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

J. Unger[1], S. Endres[3,4], N. Wannicke[1,2], A. Engel[4], M. Voss[1], G. Nausch[1], and M. Nausch[1]

[1]Leibniz Institute for Baltic Sea Research, Seestrasse 15, 18119 Rostock, Germany
[2]Leibniz Institute of Freshwater Ecology and Inland Fisheries, Alte Fischerhütte 2, 16775 Stechlin, Germany
[3]Alfred Wegener Institute for Polar and Marine Research, Am Handelshafen 12, 27570 Bremerhaven, Germany
[4]GEOMAR Helmholtz Centre for Ocean Research Kiel, Düsternbrooker Weg 20, 24105 Kiel, Germany

Correspondence to: J. Unger (juliane.unger@io-warnemuende.de)

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Abstract. Diazotrophic cyanobacteria form extensive summer blooms in the Baltic Sea driving the surrounding surface waters into phosphate limitation. One of the main bloom-forming species is the heterocystous cyanobacterium *Nodularia spumigena*. *N. spumigena* exhibits accelerated uptake of phosphate through the release of the extracellular enzyme alkaline phosphatase whose activity also serves as an indicator of the hydrolysis of dissolved organic phosphorus (DOP). The present study investigated the utilisation of DOP and its compounds (e.g., ATP) by *N. spumigena* during growth under different CO_2 concentrations, in order to estimate potential consequences of ocean acidification on the cell's supply with phosphorus (P). Cell growth, the phosphorus pool, and four DOP compounds (ATP, DNA, RNA, and phospholipids) were determined in three setups with different CO_2 concentrations (average 341 µatm, 399 µatm, and 508 µatm) during a 15-day batch experiment. The results showed stimulated growth of *N. spumigena* and a rapid depletion of dissolved inorganic phosphorus (DIP) in all pCO_2 treatments. DOP uptake was enhanced by a factor of 1.32 at 399 µatm and of 2.25 at 508 µatm compared to the lowest CO_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 total DOP were not characterised but comprised the most utilised fraction. This is demonstrated by the decrement of this fraction to 27.4 ± 9.9 % of total DOP during the growth phase with a preference at high pCO_2. Our results indicate a stimulated growth of diazotrophic cyanobacteria at increasing CO_2 concentrations which is accompanied by increasing utilisation of DOP as an alternative P source.

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 dissolved 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 utilise DOP, as indicated by the increased activity of AP which is 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). 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 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/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 on the effects of elevated pCO_2 on the marine P cycle as stated in the review of Hutchins et al. (2009). Based on available literature it seems more likely that the P cycle is not directly affected by rising ocean pCO_2 (Hutchins et al., 2009 and literature therein). However, indirect responses to the expected changes in C and N cycling 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 utilisation under three different pCO_2 concentrations (Tanaka et al., 2008); however, no statistically significant effects of pCO_2 on P biogeochemistry were determined.

Since nitrogen fixation rates are supposed to increase under higher CO_2 concentrations (Wannicke et al., 2012) additional P (or changing C : N : P ratios) are required. This will be studied as part of two other investigations based on the same experimental setup. Wannicke et al. (2012) and Endres et al. (2013) 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 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.

