

Effects of CO<sub>2</sub> perturbation on phosphorus pool sizes

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# Effects of CO<sub>2</sub> perturbation on phosphorus pool sizes and uptake in a mesocosm experiment during a low productive summer season in the northern Baltic Sea

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## Abstract

Studies investigating the effect of increasing CO<sub>2</sub> levels on the phosphorus cycle in natural waters are lacking although phosphorus often controls phytoplankton development in aquatic systems. The aim of our study was to analyze effects of elevated CO<sub>2</sub> levels on phosphorus pool sizes and uptake. Therefore, we conducted a CO<sub>2</sub>-manipulation mesocosm experiment in the Storfjärden (western Gulf of Finland, Baltic Sea) in summer 2012. We compared the phosphorus dynamics in different mesocosm treatments but also studied them outside the mesocosms in the surrounding fjord water.

In the mesocosms as well as in surface waters of Storfjärden, dissolved organic phosphorus (DOP) concentrations of  $0.26 \pm 0.03$  and  $0.23 \pm 0.04 \mu\text{molL}^{-1}$ , respectively, formed the main fraction of the total P-pool (TP), whereas phosphate (PO<sub>4</sub>) constituted the lowest fraction with mean concentration of  $0.15 \pm 0.02 \mu\text{molL}^{-1}$  and  $0.17 \pm 0.07 \mu\text{molL}^{-1}$  in the mesocosms and in the fjord, respectively. Uptake of PO<sub>4</sub> ranged between 0.6 and 3.9 nmolL<sup>-1</sup>h<sup>-1</sup> of which ~86% (mesocosms) and ~72% (fjord) were realized by the size fraction < 3 μm. Adenosine triphosphate (ATP) uptake revealed that additional P was supplied from organic compounds accounting for 25–27% of P provided by PO<sub>4</sub> only.

CO<sub>2</sub> additions did not cause significant changes in phosphorus (P) pool sizes, DOP composition, and uptake of PO<sub>4</sub> and ATP when the whole study period was taken into account. About 18% of PO<sub>4</sub> was transformed into POP, whereby the major proportion (~82%) was converted into DOP suggesting that the conversion of PO<sub>4</sub> to DOP is the main pathway of the PO<sub>4</sub> turnover.

We observed that significant relationships (e.g., between POP and Chl *a*) in the untreated mesocosms vanished under increased *f*CO<sub>2</sub> conditions. Consequently, it can be hypothesized that the relationship between POP formation and phytoplankton growth changed under elevated CO<sub>2</sub> conditions. Significant short-term effects were observed for PO<sub>4</sub> and particulate organic phosphorus (POP) pool sizes in CO<sub>2</sub> treatments > 1000 μatm during periods when phytoplankton started to grow.

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# 1 Introduction

Increasing emissions of anthropogenic CO<sub>2</sub> into the atmosphere and subsequent acidification of the ocean can potentially affect the diversity of organisms and the functioning of marine ecosystems (Eisler, 2011). The rise of the atmospheric CO<sub>2</sub> content was accelerated from 3.4±0.2PgCyr<sup>-1</sup> in the 1980s to 4.0±0.2PgCyr<sup>-1</sup> in the 2000s leading to CO<sub>2</sub> elevation in ocean surface waters the same rate (IPCC, 2013). Atmospheric CO<sub>2</sub> is predicted to rise to 750–> 1000ppm in 2100 (IPCC, 2001) corresponding with a decrease in pH by 0.3–0.5 units (Caldeira and Wickett, 2005) from the present pH of 8.1. Although this process is of global significance and all parts of the oceans are at risk, there will be regional differences in the degree of acidification (Borges et al., 2005). Thus, to determine the CO<sub>2</sub>-related changes in the oceans, multiple studies in different regions are required. Semi-enclosed coastal regions, such as the Baltic Sea, can be more sensitive to CO<sub>2</sub> elevation than open ocean waters due to high freshwater inputs resulting in a reduced buffer capacity (Orr, 2011).

In the Baltic Sea, several studies of CO<sub>2</sub> effects are done on the organismic level of fish (Frommel et al., 2013), zooplankton (Pansch et al., 2012; Vehmaa et al., 2012), macrophytes (Pajusalu et al., 2013), benthic organisms (Hiebenthal et al., 2013; Stemmer et al., 2013), and filamentous cyanobacteria (Czerny et al., 2009; Eichner et al., 2014; Wannicke et al., 2012). The impacts of elevated CO<sub>2</sub> at the ecosystem level, however, have thus far been limited to the Kiel Bay in the western Baltic Sea (Engel et al., 2014; Rossoll et al., 2013; Schulz and Riebesell, 2013), which may fundamentally differ from other parts of the Baltic Sea.

Next to nitrogen, phosphorus (P) controls the productivity of phytoplankton in the ocean (Karl, 2000; Sanudo-Wilhelmy et al., 2001; Tyrrell, 1999) and is the limiting factor in some regions (Ammerman et al., 2003). The total phosphorus (TP) pool comprises phosphate (PO<sub>4</sub>), dissolved organic phosphorus (DOP), and particulate organic phosphorus (POP). There is a continuous transformation of phosphorus between these P species due to their uptake, conversion, and release by organisms. While PO<sub>4</sub> is the

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5 preferred P-species of phyto- and bacterioplankton, DOP becomes an important P source in particular when PO<sub>4</sub> is depleted (Liebot et al., 2010; Lomas et al., 2010). DOP includes nucleic acids, phospholipids, and adenosine triphosphate (ATP) (Karl and Björkman, 2002) which are structural and functional components in all living cells, but, can be also released into the surrounding water.

10 In general, there is little knowledge on how the P cycle is affected by ocean acidification and how related changes in P availability influence the response of organisms to CO<sub>2</sub> elevation. In CO<sub>2</sub> manipulation experiments, particulate phosphorus dynamics were studied to determine effects on C : P stoichiometry of phytoplankton (Riebesell and Tortell, 2011) and PO<sub>4</sub> concentration dynamics to estimate its utilization (Bellerby et al., 2008). CO<sub>2</sub> effects on phosphorus pool sizes and PO<sub>4</sub> uptake have so far only been studied by Tanaka et al. (2008) in the Raunefjorden, Norway and by Unger et al. (2013) and Endres et al. (2013) in laboratory experiments with cultures of *Nodularia spumigena*. Thus, there is a gap of knowledge on how the phosphorus cycle may be affected under future CO<sub>2</sub> conditions. We therefore studied the impact of elevated CO<sub>2</sub> on phosphorus pool sizes, the DOP composition, and PO<sub>4</sub> uptake of northern Baltic Sea plankton community. These measurements provide important information on potential changes in P cycling under future conditions and thus will contribute to a better understanding of potential impacts of increased CO<sub>2</sub> levels in brackish water ecosystems.

