

In the laboratory experiment described herein, the DOP concentration and the composition of aged Baltic Sea water changed during the development of *N. spumigena*. Our methods allowed direct determination of dissolved ATP (Björkman and Karl, 2001), dissolved phospholipids (Suzumura and Ingall, 2001, 2004), and dissolved DNA and RNA (Karl and Bailiff, 1989) concentrations. The sum of these components amounted to 38.1–79.6 % of the total DOP during the 15-day incubation.

Dissolved ATP concentrations in the batch culture experiments of this study ranged from 399 to 1563 ng l^{-1} and constituted 0.6–3.4 % of the DOP pool and thus were higher than those reported by Azam and Hodson (1977) in surface waters at the coast of Southern California by a factor of 1.8 to 24.0. Nawrocki and Karl (1989) reported values similar to ours, i.e. between 23 and 1278 ng l^{-1}, in the upper 100 m of five stations in the Bransfield Strait whereas the concentrations measured by Björkman and Karl (2001) in the subtropical North Pacific gyre were lower (Table 2).

As noted by Suzumura (2005), information on the distribution and abundance of lipid P in marine environments is still lacking. The studies carried out thus far have been limited to the distribution and abundance of dissolved lipids, e.g. phospholipids, in the North Atlantic Ocean, Tokyo Bay, Corpus Christi Bay, and Pacific Ocean (Parrish, 1987; Suzumura and Ingall, 2001, 2004). Ours is the first such study of the Baltic Sea. Moreover, the determined concentrations of lipid P as well as the percentage of total organic P are in agreement with the values reported for the dissolved fraction in pelagic seawater by Suzumura and Ingall (2004) (Table 3).

Dissolved DNA concentrations were very low throughout the experiment, accounting for a very small proportion of DOP (~ 0.08 %). In fact, they were 8800 times lower than the measured dDNA concentrations from other marine and freshwater areas and therefore can be considered as negligible. The Northern Adriatic Sea is the only site with similarly low dDNA concentrations (Table 4). As suggested by Paul et al. (1990), actively growing phytoplankton might produce small to undetectable amounts of dDNA,

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with production occurring only in senescent phytoplankton cells. Another plausible explanation is that of Løvdal et al. (2007), who measured accelerated turnover times of dDNA under conditions of P starvation of about 1.5 h instead of 15.6 h under balanced conditions. This and the observed uptake of dDNA-P by *Nodularia* in our studies lead to the assumption that dDNA-P is rapidly used within hours, as it could not be detected with our sampling strategy.

Dissolved RNA has hardly been investigated in aquatic environments. Karl and Bailiff (1989) measured concentrations ranging from 4.03 to 51.1 $\mu g\,l^{-1}$ at several stations near Hawaii and the open Pacific Ocean in March 1988. These concentrations are in the lower range of those determined in our study (26–83 $\mu g\,l^{-1}$) (Table 4).

4.4 CO_2 effects on DOP components

Our results demonstrate that the various compounds within the DOP pool developed differentially over time and with pCO_2. The dynamic of dATP-P seems to be not or only marginally influenced by pCO_2. Dissolved ATP-P did not differ significantly between pCO_2 treatments at day 3 and day 9, despite. From day 9 to day 15, the decline in dATP-P (by a mean of 3.04 ± 0.22 nmol l^{-1}; Fig. 4e) followed the trend of the entire DOP pool. At this stage, *Nodularia* cells were in stationary phase, exhibiting the first signs of decay at day 15. Therefore, rather than the consumption of dATP-P and DOP, a release of the latter would be expected and suggests the continued need of living *Nodularia* cells for P after the more readily available DOP compounds have been consumed. We have no evidence that dATP is used by heterotophic bacteria, given that their abundances remained at the same low levels as at the start of the experiment.

Under low and medium pCO_2 conditions, dPL-P was released by *Nodularia* from day 0 to day 3, during the period of DIP uptake. Afterwards, dPL-P was utilized by the cyanobacterial cells probably because of the strengthened P demand. Thus, for these two treatments approximately the same concentrations were detected at the start and end of the experiment. In comparison, under high pCO_2 conditions a temporary dPL-P elevation was observed at day 15 and not before. We sampled in intervals of several

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days and therefore, it is possible that short time elevations of dPL-P were unascertainable before day 15. In contrast to dATP-P, dPL-P was released under high pCO_2, when *Nodularia* growth reached stationary phase or the cells became senescent (Fig. 4d).

The starting concentration of dDNA-P in all treatments was 0.29 ± 0.02 nmol l^{-1} and leveled out at 0.14 ± 0.04 nmol l^{-1} at the end of the 15-day experiment. The uptake of dDNA-P, even though in the nanomolar range, seemed to be due to the P-rich nature of DNA (Sterner and Elser, 2002) and the strengthened P demand. Throughout the experiment, dDNA-P values were generally low and did not differ significantly between the three pCO_2 treatments (Fig. 4f). The turnover of dDNA-P and other DOP compounds is very short, occurring within hours, so that shorter sampling intervals would have been necessary to estimate the variations. In addition, as mentioned above, Paul et al. (1990) reported low dDNA production during the phytoplankton growth phase. The authors assumed that phytoplankton DNA synthesis primarily occurred at night or that dDNA was released by senescent, dying, or grazed phytoplankton cells. If the synthesis and release of dDNA occur only at night, our sampling time, between 8 and 9 am, would have been unable to detect these changes in dDNA.