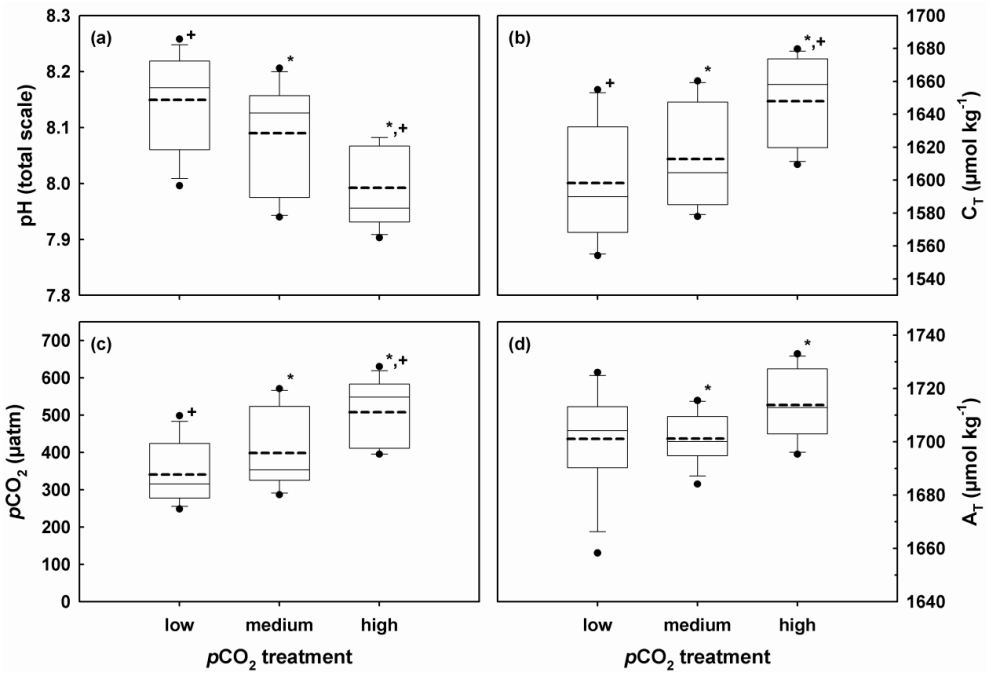

Fig. 1. Box plot ($n = 12$) of the carbonate system for three CO_2 treatments (low, medium, high). Range of the measured values: **(a)** pH, **(b)** C_T, and of the calculated values: **(c)** pCO_2, **(d)** A_T. The box plots show the range from the start to the end of the experiment (showing each outlier). Solid lines represent the median. Dashed lines represent the mean value. Statistically significant differences are highlighted using symbols: (#) between the low and the medium, (*) between the medium and the high, and (+) between the low and the high pCO_2 treatment. Values used are according to Wannicke et al. (2012, Table 1).

2 Materials and methods

2.1 Experimental setup and conditions

The experimental design and preparation is described in detail in Wannicke et al. (2012). In the following we are giving a short overview. A 15-day batch culture experiment was conducted with the diazotrophic cyanobacterium *Nodularia spumigena* in April 2010. In preparation for the experiment, 1000 l of surface water (0–10 m) from the open Baltic Sea (54.22749° N, 12.1748° E) were collected and stored in a HDPE (high-density polyethylene)-tank under cool and dark conditions. Therein the water was 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 was UV-sterilised for 5 days. Three weeks prior to the start of the experiment, axenic 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 sterilised 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 ppm, 380 ppm, and 780 ppm CO_2, 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, 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 sampling on day 3, an additional 0.35 µmol DIP l^{-1} was added because the phosphate concentration 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. On each sampling day, three bottles per pCO_2 treatment were harvested.

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 characterised as described in Wannicke et al. (2012). pH and total dissolved inorganic carbon (C_T) were measured directly after sampling. 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 analyses

2.3.1 Biomass and cell counts

A detailed description for the determination of biomass and cells counts is given in Wannicke et al. (2012). Briefly, 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, 2001). 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 the manual of Gasol and del Giorgio (2000). The cells were counted at a medium flow rate and calculations were performed using the software program "Cell Quest Pro".

2.3.2 Inorganic nutrient analyses

Inorganic nutrients were determined as reported in Wannicke et al. (2012). Briefly, 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. For a quick estimation of the DIP status of the cultures, DIP concentrations were also determined manually according to Murphy and Riley (1962). For data analysis, DIP values from both measurements were pooled.

2.3.3 Organic matter analyses

Organic matter analyses were processed as described in Wannicke et al. (2012). A brief description is given below.

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 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 $1000 \times$ g. The supernatant was aspirated and the precipitate was transferred into 50-ml Falcon tubes, centrifuged again (1.5 h, $1680 \times$ 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 in triplicates by the firefly bioluminescence assay using a Sirius Luminometer (Berthold Detection Systems). Thirty microliter subsamples 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 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 were 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 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 (Trinkler, unpublished). 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 uncharacterised 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 sub-sample 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. The activity (cpm) on the filters is that [^{33}P]PO_4 incorporated by *Nodularia*. The filtrate contained [^{33}P]PO_4 which was not taken up (or released again) and [^{33}P] released as DOP. To distinguish between these two phosphorus forms, the method described by Ammerman (1993) for the uptake of dissolved ATP was applied. To detect the total dissolved activity in the filtrate <0.2 µm (filtrate 1),

1 ml 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 to remove charcoal with the absorbed DOP on the filters. Activated charcoal absorbs dissolved organic matter including [^{33}P]DOP, [^{33}P]PO_4 remained in the dissolved fraction. One ml of the 0.45 µm filtrate (filtrate 2) was counted again. 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 Alkaline phosphatase activity

The activity of alkaline phosphatase was determined as described in detail by Endres et al. (2013) using 4-methylumbelliferyl (MUF)-phosphate (Hoppe, 1983). This fluorescent substrate analogue was added to 180- µl subsamples and incubated in duplicates for 3.5–4.5 h in the dark at 25 °C. Seven different concentrations ranging from 0 to 150 µmol l^{-1} were tested. Sample fluorescence was measured in microtiter plates with a fluorometer (FLUOstar OPTIMA, BMG Labtech, excitation 355 nm, emission 460 nm).