## 2 Material and methods

### 2.1 Experimental design and CO<sub>2</sub> manipulation

25 The study was conducted in the northwestern Gulf of Finland, in the proximity of the Tvärminne Zoological Station (TZS) (Fig. 1), between 17 June and 4 August 2012, using the KOSMOS mesocosm system (Riebesell et al., 2013). Nine mesocosms (M1–M9) were moored in the open waters of the Storfjärden (5951.5°N, 2315.5°E) at a wa-

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ter depth of  $\sim 30$  m. Only six of them were included throughout the whole study period since leakages in the remaining three rendered them unusable. Equipment and deployment procedures are described in detail by Paul et al. (2015b). Briefly, polyurethane enclosure bags of 2 m in diameter and 18.5 m in length were mounted in floating frames and lowered in such a way that  $\sim 17$  m of each bag were immersed in the water column and  $\sim 1.5$  m remained above the water surface. Large organisms were excluded from the mesocosms by a 3 mm mesh installed at the top and bottom of the bags before closure. The mesocosms were deployed 10 days prior to CO<sub>2</sub> manipulation to rinse the bags and for full water exchange. Sediment traps were mounted on the lower ends to close them water tight, while the upper ends were raised above the water surface to prevent water entry during wave action. The mesocosms were covered with a dome shaped roof to prevent nutrient input by bird and potentially significant fresh water input by rain. Salinity gradients were removed by bubbling the mesocosms with compressed air for 3.5 min, so that 5 days before the start of the experiment (day  $-5$ ) the water body was fully homogeneous.

CO<sub>2</sub> treatment started on day 0 and was repeated on subsequent 4 days by pumping various quantities of 50  $\mu$ m-filtered and CO<sub>2</sub>-enriched fjord water into seven of the mesocosms as described by Riebesell et al. (2013). The intended CO<sub>2</sub> and pH gradients were reached after the last treatment on day 4. Details are described in Paul et al. (2015b). For the two untreated (control) mesocosms, only filtered fjord water was added to adjust the water volume to that of the treated mesocosms. To compensate for outgassing, the CO<sub>2</sub> manipulation was similarly repeated in the upper 7 m layer of the mesocosms on day 16.

## 2.2 Sampling

Daily sample collection started 3 days before the first CO<sub>2</sub> injection (day  $-3$ ). Parallel samples were taken from the surrounding fjord. Sampling over the entire 17 m depth was carried out using an integrating water sampler (IWS HYDROBIOS-KIEL) that was



$A_T$  was determined using potentiometric titration and a Gran type data analysis following Dickson et al. (2007). The quality of the measurement was verified with the same reference material as used for the DIC measurements.

The  $f\text{CO}_2$  was calculated from DIC, pH, salinity, temperature, phosphate, and silicate data using the  $\text{CO}_2$  SYS program (Pierrot and Wallace, 2006).

### 2.3.2 Chlorophyll and inorganic nutrients

Subsamples of 500 mL were filtered onto GF/F-filters, which were then homogenized. Chl *a* was extracted in acetone (90%) in plastic vials by homogenisation of the filters for 5 min in a cell mill using glass beads. After centrifugation (10 min,  $800 \times g$ ,  $4^\circ\text{C}$ ) the supernatant was analysed on a fluorometer (TURNER 10-AU) at an excitation of 450 nm and an emission of 670 nm to determine Chl *a* concentrations (Jeffrey and Welschmeyer, 1997).

A segmented continuous-flow analyzer coupled with a liquid-waveguide capillary flow-cell (LWCC) of 2 m length was used to determine phosphate ( $\text{PO}_4$ ) and the sum of nitrite and nitrate ( $\text{NO}_2 + \text{NO}_3$ ) at nanomolar precision (Patey et al., 2008). The  $\text{PO}_4$  determination was based on the molybdenum blue method of Murphy and Riley (1962), and  $\text{NO}_2 + \text{NO}_3$  on the method of Morris and Riley (1963).  $\text{PO}_4$  concentrations from the same subsample were also measured manually using a 5 cm cuvette (Grasshoff et al., 1983). In most of the samplings  $\text{PO}_4$  data obtained from both methods did not differ significantly (paired  $t$  test:  $p = 0.0262$ ,  $t = 1.127$ ,  $n = 109$ ).

### 2.3.3 Dissolved organic phosphorus (DOP)

For the determination of DOP, duplicate 40 mL subsamples were filtered through pre-combusted (6 h,  $450^\circ\text{C}$ ) glass fiber filters (Whatman GF/F) and stored in 50 mL vials (Falcon) at  $20^\circ\text{C}$  until further processing. The thawed samples were oxidized in a microwave (MARSXpress, CEM, Matthews, USA) after the addition of potassium peroxydisulfate in an alkaline medium (Grasshoff et al., 1983). The P concentration, measured

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A2383) ranging from 1 to 20 nmolL<sup>-1</sup>. The detection limit of the bioluminescence assay was 2.5 pmol mL<sup>-1</sup>. The fluorescence slope of the standard concentrations was used to calculate dATP concentrations, correcting for the final sample volume. The P-content of the ATP (ATP-P) was calculated by assuming that 1 mol of ATP is equivalent to 3 mol P.

## Dissolved phospholipids

The phosphate content of the dissolved phospholipids (PL-P) was analyzed using a modified method of Suzumura and Ingall (2001, 2004). Briefly, 400 mL subsamples of the filtrate were stored at -20 °C until further processing. The samples were then thawed in a water bath at 30 °C and extracted twice with 100 mL of chloroform. The chloroform phase was collected, concentrated to 5 mL in a rotary evaporator (Heidolph Instruments, Schwabach, Germany), and then transferred into microwave tubes. The chloroform was completely evaporated by incubating the tubes in a 60 °C water bath overnight. After the addition of 20 mL of deionized water (Milli-Q, Millipore), the samples were digested with potassium peroxydisulfate in alkaline medium and microwaved as described for the DOP analysis. Six standard concentrations of phospholipids, ranging from 0 to 125 µg L<sup>-1</sup>, were prepared by adding the respective amounts of a stock solution containing 5 mg of l-phosphatidyl-dl-glycerol sodium salt (PG, Sigma Aldrich, P8318) mL<sup>-1</sup> to the aged seawater. The detection limit was 0.8 nmol L<sup>-1</sup>. The blanks contained only chloroform and were processed as for the samples.

## Dissolved DNA and RNA

Dissolved DNA and RNA (dDNA and dRNA) concentrations were determined according to Karl and Bailiff (1989) and as described by Unger et al. (2013). For each sample, 200 mL of the filtrate was gently mixed with the same volume of ethylene-diamine-tetracetic acid (EDTA, 0.1 M, pH 9.3, Merck, 1.08454) and 4 mL of cetyltrimethyl-ammonium bromide (CTAB, Sigma-Aldrich, H5882) and stored frozen at -20 °C for

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at least 24 h. After thawing the samples, the precipitate was collected onto combusted (450 °C, 6 h) glass fiber filters (25 mm, GF/F Whatman), placed into annealed vials, and stored frozen at –80 °C until further analysis.

DNA concentrations were measured using a fluorescence-spectrophotometer (Hitachi F 2000), and RNA concentrations using a dual-beam UV/VIS-spectrophotometer U3010 (Hitachi).

Coupled standards (DNA + RNA) containing 1–10 µg DNA (Sigma Aldrich, D3779) L<sup>-1</sup> and 20–120 µg RNA (Sigma Aldrich, R1753) L<sup>-1</sup> were prepared in aged seawater as described above. A reagent blank served as the reference and aged seawater as the background control. The P-contents of the DNA and RNA were calculated by multiplying the measured values by a factor of 2.06 nmol P per µg dDNA and 2.55 nmol P per µg dRNA. The latter values were determined by the microwave digestion of standard substrates.