In our study, dRNA-P was relatively constant in all pCO_2 treatments and was one of the main contributors to total DOP. However, regardless of the pCO_2 the dRNA-P concentrations were lower at the beginning of the experiment than in the following sampling days. From day 0 to day 9, dRNA-P production was highest in the medium treatment, followed by the low and high treatments (49.3, 39.1, and 20.4 nmol l^{-1}, respectively; Fig. 3b). Over the course of the experiment dRNA-P production was highest in the low treatment whereas release was lowest in the high treatment (41.7 and 12.8 nmol l^{-1} dRNA-P, respectively), suggesting that with an elevated pCO_2 the release of dRNA-P is reduced due to the strengthened P demand (Fig. 4c).

Overall, DOP is produced as long as either DIP is available or a certain intracellular P-pool exists. We found only a few trends but no significant effects of variable pCO_2 conditions on single metabolic components. Only the total DOP concentration differed

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significantly as a function of pCO_2 from day 0 to day 9, due to changes in the amounts of compounds other than those specifically detected herein.

5 Conclusions

Our results indicate that accelerated P turnover can be expected during the cyanobacterial growth period under the pCO_2 conditions predicted for the future Baltic Sea. This implies the faster utilization of DIP as well as DOP. We propose that the stimulating effect on P utilization by the filamentous cyanobacterium *Nodularia spumigena* is indirect, as it is mediated by elevated carbon fixation and is dependent on cyanobacterial growth, which induces a stronger P demand.

There is no trend towards the greater use of dissolved ATP-P, PL-P, RNA-P, and DNA-P under high pCO_2 conditions, but it remains to be confirmed in further investigations. These should include higher sampling resolution to capture the change from release to uptake. Components other than those measured in this study were taken up more intensively and dominated the decrease in the total DOP pool.

Supplementary material related to this article is available online at: http://www.biogeosciences-discuss.net/9/14709/2012/bgd-9-14709-2012-supplement.pdf.

Acknowledgement. We are grateful to Bernd Schneider for advice and Hildegard Kubsch for the analysis of the carbonate system. We also like to thank Doris Setzkorn, Sven Trinkler, Birgit Sadkowiak and Iris Liskow for technical assistance. This study was financially supported by the BMBF funded project BIOACID (03F0608F) in subproject 1.2.3 (M. Nausch).

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References

Ammerman, J. W.: Microbial cycling of inorganic and organic phosphorus in the water column, in: Kemp, P. F., Sherr, B. F., Sherr, E. B., Cole, J. J. (eds.): Handbook of methods in aquatic microbial ecology, Lewis Publishers, Boca Raton, FL, 649–660, 1993.

Azam, F. and Hodson, R. E.: Dissolved ATP in the sea and its utilisation by marine bacteria, Nature, 267, 696–698, 1977.

Bailiff, M. D. and Karl, D. M.: Dissolved DNA concentrations in the Bransfield Strait during spring bloom conditions, Eos, 68, 1686, 1987.

Barcelos e Ramos, J., Biswas, H., Schulz, K. G., LaRoche, J., and Riebesell, U.: Effect of rising atmospheric carbon dioxide on the marine nitrogen fixer *Trichodesmium*, Global Biogeochem. Cy., 21, GB2028, doi:10.1029/2006GB002898, 2007.

Benitez-Nelson, C. R.: The biogeochemical cycling of phosphorus in marine systems, Earth-Sci. Rev., 51, 109–135, 2000.

Bentzen, E. and Taylor, W. D.: Estimating organic P utilization by freshwater plankton using [^{32}P]ATP, J. Plankton Res., 13, 1223–1238, 1991.

Björkman, K. M. and Karl, D. M.: A novel method for the measurement of dissolved adenosine and guanosine triphosphate in aquatic habitats: applications to marine microbial ecology, J. Microbiol. Meth., 47, 159–167, 2001.

Blank, M. L. and Snyder, F.: Specificities of alkaline and acid phosphatases in the dephosphorylation of phospholipids, Biochemistry, 9, 5034–5036, 1970.

Breter, H. J., Kurelec, B., Müller, W. E. G., and Zahn, R. K.: Thymine content of sea water as a measure of biosynthetic potential, Mar. Biol., 40, 1–8, 1977.

Burkhardt, S., Riebesell, U., and Zondervan, I.: Effects of growth rate, CO_2 concentration, and cell size on the stable carbon isotope fractionation in marine phytoplankton, Geochim. Cosmochim. Ac., 63, 3729–3741, 1999.

Casey, J. R., Lomas, M. W., Michelou, V. K., Dyhrman, S. T., Orchard, E. D., Ammerman, J. W., and Sylvan, J. B.: Phytoplankton taxon-specific orthophosphate (P*i*) and ATP utilization in the western subtropical North Atlantic, Aquat. Microb. Ecol., 58, 31–44, 2009.

Cembella, A. D., Antia, N. J., Harrison, P. J., and Rhee, G.-Y: The Utilization of Inorganic and Organic Phosphorous Compounds as Nutrients by Eukaryotic Microalgae: A Multidisciplinary Perspective: Part 2, Crit. Rev. Microbiol., 11, 13–81, doi:10.3109/10408418409105902, 1984.