2.6 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 with a correlation coefficient $|R| > 0.6$, and $p < 0.001$. A positive correlation means that both parameters either increase or decrease in concert. A negative correlation implies that one parameter increases while the other one decreases. The operations were performed with 'Sigma Plot 11' (Systat Software Inc.).

3 Results

3.1 Carbonate system

According to Wannicke et al. (2012, Table 1) 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 ($p = 0.009$, $n = 12$) (Fig. 1a). 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 $p = 0.006$, $n = 12$) (Fig. 1b). The calculated values of pCO_2 resulted in an average of 341 ± 81 µatm pCO_2 in the low pCO_2 treatment, 399 ± 104 µatm pCO_2 in the medium pCO_2 treatment, and 508 ± 90 µatm pCO_2 in the high pCO_2 treatment. pCO_2 was significantly different between the low and high as well as the medium and high pCO_2 treatments ($p < 0.001$ and $p = 0.009$, $n = 12$) (Fig. 1c). Wannicke et al. (2012, Supplement Table S2) noted that the calculated pCO_2 was significantly different between all three pCO_2 setups ($p \leq 0.001$, $n = 12$) by testing the data with the analysis of variance (ANOVA). Calculated A_T showed an average

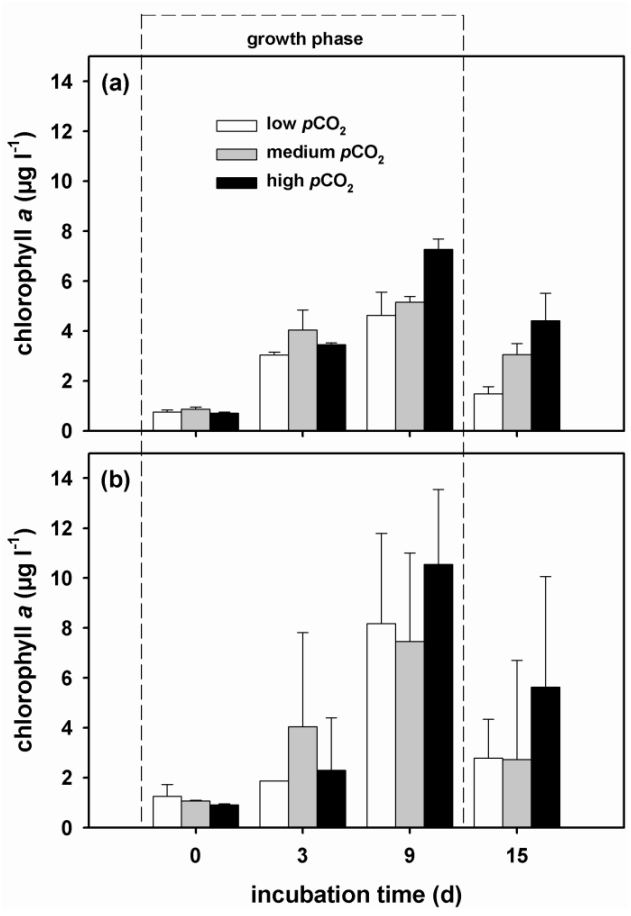

Fig. 2. Comparison of the Chlorophyll *a* distribution over incubation time and for the different pCO_2 treatments (low = white bars, medium = grey bars, high = black bars) in the 10 l batch bottles **(a)** (as shown by Wannicke et al., 2012; Endres et al., 2013), and in the 0.5 l [^{33}P] bottles **(b)** (mean values and the respective standard deviation of 3 replicates).

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). Values of DIP and DOP are according to Wannicke et al. (2012).

Date (day)	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 ($\mu mol\,l^{-1}$)
03/29/2010	Low	0.83 ± 0.03	0.63 ± 0.04	0.20 ± 0.04	0.29 ± 0.02	0.34 ± 0.04
(day 0)	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
04/01/2010	Low	0.71 ± 0.05	0.31 ± 0.02	0.43 ± 0.03	0.05 ± 0.02	0.27 ± 0.02
(day 3)	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
04/07/2010	Low	1.21 ± 0.04	0.31 ± 0.12	0.80 ± 0.21	0.01 ± 0.01	0.27 ± 0.08
(day 9)	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
04/13/2010	Low	1.20 ± 0.21	0.27 ± 0.06	0.93 ± 0.25	0.03 ± 0.01	0.24 ± 0.07
(day 15)	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

of $1701 \pm 19\,\mu mol\,kg^{-1}$ A_T (low), $1701 \pm 9\,\mu mol\,kg^{-1}$ A_T (medium), and $1714 \pm 13\,\mu mol\,kg^{-1}$ A_T (high) (Fig. 1d).