### 2.3.5 Particulate organic phosphorus, carbon, and nitrogen

Particulate organic phosphorus (POP) was analyzed using two methods in parallel. In the “aqueous method”, 40 mL of unfiltered subsamples were frozen at –20 °C and analyzed as described for DOP. The measured PO<sub>4</sub> concentration represents total phosphorus (TP). PP is the difference between the total PO<sub>4</sub> concentration in the unfiltered digested sample and the sum of DOP + PO<sub>4</sub>. In the “filter-method”, 500 mL sub pre-combusted GF/F-filters that were then placed into Schott bottles containing 40 mL of deionised water. PP was digested to PO<sub>4</sub> by the addition of oxidizing decomposition reagent (Oxisolv<sup>®</sup>, Merck) followed by heating in a pressure cooker for 30 min. The PO<sub>4</sub> concentrations of the cooled samples were determined spectrophotometrically according to Grasshoff et al. (1983). Paired *t* test revealed significant differences between both methods; however, both means of 0.19 ± 0.03 µmol L<sup>-1</sup> for the filter method and of 0.16 ± 0.04 µmol L<sup>-1</sup> for the aqueous method differed near the detection limit of the methods. Thus, solely the mean values obtained from both measurements are used in the following.

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Particulate carbon (PC) and nitrogen (PN) were analyzed by filtering 500 mL samples onto pre-combusted (450 °C, 6 h) glass fiber filters (Whatman GF/F), which were then stored frozen at -20 °C. PC and PN concentrations were measured by flash combustion of the dried (60 °C) filters using an EuroEA elemental analyser coupled with a ConFlo II interface to a Finnigan Delta<sup>Plus</sup> mass spectrometer.

### 2.3.6 Phosphate and ATP uptake

PO<sub>4</sub> uptake was measured by addition of radioactively labeled phosphate [<sup>33</sup>P]PO<sub>4</sub> (specific activity of 111 TBqmmol<sup>-1</sup>, Hartmann Analytic GmbH) at concentrations of 50 pmolL<sup>-1</sup> to 50 mL subsamples, which were then incubated under laboratory light and the in situ temperatures for ~ 2 h. For each mesocosm, three parallel samples and a blank were prepared. The blank was obtained by the addition of formaldehyde (1 % final concentration) 10 min before radiotracer addition, in order to poison the samples. At defined time intervals within the incubation, 5 mL subsamples were taken from each of the parallel samples and filtered onto polycarbonate filters pre-soaked with a cold 20 mM PO<sub>4</sub> solution to prevent non-specific [<sup>33</sup>P]PO<sub>4</sub> binding. The filters were rinsed with 5 times 1 mL of particle-free bay water and placed in 6 mL scintillation vials. Scintillation liquid (4 mL IrgaSafe; Perkin Elmer) was added and the contents of the vials were mixed using a vortex mixer. After allowing the samples to stand for at least 2 h, the radioactivity on the filters was counted in a Perkin Elmer scintillation counter. Filters of 0.2 and 3 μm pore sizes (Whatman and Millipore, respectively) were used to determine uptake by the whole plankton community and the size fraction > 3 μm, respectively. Picoplankton uptake was calculated as the difference between the activity on the 0.2 μm and 3 μm filters.

[γ<sup>33</sup>P]ATP (specific activity of 111 TBqmmol<sup>-1</sup>, Hartmann Analytic GmbH) was added to triplicate 10 mL samples and a blank, each in a 20 mL vial, at a concentration of 50 pmolL<sup>-1</sup>. The samples were incubated in the dark at in situ temperature for 1 h. The uptake was stopped by addition of 200 μL of a cold 20 mM ATP solution to

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the samples, which were then filtered and processed as described for the PO<sub>4</sub> uptake measurements.

### 2.3.7 Bacterial production (BPP)

Rates of bacterial protein production (BPP) were determined by incorporation of <sup>14</sup>[C]-leucine (<sup>14</sup>C-Leu, Simon and Azam, 1989) according to Grossart et al. (2006). Triplicates and a formalin-killed control were incubated with <sup>14</sup>C-Leu (213 mCi mmol<sup>-1</sup>; Hartmann Analytic GmbH, Germany) at a final concentration of 165 nmol L<sup>-1</sup>, which ensured saturation of uptake systems of both free and particle-associated bacteria. Incubation was performed in the dark at in situ temperature (between 7.8 °C and 15.8 °C) for 1.5 h. After fixation with 2 % formalin, samples were filtered onto 5.0 μm (attached bacteria) nitrocellulose filters (Sartorius, Germany) and extracted with ice-cold 5 % trichloroacetic acid (TCA) for 5 min. Thereafter, filters were rinsed twice with ice-cold 5 % TCA, once with ethanol (96 % v/v), and dissolved with ethylacetate for measurement by liquid scintillation counting. Afterwards the collected filtrate was filtered on 0.2 μm (free-living bacteria) nitrocellulose filters (Sartorius, Germany) and processed in the same way as the 5.0 μm filters. Standard deviation of triplicate measurements was usually < 15 %. The sum of both fractions (free-living bacteria and attached bacteria) is referred to total BPP. The amount of incorporated <sup>14</sup>C-Leu was converted into BPP by using an intracellular isotope dilution factor of 2. A conversion factor of 0.86 was used to convert the protein produced into carbon (Simon and Azam, 1989).

### 2.3.8 Statistical analyses

The Grubbs test, done online ([graphpad.com/quickcalcs/Grubbs1.cfm](http://graphpad.com/quickcalcs/Grubbs1.cfm)) was applied to identify outliers in all data sets. The outliers were removed from further statistical analyses.

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Spearman Rank correlations were carried out to describe the relationship between the development of the parameters over time in the mesocosms and in the fjord using Statistica 6 software.

Short-term CO<sub>2</sub> effects on POP concentrations at days 0–2 and 23–43 between the CO<sub>2</sub> treatments were verified with an ANCOVA analysis using the SPSS software. The “days” were treated as a covariate interacting with the treatments. Paired *t* test was applied to check the differences in PO<sub>4</sub> concentrations between the treatments.

## 3 Results

### 3.1 Development in the mesocosms

#### 3.1.1 CO<sub>2</sub>, pH, temperature and salinity

The different mesocosms were characterized based on their averaged *f*CO<sub>2</sub> and pH values from day 1 until day 43 (Fig. 2a and b):

M1 and M5: 365 and 368 μmolL<sup>-1</sup> *f*CO<sub>2</sub>, pH 8.08 and 8.07 untreated levels;

M7 and M6: 497 and 821 μmolL<sup>-1</sup> *f*CO<sub>2</sub>, pH 7.95 and 7.74 intermediate *f*CO<sub>2</sub>;

M3 and M8: 1007 and 1231 μmolL<sup>-1</sup> *f*CO<sub>2</sub>, pH 7.66 and 7.58 high *f*CO<sub>2</sub>.

Temperature development in the mesocosms was determined through temperature variations in the fjord from 7.81 to 15.86 °C. Based on this, the experiment was divided into four phases (Fig. 3): phase 0: day –3 to day 0; phase I: days 1–16, phase II: days 17–30 and phase III: day 31 until the end of the measurements. Temperature dropped from 8.71 to 7.82 °C in phase 0 and rose from 8.07 °C at the end of phase 0 to the maximum of 15.86 °C by the end of phase I. During phase II, the temperature decreased to 7.89 °C interrupted by a short reversal on days 22 and 23. During phase III, the temperature increased to 12.61 °C (Table 1).

The salinity of 5.69 ± 0.01 remained relatively stable in the mesocosms throughout the entire experimental period (Fig. 3).