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Cotner, J. B. and Wetzel, R. G.: Uptake of dissolved inorganic and organic bphosphorus compounds by phytoplankton and bacterioplankton, Limnol. Oceanogr., 37, 232–243, 1992.
Currie, D. J., and Kalff, J.: A comparison of the abilities of freshwater algae and bacteria to acquire and retain phosphorus, Limnol. Oceanogr,. 29, 298–310, 1984.
Czerny, J., Barcelos e Ramos, J., and Riebesell, U.: Influence of elevated CO_2 concentrations on cell division and nitrogen fixation rates in the bloom-forming cyanobacterium *Nodularia spumigena*, Biogeosciences, 6, 1865–1875, doi:10.5194/bg-6-1865-2009, 2009.
DeFlaun, M. F., Paul, J. H., and Davis, D.: Simplified Method for Dissolved DNA Determination in Aquatic Environments, Appl. Environ. Microb., 52, 654–659, 1986.
DeFlaun, M. F., Paul, J. H., and Jeffrey, W. H.: Distribution and molecular weight of dissolved DNA in subtropical estuarine and oceanic environments, Mar. Ecol.-Prog. Ser., 38, 65–73, 1987.
Degerholm, J., Gundersen, K., Bergman, B., and Söderbäck, E.: Phosphorus-limited growth dynamics in two Baltic Sea cyanobacteria, Nodularia sp. and Aphanizomenon sp., FEMS Microbiol. Ecol., 58: 323–332, doi:10.1111/j.1574-6941.2006.00180.x, 2006.
Doney, S. C., Fabry, V. J., Feely, R. A., and Kleypas, J. A.: Ocean acidification: the other CO_2 problem, Annu. Rev. Mar. Sci., 1, 169–192, 2009.
Dortch, Q., Roberts, T. L., Clayton, J. R. Jr., and Ahmed, S. I.: RNA/DNA ratios and DNA concentrations as indicators of growth rate and biomass in planktonic marine organisms, Mar. Ecol.-Prog. Ser., 13, 61–71, 1983.
Dyhrman, S. T. and Ruttenberg, K. C.: Presence and regulation of alkaline phosphatase activity in eukaryotic phytoplankton from the coastal ocean: Implications for dissolved organic phosphorus remineralization, Limnol. Oceanogr., 51, 1381–1390, 2006.
Elser, J. J., Bracken, M. E. S., Cleland, E. E., Gruner, D. S., Harpole, W. S., Hillebrand, H., Ngai, J. T., Seabloom, E. W., Shurin, J. B., and Smith, J. E.: Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems, Ecol. Lett., 10, 1135–1142, 2007.
Endres, S., Unger, J., Wannicke, N., Nausch, M., Voss, M., and Engel, A.: Response of *Nodularia spumigena* to pCO_2 – Part 2: Exudation and extracellular enzyme activities, Biogeosciences Discuss., 9, 5109–5151, doi:10.5194/bgd-9-5109-2012, 2012.
Finni, T., Kononen, K., Olsonen, R., and Wallström, K.: The history of Cyanobacterial blooms in the Baltic Sea, AMBIO, 30, 172–178, 2001.

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9, 14709–14750, 2012

Fu, F.-X., Warner, M. E., Zhang, Y., Feng, Y., and Hutchins, D. A.: Effects of increased temperature and CO_2 on photosynthesis, growth, and elemental ratios in marine *Synechococcus* and *Prochlorococcus* (cyanobacteria), J. Phycol., 43, 485–496, 2007.

Gasol, J. M. and del Giorgio, P. A.: Using flow cytometry for counting natural planktonic bacteria and understanding the structure of planktonic bacterial communities, Sci. Mar., 64, 197–224, doi:10.3989/scimar.2000.64n2197, 2000.

Grasshoff, K., Ehrhardt, M., and Kremling, K.: Methods of Seawater Analysis, 2nd Edn., Verlag Chemie, Weinheim, 1983.

Hansen, D. L. and Heath, R. T.: Adenosine triphosphate (ATP) as a source of phosphorus and carbon to hypolimnetic and epilimnetic freshwater plankton communities in a stratified, hardwater lake, Ann. Conf. Great Lakes Res., 48, 2005.

HELCOM: Atmospheric supply of nitrogen, lead, cadmium, mercury and lindane to the Baltic Sea over the period 1996–2000, Balt. Sea Environ. Proc., 101B, 1–75, 2005.

Hernández, I., Hwang, S.-J., and Heath, R. T.: Measurement of phosphomonoesterase activity with a radiolabeled glucose-6-phosphate. Role in the phosphorus requirement of phytoplankton and bacterioplankton in a temperate mesotrophic lake, Arch. Hydrobiol., 137, 265–280, 1996.

Hino, S.: Characterization of orthophosphate release from dissolved organic phosphorus by gel filtration and several hydrolytic enzymes, Hydrobiologia, 174, 49–55, doi:10.1007/bf00006057, 1989.

Hodson, R. E., Maccubbin, A. E., and Pomeroy, L. R.: Dissolved adenosine triphosphate utilization by free-living and attached bacterioplankton, Mar. Biol., 64, 43–51, 1981.

Hönisch, B., Hemming, N. G., Archer, D., Siddall, M., and McManus, J. F.: Atmospheric carbon dioxide concentration across the Mid-Pleistocene transition, Science, 324, 1551–1554, 2009.

Hoppe, H.-G.: Phosphatase activity in the sea, Hydrobiologia, 493, 187–200, doi:10.1023/a:1025453918247, 2003.

Howarth, R. W.: Nutrient limitation of net primary production in marine ecosystems, Annu. Rev. Ecol. Syst., 19, 89–110, 1988.

Hutchins, D. A., Fu, F. X., Zhang, Y., Warner, M. E., Feng, Y., Portune, K., Bernhardt, P. W., and Mulholland, M. R.: CO_2 control of *Trichodesmium* N_2 fixation, photosynthesis, growth rates, and elemental ratios: implications for past, present, and future ocean biogeochemistry, Limnol. Oceanogr., 52, 1293–1304, 2007.