3.2 *Nodularia* growth and bacterial occurrence

A detailed description concerning *Nodularia* growth and bacterial occurrence in response to changing pCO_2 is given in Wannicke et al. (2012). Briefly summarised, 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. POC concentrations increased by a factor of 3.8 (low), 5 (medium), and 7.7 (high) and PON concentrations showed an increase by a factor of 4.1 (low), 6.1 (medium), and 9.6 (high) until day 9. After this, POC and PON concentrations declined. A similar trend was observed for Chl *a* in the large (10 l) and in the small bottles (0.5 l) (Fig. 2). The Chl *a* concentrations increased by a factor of 6.1 (large) and 4.2 (small) at low pCO_2, 5.9 (large) and 6.2 (small) at medium pCO_2, and 10.2 (large) and 9.1 (small) 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). A comparison of the growth parameters in the large and the small bottles showed significant correlations (p <0.001, n = 36) for Chl *a* (|R|= 0.691), POC (|R|= 0.698), PON (|R|= 0.682), and *Nodularia*-P (|R|= 0.765).

Heterotrophic bacteria cell counts at the start of the experiment were below the blank value of 1000 cells l^{-1} and never exceed 1 % of cyanobacterial biomass in course of the experiments (Wannicke et al., 2012). There was no significant increase of heterotrophic bacteria cell numbers over time. Cell numbers on average were $4.69 \pm 1.64 \times 10^5$, $4.54 \pm 1.59 \times 10^5$, and $4.73 \pm 1.28 \times 10^5$ cells l^{-1} for the low, medium, and high treatment, respectively (Wannicke et al., 2012, Table 2). In Endres et al. (2013) it is discussed that if bacteria were attached to each other, to *Nodularia* or to gel particles we might have underestimated the bacterial abundance by flow cytometry. Furthermore, Wannicke et al. (2012) mentioned the possibility of staining non-viable bacteria cells with SYBR GREEN which might have been included in the enumeration. Standard deviations of cell numbers were relatively high, varying between replicates and over time probably due to methodical constraints (Endres et al., 2013). Thus, heterotrophic bacteria had only less or no influence on our measurements.

3.3 Phosphorus pool

The initial TP concentration (day 0) was 0.83 ± 0.3, 0.91 ± 0.08, and $0.87 \pm 0.08\,\mu mol\,l^{-1}$ in the low, medium, and high pCO_2 treatment, respectively. An increase of around $0.36\,\mu mol\,l^{-1}$ 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 $0.72 \pm 0.04\,\mu mol\,l^{-1}$. During the 15-day incubation, the DP concentration declined by 0.35, 0.44, and $0.50\,\mu mol\,l^{-1}$. 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 (Wannicke et al., 2012, Table 2; this publication, 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, $n = 35$) which indicates the strong need of DIP for the biomass development. *Nodularia*-P

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

increased in all treatments in 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, respectively. 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 ($|R| = 0.824$, $p < 0.001$, $n = 36$).

DOP concentrations (Wannicke et al., 2012, Table 2; this publication, Table 1) decreased from day 0 onwards. During the growth phase, DOP utilisation seemed to vary as a function of pCO_2, with concentrations declined by 0.06, 0.09, and 0.14 µmol l^{-1} at low, medium, and high pCO_2, respectively (Fig. 3b), but the differences were not significant due to high variations between replicates (Endres et al., 2013; Fig. 7).

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 utilisation of both pools, as confirmed by the positive correlation between both ($|R| = 0.675$; $p < 0.001$, $n = 35$).

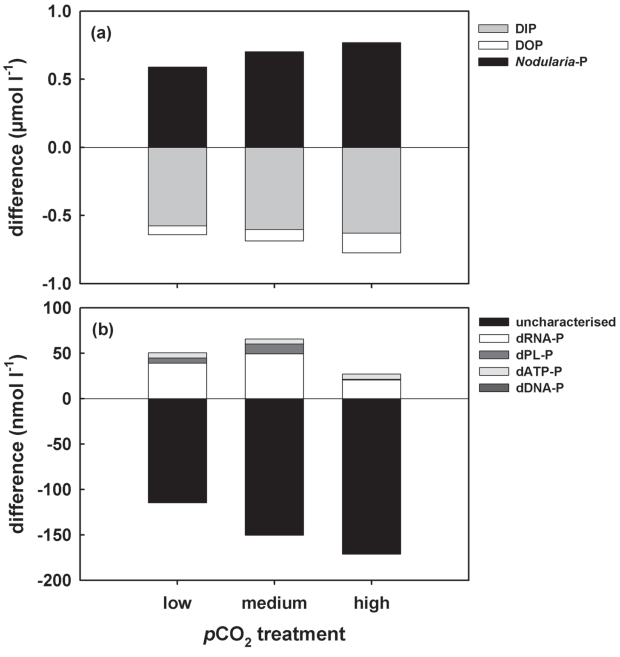

Fig. 3. Changes of the P pool **(a)** and the DOP pool **(b)** between day 9 and day 0 of the 15-day incubation experiment for the three different pCO_2 treatments