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### 3.1.2 Phytoplankton biomass

Chlorophyll *a* (Chl *a*) reached maximum concentrations of 2.06–2.48  $\mu\text{g L}^{-1}$  at day 5 (Fig. 4). Average concentrations of  $1.94 \pm 0.23 \mu\text{g L}^{-1}$  in phase I exceeded those in phases II and III when Chl *a* decreased to a mean of  $1.08 \pm 0.16 \mu\text{g L}^{-1}$ . The increase in Chl *a* in the high  $\text{CO}_2$  mesocosms by 24% in phase III was statistically significant (Paul et al., 2015b), and differences of  $0.27 \mu\text{g L}^{-1}$  were only marginal.

We observed a significant relationship between Chl *a* and  $\text{PO}_4$  in the untreated and intermediate treated mesocosms that was diminished with increasing  $f\text{CO}_2$  indicated by lower  $p$  values. The significance got lost in the highest  $f\text{CO}_2$  mesocosms (Table 2).

### 3.1.3 Phosphorus Pools

Total phosphorus (TP) concentrations in the mesocosms ranged between 0.49 and  $0.68 \mu\text{mol L}^{-1}$  (Fig. 5a) without significant differences between the different  $\text{CO}_2$  treatments. Shortly after the bags were closed, the decline in TP concentrations began and continued until the beginning of phase II. On average, TP concentrations decreased from  $0.63 \pm 0.02 \mu\text{mol L}^{-1}$  on day -3 to  $0.51 \pm 0.01 \mu\text{mol L}^{-1}$  on day 21. Thereafter, the mean TP remained constant at  $0.54 \pm 0.03 \mu\text{mol L}^{-1}$  until the end of the measurements. Thus, the loss of phosphorus ( $116 \text{ nmol L}^{-1}$ ) from the 17 m layer during the 29 day measurement period was calculated to be  $4.0 \text{ nmol L}^{-1} \text{ day}^{-1}$ . The decline in TP can be explained by loss through sedimentation of POP (Paul et al., 2015b).

Particulate organic phosphorus (POP) concentrations varied from 0.10 to  $0.23 \mu\text{mol L}^{-1}$  in all  $\text{CO}_2$  treatments (Figs. 5b and 6). We expected that the decrease in TP was reflected in POP. However, parallel changes occurred only periodically. POP concentrations increased during the first 5 days after the bags were closed. This increase was stimulated by the  $\text{CO}_2$  treatments from day 0 to day 2 (ANCOVA:  $p = 0.004$ ,  $F = 20.811$ ) (Fig. 7a). Subsequently, POP declined in parallel with TP until day 21, albeit with a lower amount. Averaged over all mesocosms, TP decreased by  $0.12 \mu\text{mol L}^{-1}$ , whereas POP declined only by  $0.06 \mu\text{mol L}^{-1}$  during this period. From





times were longest on day 9 and shortest on day 4 (Fig. 9c). Between 0.05 and 0.36 nmol ATP L<sup>-1</sup> h<sup>-1</sup> (mean 0.14 ± 0.08 nmol L<sup>-1</sup> h<sup>-1</sup>, *n* = 36) were degraded, corresponding to a P supply of 0.14 and 1.08 nmol L<sup>-1</sup> h<sup>-1</sup> (mean 0.44 ± 0.25 nmol L<sup>-1</sup> h<sup>-1</sup>, *n* = 36). Thus, phosphorus additionally supplied from ATP accounted for ~ 25 % of that provided by PO<sub>4</sub>. The picoplankton size fraction (< 3 μm) was responsible for 90–99 % of ATP uptake, with only a marginal portion (1.6–9.5 %) attributable to the phytoplankton fraction > 3 μm (Table 4).

## 3.2 Development in the fjord

### 3.2.1 In situ CO<sub>2</sub> and pH conditions

Large variations in *f*CO<sub>2</sub> and pH occurred in fjord water during the period of investigation (Table 1). The relationship of *f*CO<sub>2</sub> with temperature and salinity indicated that the CO<sub>2</sub> conditions were influenced predominantly by changes in the water masses, specifically by upwelling which affected both the relationship of *f*CO<sub>2</sub> with PO<sub>4</sub> and probably the correlation of *f*CO<sub>2</sub> with Chl *a* and PC (Table 2). *f*CO<sub>2</sub> ranged from 207 μatm (Fig. 2a) at days 12–16 when temperatures were highest to 800 μatm at day 33 when deep water input occurred which was indicated by low pH (7.75).

### 3.2.2 Phytoplankton biomass

Chl *a* concentrations in the fjord were between 1.12 and 5.46 μg L<sup>-1</sup> (mean 2.29 ± 1.11 μg L<sup>-1</sup>; *n* = 38), with distinct phases similar to those of temperature and salinity. However, the Chl *a* maximum occurred at the beginning of phase II, which was 1–2 days after the maximum temperature. Shortly thereafter, Chl *a* decreased to its lowest level before it increased again, albeit only marginally to 1.93 μg L<sup>-1</sup> during phase III (Fig. 4). Chl *a* concentrations correlated positively with temperature and pH (Table 5). The correlations of Chl *a* with PC and BPP suggested that phytoplankton determined the development of PC and was associated with bacterial growth.

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### 3.2.3 Phosphorus-Pools

TP concentrations from day -3 until day 29 ranged between 0.54 and 0.70  $\mu\text{molL}^{-1}$  (mean  $0.61 \pm 0.04 \mu\text{molL}^{-1}$ ;  $n = 19$ ) (Figs. 5a and 6a). The progression of TP differed from that of the hydrographic parameters or the Chl *a* concentrations. With a general decreasing tendency, TP undulated with a frequency of about 10 days in the period of phases 0 to the first half of phase I and of 6 days in the second half of phase I to II. For the period under investigation, the TP fractions had the following characteristics:

POP concentrations varied from 0.13 to 0.30  $\mu\text{molL}^{-1}$  (mean  $0.20 \pm 0.04 \mu\text{molL}^{-1}$ ;  $n = 29$ ), thus accounting for 23.4–51.8% (mean  $34.7 \pm 7.9\%$ ;  $n = 19$ ) of the TP pool. The development of POP over time did not follow that of TP (Fig. 6b). POP concentrations were highest between days 8 and 19, when the accumulation of POP in the biomass was reflected in declining C : P ratios from 180 to 107 and thereafter remained at the low ratio until the end of the measurements. The POP increase in phase III occurred in parallel to Chl *a* and to the  $\text{PO}_4$  decrease (Table 5). Thus  $\text{PO}_4$  was transformed into POP via biomass production. The calculated P content of phytoplankton was 0.05–0.15 (mean 0.1)  $\mu\text{mol POP} (\mu\text{g Chl } a)^{-1}$ .

DOP substantially contributed (26–45%) to the TP pool (Fig. 6). Concentrations ranged between 0.19 and 0.29  $\mu\text{molL}^{-1}$  (mean  $0.24 \pm 0.03 \mu\text{molL}^{-1}$ ;  $n = 17$ ), with high concentrations occurring in parallel to those of TP in phases I and II (Fig. 5c). The very low DOP value of 0.11  $\mu\text{molL}^{-1}$ , on day 29, was an outlier (Grubbs test) and was excluded from the calculation. For the whole study period, DOP concentrations correlated positively with both POP and  $\text{PO}_4$  turnover times and inversely with  $\text{PO}_4$  concentrations (Table 5). A similar behavior between DOP and Chl *a* was restricted to phases 0 and I, whereas the relationship was inverse in phase II (Fig. 8b) indicating that upwelling of deep water did not change the DOP concentrations in surface water. As shown in Fig. 8a, the DOP and BPP levels alternated with the same rhythm, but inversely, in phases 0 and I and changed to a parallel development in phase II. Statistical analysis

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was not feasible because DOP and BPP were not always sampled on the same day and only very few data pairs were available.