BGD
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Hutchins, D. A., Mulholland, M. R., and Fu, F.: Nutrient cycles and marine microbes in a CO_2-enriched ocean, Oceanography, 22, 128–145, 2009.
IPCC: Climate Change 2007: Impacts, Adaptation and Vulnerability. Contribution of working group II to the fourth assessment report of the Intergovernmental Panel on Climate Change, Cambridge, 2007.
Jeffrey, S. W. and Welschmeyer, N. A.: Spectrophotometric and fluorometric equations in common use in oceanography, in: Phytoplankton Pigments in Oceanography, edited by: Jeffrey, S. W., Mantoura, R. F. C., Wright, S. W., UNESCO Publishing, Paris, 597–615, 1997.
Johnson, K. M., Wills, K. D., Butler, D. B., Johnson, W. K., and Wong, C. S.: Coulometric total carbon dioxide analysis for marine studies: Maximizing the performance of an automated gas extraction system and coulometric detector, Mar. Chem., 44, 167–187, 1993.
Kahru, M., Horstmann, U., and Rud, O.: Satellite detection of increased cyanobacteria blooms in the Baltic Sea: natural fluctuations or ecosystem changes?, AMBIO, 23, 469–472, 1994.
Karl, D. M.: Aquatic ecology: phosphorus, the staff of life, Nature, 406, 31–33, 2000.
Karl, D. M. and Bailiff, M. D.: The measurement and distribution of dissolved nucleic acids in aquatic environments, Limnol. Oceanogr., 34, 543–558, 1989.
Karl, D. M. and Björkman, K. M.: Dynamics of dissolved organic phosphorus, in: Biogeochemistry of Marine Dissolved Organic Matter, edited by: Hansell, D. A., Carlson, C. A., Academic Press, Amsterdam, 249–366, 2002.
Kolowith, L., Ingall, E., and Benner, R.: Composition and cycling of marine organic phosphorus, Limnol. Oceanogr., 46, 309–320, 2001.
Labry, C., Delmas, D., and Herbland, A.: Phytoplankton and bacterial alkaline phosphatase activities in relation to phosphate and DOP availability within the Gironde plume waters (Bay of Biscay), J. Exp. Mar. Biol. Ecol., 318, 213–225, 2005.
Levitan, O., Rosenberg, G., Setlik, I., Setlikova, E., Grigel, J., Klepetar, J., Prasil, O., and Berman-Frank, I.: Elevated CO_2 enhances nitrogen fixation and growth in the marine cyanobacterium *Trichodesmium*, Glob. Change Biol., 13, 531–538, doi:10.1111/j.1365-2486.2006.01314.x, 2007.
Lewis, E. and Wallace, D. W. R.: Program developed for CO_2 system calculations, ORNL/CDIAC-105, Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, US Department of Energy, Oak Ridge, Tennessee, 1998.

BGD
9, 14709–14750, 2012

Løvdal, T., Tanaka, T., and Thingstad, F. T.: Algal–bacterial competition for phosphorus from dissolved DNA, ATP, and orthophosphate in a mesocosm experiment, Limnol. Oceanogr., 52, 1407–1419, 2007.
Miyata, K. and Hattori, A.: A simple fractionation method for determination of phosphorus components in phytoplankton: application to natural populations of phytoplankton in summer surface waters of Tokyo Bay, J. Oceanogr., 42, 255–265, 1986.
Murphy, J. and Riley, J. P.: A modified single solution method for the determination of phosphate in natural waters, Anal. Chim. Acta, 27, 31–36, 1962.
Nausch, M.: Alkaline phosphatase activities and the relationship to inorganic phosphate in the Pomeranian Bight (southern Baltic Sea), Aquat. Microb. Ecol., 16, 87–94, 1998.
Nausch, M. and Nausch, G.: Bacterial utilization of phosphorus pools after nitrogen and carbon amendment and its relation to alkaline phosphatase activity, Aquat. Microb. Ecol., 37, 237–245, 2004.
Nausch, M. and Nausch, G.: Dissolved phosphorus in the Baltic Sea – occurrence and relevance, J. Mar. Syst., 87, 37–46, 2011.
Nawrocki, M. P. and Karl, D. M.: Dissolved ATP turnover in the Bransfield Strait, Antarctica during a spring bloom, Mar. Ecol.-Prog. Ser., 57, 35–44, 1989.
Orchard, E. D., Ammerman, J. W., Lomas, M. W., and Dyhrman, S. T.: Dissolved inorganic and organic phosphorus uptake in *Trichodesmium* and the microbial community: the importance of phosphorus ester in the Sargasso Sea, Limnol. Oceanogr., 55, 1390–1399, 2010.
Orr, J. C., Fabry, V. J., Aumont, O., Bopp, L., Doney, S. C., Feely, R. A., Gnanadesikan, A., Gruber, N., Ishida, A., Joos, F., Key, R. M., Lindsay, K., Maier-Reimer, E., Matear, R., Monfray, P., Mouchet, A., Najjar, R. G., Plattner, G.-K., Rodgers, K. B., Sabine, C. L., Sarmiento, J. L., Schlitzer, R., Slater, R. D., Totterdell, I. J., Weirig, M.-F., Yamanaka, Y., and Yool, A.: Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms, Nature, 437, 681–686, 2005.
Paasche, E. and Erga, S. R.: Phosphorus and nitrogen limitation of phytoplankton in the inner Oslofjord (Norway), Sarsia, 73, 229–243, 1988.
Parrish, C. C.: Time series of particulate and dissolved lipid classes during spring phytoplankton blooms in Bedford Basin, a marine inlet, Mar. Ecol.-Prog. Ser., 35, 129–139, 1987.
Paul, J. H., Jeffrey, W. H., and Cannon, J. P.: Production of dissolved DNA, RNA, and protein by microbial populations in a Florida reservoir, Appl. Environ. Microb., 56, 2957–2962, 1990.