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 5.6, 5.2, and 5.6 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 ($|R| = -0.736$, $p < 0.001$, $n = 35$).

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

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 ($\mu g\,l^{-1}$)	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.

3.4.2 Dissolved phospholipids

The initial concentrations of dPL-P were 6.7 ± 5.7, 10.5 ± 4.3, and $15.2 \pm 9.2\,nmol\,l^{-1}$ at low, medium, and high pCO_2, accounting for 2.0, 3.2, and 4.0 % 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 10.1 and $14.5\,nmol\,l^{-1}$, respectively. In contrast, dPL-P concentrations in the high pCO_2 treatment were constant at around $15.9 \pm 0.6\,nmol\,l^{-1}$ until day 9, with a slight increase by $2.9\,nmol\,l^{-1}$ at day 15 (Fig. 4d). By this time, the

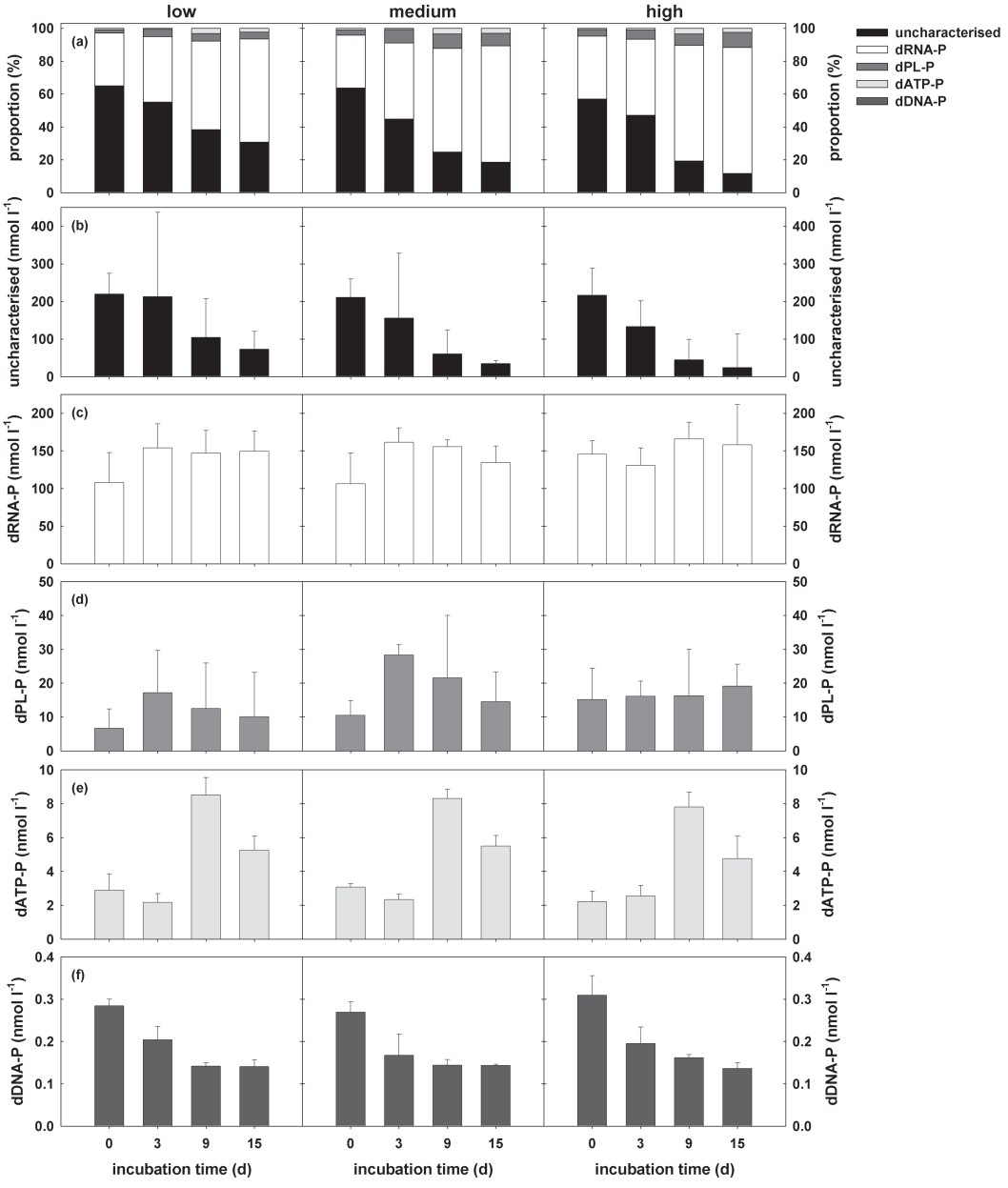

Fig. 4. Composition of DOP as %-proportion **(a)**,and absolute values for the uncharacterised fraction, black **(b)**, dRNA-P, white **(c)**, dPL-P, mid-grey **(d)**, dATP-P, light-grey **(e)**, and dDNA-P, dark-grey **(f)** over incubation time and for the three pCO_2 treatments (low, medium, and high).

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) compared to the starting proportion, 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 ± 0.02, 0.27 ± 0.03, and 0.31 ± 0.05 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 ± 0.01, 0.14 ± 0.01, and 0.16 ± 0.01 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-P at low, medium, and high pCO_2 were 108 ± 40, 106 ± 41, and 145 ± 18 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 ± 3 nmol l^{-1} (low) and 151 ± 14 nmol l^{-1} (medium). In contrast, the dRNA concentration of the high pCO_2 treatment was constant at around 138 ± 20 nmol l^{-1} on day 0 and day 3. At day 9 the concentration slightly increased by 35 nmol l^{-1}, remaining at the same level thereafter (Fig. 4c). Thus, the dRNA-P concentrations increased slightly 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}) but differences were not significant. Due to the decrease of DOP, the proportions of total DOP contributed by dRNA-P increased with 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 Uncharacterised DOP