Phosphorus, derived from the sum of ATP, PL, RNA, and DNA, constituted 42.8–72.0% (mean  $59.7 \pm 10.7\%$ ;  $n = 7$ ) of the DOP pool (Table 3). Thus, 27.8–57.2% of the DOP remained unidentified. Concentrations of  $1.4\text{--}4.6\text{ nmol ATP L}^{-1}$ ,  $0.6\text{--}4.5\text{ nmol PLL}^{-1}$ ,  $42.2\text{--}163\text{ }\mu\text{g RNAL}^{-1}$ , and  $0.03\text{--}0.06\text{ }\mu\text{g DNAL}^{-1}$  were measured, yielding  $3.1\text{--}13.8\text{ nmol ATP-PL}^{-1}$ ,  $0.6\text{--}4.5\text{ nmol PL-PL}^{-1}$ ,  $42.2\text{--}163\text{ nmol RNA-PL}^{-1}$ , and  $0.06\text{--}0.13\text{ nmol DNA-PL}^{-1}$ . Thus, the contribution of RNA to the DOP pool was the highest, whereas the contributions of ATP, PL, and DNA were relatively small (Table 3). The changes in all of these components over time were not related to changes in the total DOP pool (Fig. 10).

$\text{PO}_4$  concentrations ranged between  $0.06$  and  $0.41\text{ }\mu\text{mol L}^{-1}$  (mean  $0.21 \pm 0.09\text{ }\mu\text{mol L}^{-1}$ ,  $n = 21$ ), thus comprising  $24.3 \pm 11.2\%$  ( $n = 21$ ) of the TP pool (Fig. 6). With a few exceptions,  $\text{PO}_4$  concentrations declined from the beginning of the study period until the end of phase I and increased during phase II and the beginning of phase III. These changes were caused by upwelling of  $\text{PO}_4$  enriched deep water of higher salinity and lower temperatures. The subsequent decline in  $\text{PO}_4$  between days 33 and 40 was caused by the stimulation of phytoplankton production, as indicated by the increase in Chl *a* concentration (Fig. 4). For the whole experimental period, the Spearman rank correlation showed an inverse relationship between  $\text{PO}_4$  and particulate organic matter such as Chl *a*, PC, and PN (Table 5).

### 3.2.4 Uptake of $\text{PO}_4$ and ATP

Applying [ $^{33}\text{P}$ ] $\text{PO}_4$ ,  $\text{PO}_4$  turnover times in the fjord were in the range of 30–379 h (1.2–15 days) (mean  $139 \pm 98\text{ h}$ ,  $n = 18$ ) (Fig. 9a), corresponding to uptake rates of  $0.73\text{--}3.37\text{ nmol L}^{-1}\text{ h}^{-1}$  (mean  $1.64 \pm 0.82\text{ nmol L}^{-1}\text{ h}^{-1}$ ,  $n = 18$ ) (Table 4). These rates were influenced by multiple factors, including temperature, phytoplankton biomass, and DP, POP, and  $\text{PO}_4$  concentrations as deduced from Table 5. Despite the paucity of

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data pairs, the total PO<sub>4</sub> uptake rate correlated with total BPP and with BPP in the fraction < 5 μm ( $r = 0.886$ ;  $p = 0.0188$ ;  $n = 6$  for each relationship).

Within the experimental period, the turnover times shortened on days 15–17 (Fig. 9a), when temperature and Chl *a* (Figs. 3 and 4) reached a maximum and PO<sub>4</sub> concentrations were lowest (Figs. 5d). Although the shortest turnover times were expected to be coupled with the highest uptake rates, the latter were estimated 2 days later, between days 17 and 19. The day-to-day variations conformed to the small changes in temperature and PO<sub>4</sub> concentrations. Uptake was dominated by the size fraction < 3 μm in most of the measurements (Table 4), which accounted for 17.4–92.3 % (mean  $72.2 \pm 20.6$  %) of the total uptake rate. The mean contribution of the size fractions > 3 μm was  $27.8 \pm 20.6$  %. Assuming that autotrophic organisms were largely responsible for the uptake by this fraction, the specific PO<sub>4</sub> uptake rates of phytoplankton, calculated from the size fraction > 3 μm and the Chl *a* concentration, were 0.02 and 0.46 nmol (μg Chl *a*)<sup>-1</sup> h<sup>-1</sup>.

The turnover times of ATP (7.5–62 h, or 0.3–2.6 days; mean  $23 \pm 15$  h or  $0.96 \pm 0.59$  days;  $n = 15$ ) were significantly shorter than those of PO<sub>4</sub> (Fig. 9c), without any apparent relationship between the two. The longest ATP turnover times of 2.6 and 1.7 days occurred on days 9 and 15, respectively, when PO<sub>4</sub> turnover times were short. Based on measured ATP concentrations, 0.03–0.15 nmol ATP L<sup>-1</sup> h<sup>-1</sup> was converted, thus delivering 0.13–0.46 nmol P L<sup>-1</sup> h<sup>-1</sup> (Table 4). The size fraction < 3 μm utilized 85.0–97.8 % (mean  $92.4 \pm 4.7$  %) of the ATP whereas only a small portion (2.2–15 %) could be attributed to the size fraction > 3 μm. ATP uptake rates and concentrations did not correlate with any of the other measured parameters (data not shown), with the exception that ATP turnover time correlated with BPP > 5 μm ( $r = 0.943$ ,  $p = 0.048$ ,  $n = 6$ ).

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## 4 Discussion

An increase in CO<sub>2</sub> in marine waters and the associated acidification may potentially have multiple effects on organisms and biogeochemical element cycling (Gattuso and Hansson, 2011). However, reported findings indicate wide ranging responses, probably depending on the investigated species and growth conditions. For example, CO<sub>2</sub> stimulation as well as lack of stimulation were found for primary production and carbon fixation (Beardall et al., 2009; Boettjer et al., 2014), DOC release (Engel et al., 2014; MacGilchrist et al., 2014) and phytoplankton growth (Riebesell and Tortell, 2011). Thus, the responses of organisms and ecosystems to enhanced CO<sub>2</sub> concentrations are complex and still poorly understood. The present study is the first to determine the effects of increased CO<sub>2</sub> levels on the phosphorus cycle in a brackish water ecosystem.

### 4.1 Response of P-pools and P-uptake to enhanced CO<sub>2</sub> in the mesocosms

The Finish side of the Gulf of Finland is one of the most important upwelling regions in the Baltic Sea. During our investigation in 2012, surface temperatures, obtained from the NOAA satellite (Siegel and Gerth, 2013) showed that upwelling persisted during the whole study period but with varying intensity. The intensity of upwelling shaped the pattern of temperature in the fjord and in the mesocosms varying from 7.8 to 15.9°C. Such variations in temperature influence the phosphorus transformation and interleave with CO<sub>2</sub> effects.