BGD
9, 14709–14750, 2012

Paytan, A. and McLaughlin, K.: The oceanic phosphorus cycle, Chem. Rev., 107, 563–576, 2007.
Pillai, T. N. V. and Ganguly, A. K.: Nucleic acid in the dissolved constituents of sea water, J. Mar. Biol. Assoc. India, 14, 384–390, 1972.
Riebesell, U.: Effects of CO_2 enrichment on marine phytoplankton, J. Oceanogr., 60, 719–729, 2004.
Riemann, L., Holmfeldt, K., and Titelman, J.: Importance of viral lysis and dissolved dna for bacterioplankton activity in a P-limited estuary, Northern Baltic Sea, Microb. Ecol., 57, 286–294, 2009.
Rohde, K. H. and Nehring, D.: Ausgewählte Methoden zur Bestimmung von Inhaltsstoffen im Meer- und Brackwasser, Geod. Geoph. Veröff., Reihe IV, 27, 1–68, 1979.
Ruttenberg, K. C. and Dyhrman, S. T.: Temporal and spatial variability of dissolved organic and inorganic phosphorus, and metrics of phosphorus bioavailability in an upwelling-dominated coastal system, J. Geophys. Res., 110, C10S13, doi:10.1029/2004JC002837, 2005.
Sebastián, M., Arístegui, J., Montero, M. F., Escanez, J., and Niell, F. X.: Alkaline phosphatase activity and its relationship to inorganic phosphorus in the transition zone of the North-western African upwelling system, Prog. Oceanogr., 62, 131–150, 2004.
Siegenthaler, U., Stocker, T. F., Monnin, E., Lüthi, D., Schwander, J., Stauffer, B., Raynaud, D., Barnola, J.-M., Fischer, H., Masson-Delmotte, V., and Jouzel, J.: Stable carbon cycle–climate relationship during the Late Pleistocene, Science, 310, 1313–1317, 2005.
Sivonen, K., Kononen, K., Carmichael, W. W., Dahlem, A. M., Rinehart, K. L., Kiviranta, J., and Niemela, S. I.: Occurrence of the hepatotoxic cyanobacterium *Nodularia spumigena* in the Baltic Sea and structure of the toxin, Appl. Environ. Microbiol., 55, 1990–1995, 1989.
Smith, S. V.: Phosphorus versus nitrogen limitation in the marine environment, Limnol. Oceanogr., 29, 1149–1160, 1984.
Sokal, R. R. and Rohlf, F. J.: Biometry: The Principles and Practice of Statistics in Biological Research, 3rd Ed., W. H. Freeman, New York, 1995.
Sterner, R. W. and Elser, J. J.: Ecological Stoichiometry: the Biology of Elements from Molecules to the Biosphere, Princeton University Press, Princeton, NJ, 2002.
Suzumura, M.: Phospholipids in marine environments: a review, Talanta, 66, 422–434, 2005.
Suzumura, M. and Ingall, E. D.: Concentrations of lipid phosphorus and its abundance in dissolved and particulate organic phosphorus in coastal seawater, Mar. Chem., 75, 141–149, 2001.

BGD
9, 14709–14750, 2012

Suzumura, M. and Ingall, E. D.: Distribution and dynamics of various forms of phosphorus in seawater: insights from field observations in the Pacific Ocean and a laboratory experiment, Deep-Sea Res. Pt. I, 51, 1113–1130, 2004.

Tanaka, T., Thingstad, T. F., Løvdal, T., Grossart, H.-P., Larsen, A., Allgaier, M., Meyerhöfer, M., Schulz, K. G., Wohlers, J., Zöllner, E., and Riebesell, U.: Availability of phosphate for phytoplankton and bacteria and of glucose for bacteria at different pCO_2 levels in a mesocosm study, Biogeosciences, 5, 669–678, doi:10.5194/bg-5-669-2008, 2008.

Utermöhl, H.: Zur Vervollkommnung der quantitativen Phytoplankton-Methodik, Ass. Intern. Limnol. Theor., 9, 1–38, 1958.

Vahtera, E., Laanemets, J., Pavelson, J., Huttunen, M., and Kononen, K.: Effect of upwelling on the pelagic environment and bloom-forming cyanobacteria in the western Gulf of Finland, Baltic Sea, J. Mar. Syst., 58, 67–82, 2005.

Vahtera, E., Laamanen, M., and Rintala, J.-M.: Use of different phosphorus sources by the bloom-forming cyanobacteria *Aphanizomenon flos-aquae* and *Nodularia spumigena*, Aquat. Microb. Ecol., 46, 225–237, 2007.

Wannicke, N., Endres, S., Engel, A., Grossart, H.-P., Nausch, M., Unger, J., and Voss, M.: Response of *Nodularia spumigena* to pCO_2 – Part 1: Growth, production and nitrogen cycling, Biogeosciences, 9, 2973–2988, doi:10.5194/bg-9-2973-2012, 2012.

Wasmund, N.: Occurrence of cyanobacterial blooms in the Baltic Sea in relation to environmental conditions, Int. Rev. ges. Hydrobio., 82, 169–184, 1997.

Wu, Z., Zeng, B., Li, R., and Song, L.: Physiological regulation of Cylindrospermopsis raciborskii (Nostocales, Cyanobacteria) in response to inorganic phosphorus limitation, Harmful Algae, 15, 53–58, doi:10.1016/j.hal.2011.11.005, 2012.

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Table 1. The phosphorus pool fractions at the four sampling times. Values are means and standard deviations of three replicates except for DIP where one replicate bottle was excluded (low-II at day 9).