At the beginning of the experiment, uncharacterised DOP accounted for the majority of 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 uncharacterised DOP declined by 115 ± 50, 150 ± 15, and 171 ± 22 nmol l^{-1}, respectively. This implied a decrease, albeit not significant, by a factor of 1.3 at medium pCO_2 and 1.5 at high pCO_2 compared to the low pCO_2 treatment. Until day 15, the decrement proceeded, progressively reducing the proportions of uncharacterised DOP to 30.8 % (low), 18.6 % (medium), and 11.7 % (high) of total DOP (Fig. 4b). The uncharacterised 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 uncharacterised 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 the mean at day 3 of 74 % and 58 %, respectively, 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 decline of the cyanobacteria biomass at day 15 (Fig. 2) was accompanied by a strong decrease in 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, the transition of *Nodularia* to the senescent state rather occurred under the medium treatment than in the other treatments. [^{33}P] was released from *Nodularia* predominantly as DIP either directly or after processing by APA; only a small proportion occurred as DOP (Fig. 5). The 81 % decrease in *Nodularia*-P detected in the medium treatment was combined with a release of 78 % as DIP and 3 % as DOP. The decrease of *Nodularia*–P by 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. 3a).

Table 5. Quantity of DIP (nmol l^{-1}) transformed to DOP by *N. spumigena*. Calculation was done using the proportion of [^{33}P]DOP to total [^{33}P] at each sampling day considering the initial DIP concentration + DIP concentration added 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

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., 2013). 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.

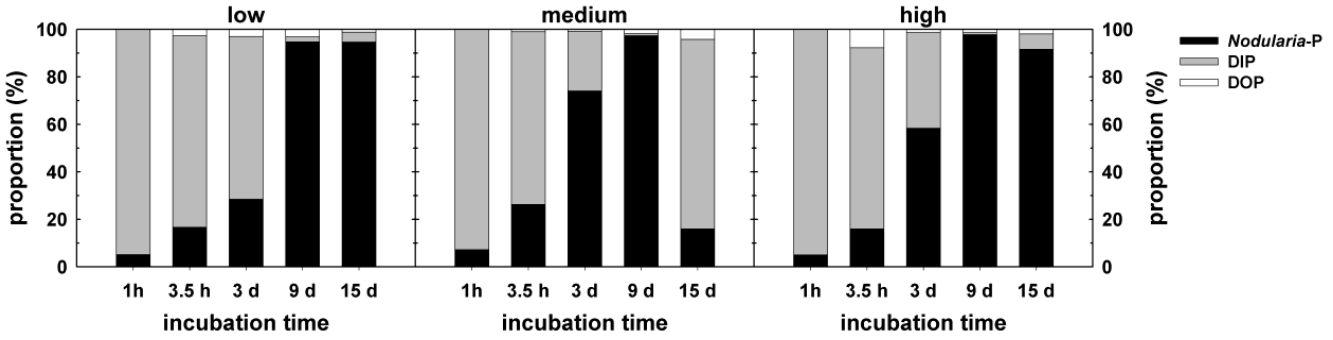

Fig. 5. Proportion of [^{33}P]PO_4 in the three fractions: *Nodularia*–P, DIP and DOP over the course of the experiment for the low, medium, and high pCO_2 treatment.