While nutrients were added in previous mesocosms experiments (Riebesell et al., 2008; Schulz et al., 2008), no amendments were undertaken in this study in order to be close to natural conditions. Initial PO<sub>4</sub> concentrations of only  $0.17 \pm 0.01 \mu\text{mol L}^{-1}$  were measured, however, PO<sub>4</sub> was never exhausted (Figs. 5 and 6). Cellular C:P and N:P ratios were close to the Redfield ratio. Therefore, phosphorus limitation unlikely occurred in this experiment. Simultaneous low nitrate and ammonium concentrations (Paul et al., 2015b) formed nutrient conditions that benefit the growth of diazotrophic cyanobacteria. However, any cyanobacteria bloom failed to appear, despite the low-

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level presence of *Aphanizomenon sp.* and *Anabaena sp.* (Paul et al., 2015a) as potential seed stock. For Baltic Sea summer conditions, the phytoplankton development with maximum Chl *a* concentrations of 2.2–2.5  $\mu\text{g L}^{-1}$  remained relatively low with the highest contribution of cryptophytes and chlorophytes in phase I and at the beginning of phase II. Picoplankton was mostly the dominating size fraction amounting  $\sim 20$ – $70\%$  of Chl *a* in phase I and rising to  $\sim 85\%$  in phase III (Paul et al., 2015b). However, a positive correlation of  $f\text{CO}_2$  with the Chl *a* size fraction  $> 20\mu\text{m}$  was estimated. The abundance of diatoms that could be a part of this fraction increased from  $\sim$  day 23 to day 30 and might have an influence on this relationship.

Against this background, the CO<sub>2</sub> perturbation did not cause significant changes in phosphorus pool sizes, DOP composition, and P-uptake rates from PO<sub>4</sub> and ATP when the whole study period was considered. However, small but nevertheless significant, short-term effects on PO<sub>4</sub> and POP pool sizes were observed in phases I and III (Fig. 7). CO<sub>2</sub> elevation stimulated the formation of POP until day 3 (Fig. 5b) when chlorophytes, cyanobacteria, prasinophytes and the pico-cyanobacteria started to grow (Paul et al., 2015b).

The effects of CO<sub>2</sub> addition on PO<sub>4</sub> and POP pool sizes were evident from day 23 onwards (Figs. 5 and 7). PO<sub>4</sub> concentrations were slightly, but significantly lower in the high CO<sub>2</sub> treatment than in the untreated mesocosms, accompanied by significantly elevated POP concentrations indicating that the transformation of PO<sub>4</sub> into POP was likely stimulated under high CO<sub>2</sub> conditions. Since Chl *a* was elevated as well at similar POP : Chl *a* ratios, the PO<sub>4</sub> taken up was used for new biomass formation. However, the elevated transformation of PO<sub>4</sub> into POP was not detected in the PO<sub>4</sub> uptake rates which can be seen as gross uptake rates. Thus, it is likely that not the gross uptake but rather the net uptake was modified, e.g. via reduction in P-release from biomass under CO<sub>2</sub> elevation.

It is hard to assess the short-term effects that we have found in phase I. Uptake and release are assumed to be continuous processes and can alter the P pool sizes on timescales shorter than one day. Thus, variations and differences in the treatments

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can be overseen at daily sampling. Unger et al. (2013) demonstrated that an accelerated PO<sub>4</sub> uptake by the cyanobacterium *Nodularia spumigena* under elevated CO<sub>2</sub> incubations could only be observed during the first hours. Thereafter, the differences were balanced and the same level of radiotracer labeling was reached in all treatments.

5 An acceleration in formation of particulate P concentrations under CO<sub>2</sub> elevation without any changes of PO<sub>4</sub> turnover times was also observed by Tanaka et al. (2008). They observed an increase of the POP amount and an earlier appearance of the POP maximum under CO<sub>2</sub> elevation.

Correlations calculated by using the Spearman rank test between P pools or up-  
take rates and other parameters for each mesocosm are presented in Table 2. The  
relationships between POP and TP with Chl *a* disappeared at elevated *f*CO<sub>2</sub>, whereas  
correlations developed between POP and PC as well as between the PO<sub>4</sub> uptake by  
phytoplankton > 3 μm and the POP : Chl *a* ratio (Table 2). These shifts could be caused  
by changes in the phytoplankton composition deduced from CO<sub>2</sub> effects on the pigment  
composition (Paul et al., 2015b).

Independent of the CO<sub>2</sub> treatment, TP decreased by 2.6 nmol L<sup>-1</sup> day<sup>-1</sup> in all meso-  
cosms over the course of the experiment, in agreement with the measured sedimenta-  
tion rates (Paul et al., 2015b). The strongest decrease (~ 3.2 nmol L<sup>-1</sup> day<sup>-1</sup>) oc-  
curred during phase I. Of the total TP removal during this phase (48 nmol L<sup>-1</sup>), 84 %  
20 (~ 40.5 nmol L<sup>-1</sup>) could be explained by the decrease in POP and 16 % (~ 8 nmol L<sup>-1</sup>)  
by changes in the dissolved pool. However, the PO<sub>4</sub> decline (~ 34.5 nmol L<sup>-1</sup>) was  
stronger than that of the total dissolved P pool since DOP increased in parallel by  
~ 26.5 nmol L<sup>-1</sup>. Thus, about 77 % of the PO<sub>4</sub> reduction was retrieved as DOP and  
remained in the dissolved P-pool being the main pathway of PO<sub>4</sub> transformation.

## 4.2 Phosphorus dynamics in the Storfjärden

Measurements of P-pool sizes and P uptake in the fjord provided new information  
about the phosphorus dynamics in a Baltic Sea upwelling system and in times when  
diazotrophic cyanobacteria did not dominate the phytoplankton community. Nutrient









measurements. We appreciate the assistance of Jehane Ouriqua in the nutrient analysis and that of many other participants who carried out the samplings. We also appreciate the collegial atmosphere during the work and thank everyone who contributed to it. We would also like to acknowledge the staff of the Tvärminne Zoological Station for their hospitality and support, for allowing us to use the experimental facilities, and for providing CTD data for the summers of 2008–2011. Finally, we thank Jana Woelk for analysing the phosphorus samples in the IOW. This study was funded by the BMBF project BIOACID II (FKZ 03F06550).

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**Table 1.** Minimum, maximum and mean values of hydrographical parameters and  $f\text{CO}_2$  for the different phases in the fjord. Temperatures in the mesocosms were identical with those in surrounding fjord water.

| phase                  | min  | max   | mean  |
|------------------------|------|-------|-------|
| water temperature (°C) |      |       |       |
| 0                      | 7.82 | 8.71  | 8.20  |
| I                      | 9.66 | 15.86 | 12.27 |
| II                     | 7.89 | 14.79 | 11.68 |
| III                    | 8.35 | 12.61 | 10.83 |
| salinity               |      |       |       |
| 0                      | 5.72 | 5.85  | 5.78  |
| I                      | 5.46 | 5.85  | 5.65  |
| II                     | 5.67 | 6.04  | 5.82  |
| III                    | 5.9  | 6.05  | 5.98  |
| pH                     |      |       |       |
| 0                      | 8.09 | 8.23  | 8.16  |
| I                      | 8.11 | 8.30  | 8.17  |
| II                     | 7.81 | 8.30  | 8.00  |
| III                    | 7.75 | 7.93  | 7.83  |
| $f\text{CO}_2$ (µatm)  |      |       |       |
| 0                      | 250  | 347   | 298   |
| I                      | 207  | 336   | 283   |
| II                     | 208  | 679   | 465   |
| III                    | 521  | 800   | 668   |