Time	pCO_2 treatment	TP ($\mu mol\,l^{-1}$)	DP ($\mu mol\,l^{-1}$)	*Nodularia*-P ($\mu mol\,l^{-1}$)	DIP ($\mu mol\,l^{-1}$)	DOP ($\mu mol\,l^{-1}$)
Day 0	Low	0.83 ± 0.03	0.63 ± 0.04	0.20 ± 0.04	0.29 ± 0.02	0.34 ± 0.04
	Medium	0.91 ± 0.08	0.65 ± 0.02	0.26 ± 0.06	0.32 ± 0.08	0.33 ± 0.06
	High	0.87 ± 0.08	0.72 ± 0.04	0.15 ± 0.07	0.34 ± 0.02	0.38 ± 0.06
Day 3	Low	0.71 ± 0.05	0.31 ± 0.02	0.43 ± 0.03	0.05 ± 0.02	0.39 ± 0.20
	Medium	0.77 ± 0.05	0.27 ± 0.01	0.39 ± 0.15	0.03 ± 0.01	0.24 ± 0.02
	High	0.68 ± 0.03	0.32 ± 0.08	0.36 ± 0.09	0.04 ± 0.00	0.28 ± 0.07
Day 9	Low	1.21 ± 0.04	0.31 ± 0.12	0.80 ± 0.21	0.01 ± 0.01	0.27 ± 0.08
	Medium	1.20 ± 0.06	0.27 ± 0.10	0.93 ± 0.15	0.02 ± 0.02	0.25 ± 0.09
	High	1.20 ± 0.02	0.25 ± 0.05	0.96 ± 0.06	0.01 ± 0.00	0.23 ± 0.05
Day 15	Low	1.20 ± 0.21	0.27 ± 0.06	0.93 ± 0.25	0.03 ± 0.01	0.24 ± 0.07
	Medium	1.22 ± 0.06	0.21 ± 0.03	1.01 ± 0.06	0.02 ± 0.01	0.19 ± 0.02
	High	1.27 ± 0.07	0.22 ± 0.05	1.05 ± 0.06	0.02 ± 0.01	0.21 ± 0.04

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Table 2. Observed dATP values based on literature data from different oceanic regions.

Sample location	dATP (ng l^{-1})	Reference
Field observations		
Coast, Southern California (SIO pier to Point Loma)	65–218	Azam and Hodson (1977)
Saanich Inlet, British Columbia	466	Azam and Hodson (1977)
Gulf stream, Florida (range of several stations; 5 m)	22–306	Hodson et al. (1981)
Bransfield Strait, Antarctica (Jan 87; range of 5 stations, 0–100 m)	23–1278	Nawrocki and Karl (1989)
Subtropical North Pacific gyre (range of several stations, 5–125 m)	14.9–41.3	Björkman and Karl (2001)
Laboratory observations		
Aged Baltic Sea water (batch experiment)	356–1594	Present study

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Table 3. Lipid P concentrations and percentages of total organic P as shown by Suzumura (2005).

Sample	Sample description	Concentration	% of total organic P	Reference
Seawater (coastal)	Particulate (> 0.7 µm)	90–750 nM P	5.6–11.6	Miyata and Hattori (1986)
Seawater (coastal)	Dissolved (< 0.7 µm)	0.7–6.0 nM P	0.1–0.9	Suzumura and Ingall (2001)
	Particulate (> 0.7 µm)	31–294 nM P	3.0–13.5	Suzumura and Ingall (2001)
Seawater (pelagic)	Dissolved (< 0.7 µm)	4.0–17.9 nM P	1.7–17.6	Suzumura and Ingall (2004)
	Particulate (> 0.7 µm)	0.05–1.72 nM P	0.8–34.4	Suzumura and Ingall (2004)
Seawater (aged, batch experiment)	Dissolved (< 0.2 µm)	6.7–28.3 nM P	1.7–12.4	Present study

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Table 4. dDNA and dRNA values for marine and freshwater stations adapt from Karl and Bailiff (1989).

Sample location	dDNA ($\mu g\,l^{-1}$)	dRNA	Reference
Marine: coastal/estuarine			
Bombay Harbor, India (range of four stations)	13.4–80.6	–[b]	Pillai and Ganguly (1972)
Northern Adriatic Sea (range of two stations; 0–30 m)	0.05–0.8[a]	–	Breter et al. (1977)
Bayboro Harbor, Florida (Mar 86)	18.32 (±1.78)	–	DeFlaun et al. (1986)
Bransfield Strait, Antarctica (Dec 86; range of 69 stations)	6–15	–	Bailiff and Karl (1987)
Kaneohe Bay, Hawaii (Mar 88; range of four stations)	2.66–3.15	20.6–31.9	Karl and Bailiff (1989)
Mamala Bay, Hawaii (Mar 88)	1.02 (±0.08)	6.67 (±2.67)	Karl and Bailiff (1989)
Kahana Bay, Hawaii (Mar 88; water column)	4.70	51.1	Karl and Bailiff (1989)
Northern Baltic Sea, Sweden (Jun 06, range of four stations, 1 m)	1.3–2.6	–[b]	Riemann et al. (2009)
Marine: offshore			
N. Pacific Ocean (33° HN, 139° W; 0–400 m)	0.56–1.39	4.03–13.9	Karl and Bailiff (1989)
Freshwater			
Quarry Pond, Hawaii	3.54 (±0.03)	23.0 (±0.16)	Karl and Bailiff (1989)
Krauss Pond, Hawaii	88	871	Karl and Bailiff (1989)
Laboratory: batch experiments			
Aged Baltic Sea water, Germany (batch experiment)	0.01–0.04	26–83	Present study

[a] Assumes DNA is 10% thymine, by weight.
[b] No data available.