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 growth rates in terms of biomass increase (Chl *a*, POC, PON, and filament abundances) (Wannicke et al., 2012; Fig. 5). Carbon and nitrogen fixation rates were stimulated as well (Wannicke et al., 2012; Fig. 6). The response of *N. spumigena* to pCO_2 elevation was similar to that reported for the oceanic filamentous cyanobacterium *Trichodesmium* (Barcelos e 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 most pronounced at high pCO_2. Dissolved phosphorus (DIP and DOP) uptake was reflected in the P content of *Nodularia* (Fig. 3a, 5), but normalised to filament abundance *Nodularia*-P was lowest at high pCO_2 indicating a more efficient P utilisation (Wannicke et al., 2012, Fig. 3). In the [^{33}P]-experiments, nearly all of the DIP (95–98 %) was fixed in biomass during the growth phase of *Nodularia*. The uptake seemed to be faster in the medium and high pCO_2 treatment compared to the low treatment, but a higher sampling frequency would be necessary to make a clear statement. The subsequent release of DIP from *Nodularia* was delayed in both, the low and the high pCO_2 treatment compared to the medium treatment. However, we hypothesise that the mechanisms behind this parallel delay in P release may differ in the treatments tested here. We have the following explanation: 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 contrast, in the high pCO_2 treatment, the greater P demand of *Nodularia* could have caused the persistence of P in the cells. This hypothesis needs confirmation by further experiments, but if it is correct, an increase in the P demand of *Nodularia* will be likely under the conditions predicted for the Baltic Sea. The slight elevation in pCO_2 in our experiment, from 341 to 399 µatm, already showed a stimulating growth effect. Accordingly, small variations in the present pCO_2 (spatially and seasonally) can be expected to result in changes of *Nodularia* growth, nitrogen fixation, and P demand. Currently, the pCO_2 in the central Baltic Sea ranges between 100 and 300 µatm during the summer season (Schneider et al., 2006) and its increase within the next decades could change the behaviour of *Nodularia spumigena*.

4.2 DOP as phosphorus source

The ability of phytoplankton to utilise 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; Vahtera, 2007). Accordingly, a high alkaline phosphatase activity (APA) indicates DOP utilisation (Cembella et al., 1984; Nausch, 1998; Hoppe, 2003) and may even be an indicator of P stress in cyanobacteria (Paasche and Erga, 1988; Wu et al., 2012).

In this joint experiment, DIP depletion and DOP decrease occurred in parallel with enhanced APA, as previously reported by Endres et al. (2013). We not only confirmed the negative correlation between APA and DIP ($|R| = -0.852$, $n = 35$, $p < 0.001$) as well as DOP ($|R| = -0.635$, $n = 36$, $p < 0.001$) but also found changes of similar range in both, 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. According to Endres et al. (2013), we can assume that (1) AP was mainly attached to *Nodularia* cell surface and (2) the uptake of DOP was driven by *Nodularia* while heterotrophic degradation of DOP was negligible.

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 released from the terminal of phosphodiesters of DNA and RNA by AP (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 uncharacterised 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 the uncharacterised DOP derives from the fact that it presumably accounted for the bulk P-nutrition in *Nodularia* in this study. On the one hand the positive correlation with DOP ($|R| = 0.932$, $p < 0.001$, $n = 36$) suggested that the uncharacterised DOP fraction satisfies *Nodularia*'s P demand. Moreover, this relationship is supported by the negative correlation with *Nodularia*-P and APA ($|R| = -0.82$ and -0.681, $p < 0.001$, $n = 36$) which is an indication for the need of P for growth and the enzymatic degradation of this pool (Endres et al., 2013).