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**Table 2.** Mesocosms in which the Spearman Rank correlation between P-pools or uptake rates and other parameters was significant. The relationship of POP with TP and Chl *a* was significant only in the untreated mesocosms while the correlation to PC was also significant in the mesocosms with intermediate CO<sub>2</sub> levels. DOP was related to temperature only in the high CO<sub>2</sub> treatments. Under high *f*CO<sub>2</sub> conditions, the PO<sub>4</sub> uptake in the size fraction > 3µm correlated with Chl *a* and the P content of phytoplankton.

| Relationship between                          | <i>f</i> CO <sub>2</sub><br>(µatm) | significant responses |          |          |
|---|------------------------------------|-----------------------|----------|----------|
|   |                                    | <i>r</i>              | <i>p</i> | <i>n</i> |
| POP–TP  | 365                                | 0.599                 | 0.008    | 18       |
|   | 368                                | 0.515                 | 0.029    | 18       |
| POP–Chl <i>a</i>                              | 365                                | 0.479                 | 0.0130   | 25       |
|   | 368                                | 0.584                 | 0.0022   | 25       |
| PO <sub>4</sub> –Chl <i>a</i>                 | 365                                | –0.832                | < 0.0001 | 21       |
|   | 368                                | –0.756                | 0.0011   | 20       |
|   | 497                                | –0.674                | 0.0008   | 21       |
|   | 821                                | –0.524                | 0.0147   | 21       |
|   | 1007                               | –0.634                | 0.0027   | 20       |
| POP–PC  | 365                                | 0.542                 | 0.0061   | 24       |
|   | 368                                | 0.625                 | 0.0011   | 24       |
|   | 497                                | 0.404                 | 0.0490   | 24       |
|   | 821                                | 0.551                 | 0.0052   | 24       |
| DOP–temperature                               | 1007                               | 0.488                 | 0.0470   | 17       |
|   | 1231                               | 0.525                 | 0.0310   | 17       |
| PO <sub>4</sub> uptake > 3µm–Chl <i>a</i>     | 497                                | 0.743                 | 0.0056   | 12       |
|   | 821                                | 0.674                 | 0.0081   | 14       |
|   | 1231                               | 0.476                 | 0.0310   | 14       |
| PO <sub>4</sub> uptake > 3µm–POP/Chl <i>a</i> | 497                                | –0.601                | 0.0380   | 12       |
|   | 821                                | –0.631                | 0.0160   | 14       |
|   | 1231                               | –0.626                | 0.0165   | 14       |

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**Table 3.** Contribution of different phosphorus components to DOP in the mesocosms and in the fjord.

| $f\text{CO}_2$<br>( $\mu\text{atm}$ ) | ATP-P | PL-P | contribution to DOP (%) |       |       | unidentified P |
|---------------------------------------|-------|------|-------------------------|-------|-------|----------------|
|                                       |       |      | DNA-P                   | RNA-P | sum   |                |
| Fjord                                 | 0.7   | 0.7  | 0.04                    | 69.4  | 70.84 | 29.16          |
| 365                                   | 0.7   | 0.5  | 0.03                    | 44.1  | 45.33 | 54.67          |
| 368                                   | 0.6   | 0.5  | 0.03                    | 46.9  | 48.03 | 51.97          |
| 497                                   | 0.6   | 0.4  | 0.04                    | 49.5  | 50.54 | 49.46          |
| 821                                   | 0.6   | 0.4  | 0.03                    | 41.8  | 42.83 | 57.17          |
| 1003                                  | 0.8   | 0.4  | 0.04                    | 60.1  | 61.34 | 38.66          |
| 1231                                  | 0.5   | 0.4  | 0.03                    | 48.6  | 49.53 | 50.47          |

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**Table 4.** PO<sub>4</sub>- and ATP uptake rates in the fjord and in the mesocosms. Minimum, maximum and mean values as well as the contribution of the size fraction < 3 μm to the total activity are given for the whole period of investigation (each: *n* = 16 for PO<sub>4</sub> and *n* = 6 for ATP uptake).

| fCO <sub>2</sub><br>(μatm) | total PO <sub>4</sub> uptake (nmol L <sup>-1</sup> h <sup>-1</sup> ) |      |             | portion (%)<br>< 3 μm | total ATP-P uptake (nmol L <sup>-1</sup> h <sup>-1</sup> ) |      |             | portion (%)<br>< 3 μm |
|----------------------------|--|------|-------------|-----------------------|--|------|-------------|-----------------------|
|                            | min  | max  | mean        |                       | min  | max  | mean        |                       |
| Fjord                      | 0.87   | 2.81 | 1.63 ± 0.58 | 76 ± 15               | 0.04   | 0.51 | 0.26 ± 0.15 | 92 ± 5                |
| 365                        | 0.82   | 3.89 | 1.67 ± 0.82 | 81 ± 11               | 0.14   | 1.08 | 0.43 ± 0.33 | 96 ± 2                |
| 368                        | 0.65   | 2.74 | 1.61 ± 0.58 | 86 ± 7                | 0.16   | 0.97 | 0.47 ± 0.27 | 96 ± 2                |
| 497                        | 0.61   | 3.03 | 1.52 ± 0.59 | 86 ± 6                | 0.20   | 1.07 | 0.54 ± 0.28 | 96 ± 2                |
| 821                        | 0.91   | 2.83 | 1.60 ± 0.59 | 88 ± 8                | 0.14   | 0.71 | 0.36 ± 0.21 | 97 ± 2                |
| 1003                       | 0.67   | 3.79 | 1.73 ± 0.85 | 86 ± 6                | 0.22   | 0.69 | 0.39 ± 0.15 | 97 ± 1                |
| 1231                       | 0.87   | 2.23 | 1.53 ± 0.43 | 87 ± 6                | 0.17   | 0.67 | 0.44 ± 0.17 | 97 ± 2                |

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**Table 5.** Significance level ( $p$ ) deduced from of Spearman Rank correlations that were calculated between the parameters in fjord water listed in the table.