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Table 5. Quantity of DIP (nmol l^{-1}) transformed into DOP by *N. spumigena* calculated from ^{33}P-experiments and DIP concentrations at the start and the additional supply at day 3.

Time	pCO_2 treatment		
	Low	Medium	High
3 days	8.4 ± 1.7	2.4 ± 1.5	4.1 ± 3.9
9 days	21.4 ± 12	13.9 ± 0.7	10.2 ± 2.4
15 days	7.8 ± 4.5	35.5 ± 8.8	18.5 ± 8.8

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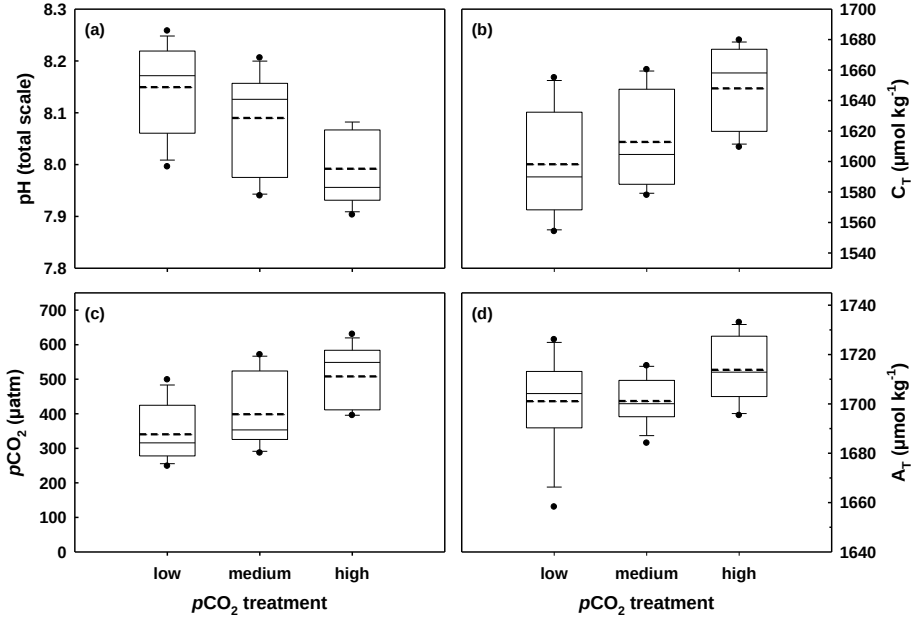

Fig. 1. Box plot (n = 12) of the carbonate system for three CO_2 treatments (low, medium, high). Range of the measured values: **(a)** pH, **(b)** C_T, and of the calculated values: **(c)** pCO_2, **(d)** A_T. The box plots show the range from the start to the end of the experiment (showing each outlier). Solid lines represent the median. Dashed lines represent the mean value.

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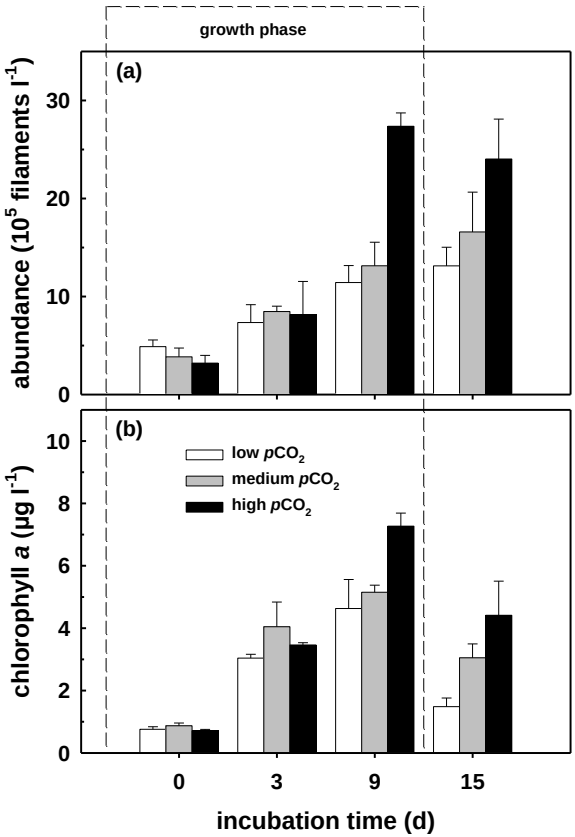

Fig. 2. Development of *Nodularia spumigena* over the sampling time and for the different pCO_2 treatments (low = white bars, medium = grey bars, high = black bars), abundance of *Nodularia spumigena* **(a)**, chlorophyll *a* distribution **(b)** (mean values and the respective standard deviation of 3 replicates).