Phytoplankton is assumed to be the main producer of DOP as supported by, for example, high DOP concentrations detected during a spring bloom (Lomas et al., 2010). Deduced from the [^{33}P]-experiments, *Nodularia spumigena* released DOP only in nanomolar concentrations (Table 5) under DIP depleted conditions, which can hardly be detected by pool size measurements. Therefore, the CO_2 dependent variations of DOP utilisation seem to be influenced rather by the P demand of *Nodularia* which become evident by the enhanced [^{33}P]PO_4 incorporation into biomass in the medium and high treatment compared to the low on day 3 (Fig. 5). The higher formation of [^{33}P]DOP in these treatments at day 15 might result from a more pronounced senescence, as visible by *Nodularia* growth, whereas in the low treatment DIP incorporated in *Nodularia* biomass was still high (Fig. 5). Thus, the influence of CO_2 on DOP formation seems to be of indirect nature.

4.3 Composition of DOP under *Nodularia spumigena* growth

In the laboratory experiment described here, the DOP concentration and the composition of aged and UV-light treated Baltic Sea water changed during the development of *N. spumigena*. The sum of the determined DOP components accounted for 38.1–79.6 % of total DOP during the 15-day incubation.

Dissolved ATP concentrations in the batch culture experiments ranged from 399 to 1563 ng l^{-1} and constituted 0.6–3.4 % of the DOP pool and thus were by a factor of 1.8 to 24 higher than those reported by Azam and Hodson (1977) in surface waters at the coast of Southern California. 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 so 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 study focusing on 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 four magnitudes 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, 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 decrease of dDNA-P in our study lead to the assumption that dDNA-P is rapidly used within hours, as it could not be detected with our sampling strategy of 3 or 6 days. Furthermore, other enzymes than AP, e.g. nucleases or phosphodiesterases, could be responsible for the cleavage of dDNA making it easier for AP to release phosphate (Hino, 1989). These enzymes were not determined in this study.

Dissolved RNA has hardly been investigated in aquatic environments. Karl and Bailiff (1989) measured concentrations ranging from 4.03 to 51.1 µg 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 µg l^{-1}) (Table 4).

In summary, our determined DOP compounds fit in the range of values demonstrated for other marine areas. However, further investigations have to confirm the results especially under natural Baltic Sea conditions.

4.4 Effects of CO_2 treatments on individual DOP components

Our results demonstrate that the various compounds within the DOP pool developed differentially over time and with pCO_2 (Fig. 4). 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 0 and day 3. A trend towards a higher release at low pCO_2 than at medium and high pCO_2 (by a factor of 1.06 and 1.20, respectively) was noted at day 9. From day 9 to day 15, the decline in dATP-P (by a mean of $3.04 \pm 0.22\,\mathrm{nmol\,l^{-1}}$; Fig. 4e) followed the trend of total DOP (by a mean of $40 \pm 15\ \mathrm{nmol\,l^{-1}}$). At this stage (day 15), *Nodularia* cells were in stationary phase, exhibiting the first signs of decay. During decay the release of DOP, including dATP, would be expected. Instead, the opposite was observed which suggests the possibility of DOP and dATP utilisation in this phase. However, we have no evidence that dATP is used significantly by heterotrophic bacteria, because their abundances remained at the same low levels as at the start of the experiment (Wannicke et al., 2012, Table 2).

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 utilised 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 days and therefore, it is possible that short time elevations of dPL-P were not detected before day 15. In contrast to dATP-P, dPL-P was released under high pCO_2 (Fig. 4d), when *Nodularia* growth reached stationary phase or the cells became senescent (Wannicke et al., 2012, Fig. 2).

The starting concentration of dDNA-P in all treatments was $0.29 \pm 0.02\,\mathrm{nmol\,l^{-1}}$ and leveled out at $0.14 \pm 0.04\,\mathrm{nmol\,l^{-1}}$ 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 (Paul et al., 1987) and other DOP compounds (e.g., dATP, Azam and Hodson, 1977; Björkman and Karl, 2005) 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. Furthermore, they 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 08:00 and 09:00 a.m., 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 ($49.3\,\mathrm{nmol\,l^{-1}}$), followed by the low and high treatments (39.1, and $20.4\,\mathrm{nmol\,l^{-1}}$, respectively; Fig. 3b). Over the course of the experiment, dRNA-P release was highest in the low treatment whereas it was lowest in the high treatment (41.7 and $12.8\,\mathrm{nmol\,l^{-1}}$ 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 a viable P source besides DIP and an intracellular P pool. Although, with our sampling intervals we found no significant effects of variable pCO_2 conditions on single metabolic components, some trends were visible.

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 utilisation of DIP as well as DOP. We propose that the stimulating effect on P utilisation 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. These components may be studied in further experiments where a higher sampling resolution is applied to capture the changes from release to uptake processes. Components of the DOP pool other than those quantified in this study were consumed more intensively and dominated the decrease in the DOP pool.

Supplementary material related to this article is available online at: http://www.biogeosciences.net/10/1483/2013/bg-10-1483-2013-supplement.pdf.

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