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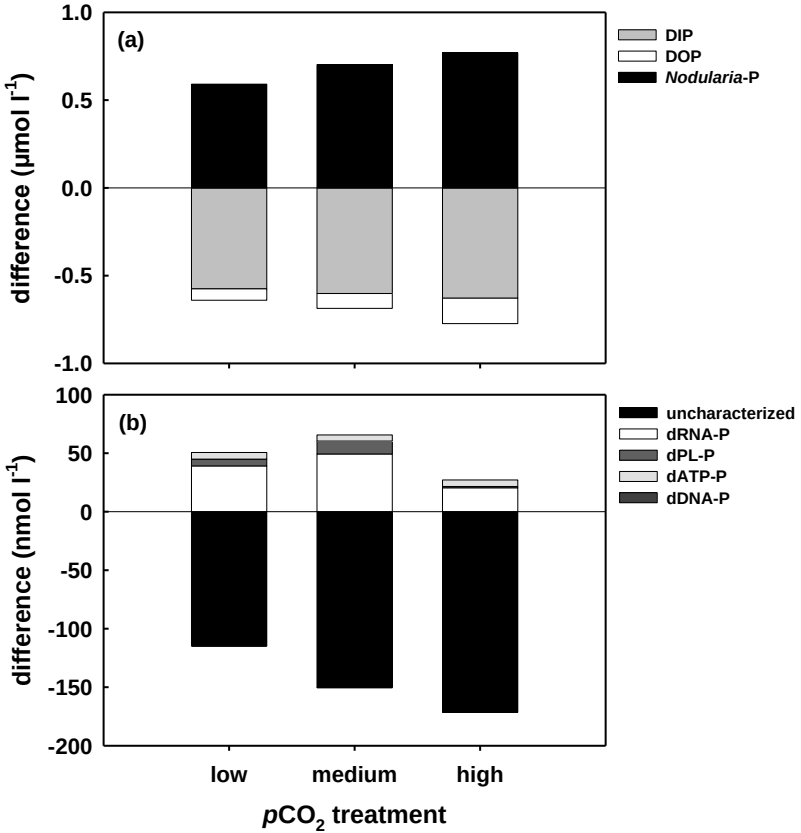

Fig. 3. Changes of the P pool **(a)** and the DOP pool **(b)** between day 9 and day 0 of the 15-day incubation experiment for the three different pCO_2 treatments.

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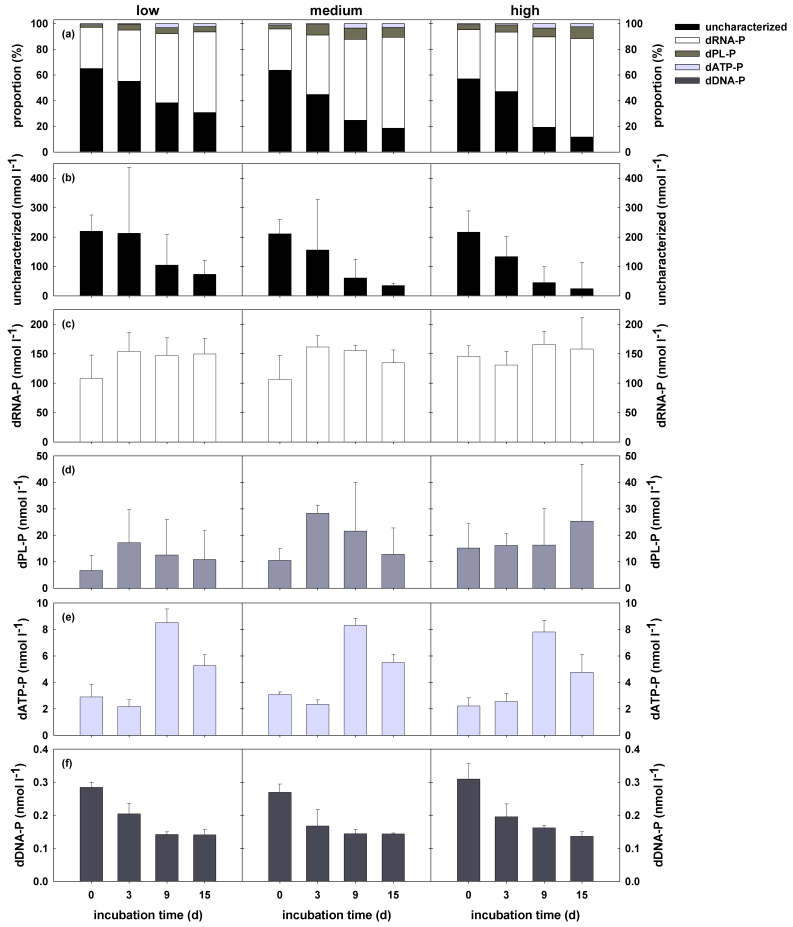

Fig. 4. Composition of DOP as %-proportion **(a)**, and absolute values for the uncharacterized fraction, black **(b)**, dRNA-P, white **(c)**, dPL-P, mid-grey **(d)**, dATP-P, light-grey **(e)**, and dDNA-P, dark-grey **(f)** over incubation time and for the three pCO_2 treatments (low, medium, and high).

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Phosphorus turnover as a function of pCO_2

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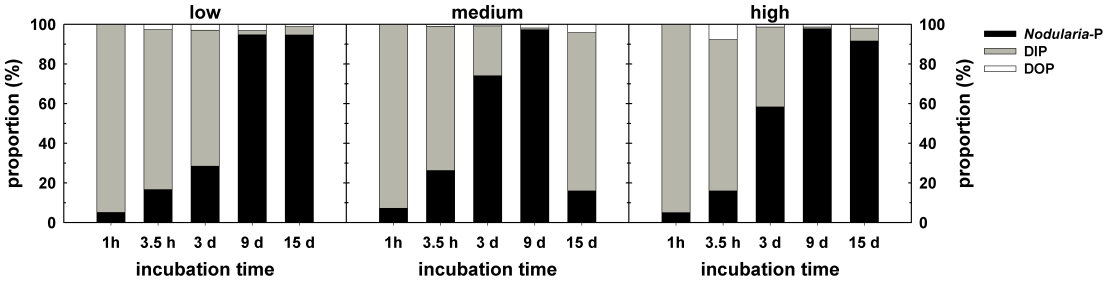

Fig. 5. Proportion of [^{33}P]PO_4 in the three fractions: *Nodularia* – P, DIP and DOP over the course of the experiment for the low, medium, and high pCO_2 treatment.

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