| Variable   | $T$                   | $S$                   | $f\text{CO}_2$        | $\text{PO}_4$         | POP                   | DOP                 | Chl $a$               | PC                    | C/P                 |
|--|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|---------------------|-----------------------|-----------------------|---------------------|
| $T$ (°C)   |                       | 0.0001 <sup>-</sup>   | 0.0061 <sup>-</sup>   | < 0.0001 <sup>-</sup> | < 0.0001 <sup>+</sup> | n.s.                | < 0.0001 <sup>+</sup> | 0.0083 <sup>+</sup>   | n.s.                |
| $S$  | 0.0001 <sup>-</sup>   |                       | < 0.0001 <sup>+</sup> | < 0.0001 <sup>+</sup> | 0.0030 <sup>-</sup>   | 0.0445 <sup>-</sup> | < 0.0001 <sup>-</sup> | < 0.0001 <sup>-</sup> | 0.0181 <sup>-</sup> |
| $p\text{CO}_2$   | 0.0061 <sup>-</sup>   | < 0.0001 <sup>+</sup> |                       | < 0.0001 <sup>+</sup> | 0.0288 <sup>-</sup>   | n.s.                | < 0.0001 <sup>-</sup> | < 0.0001 <sup>-</sup> | 0.0104 <sup>-</sup> |
| $\text{PO}_4$ ( $\mu\text{mol L}^{-1}$ )                                     | < 0.0001 <sup>-</sup> | < 0.0001 <sup>+</sup> | < 0.0001 <sup>+</sup> |                       | < 0.0001 <sup>-</sup> | 0.0049 <sup>-</sup> | < 0.0001 <sup>-</sup> | 0.0008 <sup>-</sup>   | n.s.                |
| POP ( $\mu\text{mol L}^{-1}$ )   | < 0.0001 <sup>+</sup> | 0.0030 <sup>-</sup>   | 0.0288 <sup>-</sup>   | < 0.0001 <sup>-</sup> |                       | 0.0345 <sup>+</sup> | < 0.0001 <sup>+</sup> | 0.0016 <sup>+</sup>   | n.s.                |
| DOP ( $\mu\text{mol L}^{-1}$ )   | n.s.                  | 0.0445 <sup>-</sup>   | n.s.                  | 0.0049 <sup>-</sup>   | 0.0345 <sup>+</sup>   |                     | 0.0271 <sup>+</sup>   | n.s.                  | n.s.                |
| Chl $a$ ( $\mu\text{g L}^{-1}$ )   | < 0.0001 <sup>+</sup> | < 0.0001 <sup>-</sup> | < 0.0001 <sup>-</sup> | < 0.0001 <sup>-</sup> | < 0.0001 <sup>+</sup> | 0.0271 <sup>+</sup> |                       | 0.0003 <sup>+</sup>   | n.s.                |
| PC ( $\mu\text{mol L}^{-1}$ )  | 0.0083 <sup>+</sup>   | < 0.0001 <sup>-</sup> | < 0.0001 <sup>-</sup> | 0.0008 <sup>-</sup>   | 0.0016 <sup>+</sup>   | n.s.                | 0.0003 <sup>+</sup>   |                       | 0.0014 <sup>+</sup> |
| PN ( $\mu\text{mol L}^{-1}$ )  | 0.0007 <sup>+</sup>   | < 0.0001 <sup>-</sup> | < 0.0001 <sup>-</sup> | 0.0001 <sup>-</sup>   | < 0.0001 <sup>+</sup> | n.s.                | < 0.0001 <sup>+</sup> | < 0.0001 <sup>+</sup> | 0.0156 <sup>+</sup> |
| C/P  | n.s.                  | 0.0181 <sup>-</sup>   | 0.0104 <sup>-</sup>   | n.s.                  | n.s.                  | n.s.                | n.s.                  | 0.0014 <sup>+</sup>   |                     |
| $\text{PO}_4$ -TO time (d)   | 0.0001 <sup>-</sup>   | < 0.0001 <sup>+</sup> | 0.0250 <sup>+</sup>   | < 0.0001 <sup>+</sup> | 0.0048 <sup>-</sup>   | 0.0099 <sup>+</sup> | 0.0006 <sup>-</sup>   | n.s.                  | n.s.                |
| $\text{PO}_4$ total uptake ( $\text{nmol L}^{-1}\text{h}^{-1}$ )             | n.s.                  | 0.0244 <sup>-</sup>   | 0.0403 <sup>-</sup>   | 0.0455 <sup>-</sup>   | n.s.                  | n.s.                | n.s.                  | n.s.                  | n.s.                |
| $\text{PO}_4$ uptake > 3 $\mu\text{m}$ ( $\text{nmol L}^{-1}\text{h}^{-1}$ ) | n.s.                  | 0.0284 <sup>-</sup>   | 0.0006 <sup>-</sup>   | n.s.                  | n.s.                  | n.s.                | n.s.                  | 0.0386 <sup>+</sup>   | n.s.                |
| $\text{PO}_4$ uptake < 3 $\mu\text{m}$ ( $\text{nmol L}^{-1}\text{h}^{-1}$ ) | n.s.                  | n.s.                  | n.s.                  | n.s.                  | n.s.                  | n.s.                | n.s.                  | n.s.                  | n.s.                |
| BBP total ( $\mu\text{g C L}^{-1}\text{h}^{-1}$ )                            | 0.0005 <sup>+</sup>   | 0.0037 <sup>-</sup>   | n.s.                  | 0.0018 <sup>-</sup>   | 0.0022 <sup>+</sup>   | n.s.                | < 0.0001 <sup>+</sup> | 0.0092 <sup>+</sup>   | n.s.                |
| BBP > 5 $\mu\text{m}$ ( $\mu\text{g C L}^{-1}\text{h}^{-1}$ )                | 0.0088 <sup>+</sup>   | 0.0370 <sup>-</sup>   | n.s.                  | 0.0353 <sup>-</sup>   | 0.0056 <sup>+</sup>   | n.s.                | 0.0027 <sup>+</sup>   | 0.0479 <sup>+</sup>   | n.s.                |
| BBP 0.2–5 $\mu\text{m}$ ( $\mu\text{g C L}^{-1}\text{h}^{-1}$ )              | 0.0012 <sup>+</sup>   | 0.0072 <sup>-</sup>   | n.s.                  | 0.0041 <sup>-</sup>   | 0.0102 <sup>+</sup>   | n.s.                | < 0.0001 <sup>+</sup> | 0.0114 <sup>+</sup>   | n.s.                |

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**Table 5.** Continued.

| Variable   | PO <sub>4</sub><br>TO-time | total PO <sub>4</sub><br>uptake | > 3μm PO <sub>4</sub><br>uptake | < 3μm PO <sub>4</sub><br>uptake | total<br>BPP          | > 5μm<br>BPP          | 0.2–5μm<br>BPP        |
|--|----------------------------|---------------------------------|---------------------------------|---------------------------------|-----------------------|-----------------------|-----------------------|
| <i>T</i> (°C)  | 0.0001 <sup>-</sup>        | n.s.                            | n.s.                            | n.s.                            | 0.0005 <sup>+</sup>   | 0.0088 <sup>+</sup>   | 0.0012 <sup>+</sup>   |
| <i>S</i>   | < 0.0001 <sup>+</sup>      | 0.0244 <sup>-</sup>             | 0.0284 <sup>-</sup>             | n.s.                            | 0.0037 <sup>-</sup>   | 0.0370 <sup>-</sup>   | 0.0072 <sup>-</sup>   |
| <i>p</i> CO <sub>2</sub>   | 0.0250 <sup>+</sup>        | 0.0403 <sup>-</sup>             | 0.0006 <sup>-</sup>             | n.s.                            | n.s.                  | n.s.                  | n.s.                  |
| PO <sub>4</sub> (μmol L <sup>-1</sup> )                              | < 0.0001 <sup>+</sup>      | 0.0455 <sup>-</sup>             | n.s.                            | n.s.                            | 0.0018 <sup>-</sup>   | 0.0353 <sup>-</sup>   | 0.0041 <sup>-</sup>   |
| POP (μmol L <sup>-1</sup> )  | 0.0048 <sup>-</sup>        | n.s.                            | n.s.                            | n.s.                            | 0.0022 <sup>+</sup>   | 0.0056 <sup>+</sup>   | 0.0102 <sup>+</sup>   |
| DOP (μmol L <sup>-1</sup> )  | 0.0099 <sup>+</sup>        | n.s.                            | n.s.                            | n.s.                            | n.s.                  | n.s.                  | n.s.                  |
| Chl <i>a</i> (μg L <sup>-1</sup> )                                   | 0.0006 <sup>-</sup>        | n.s.                            | n.s.                            | n.s.                            | < 0.0001 <sup>+</sup> | 0.0027 <sup>+</sup>   | < 0.0001 <sup>+</sup> |
| PC (μmol L <sup>-1</sup> )   | n.s.                       | n.s.                            | 0.0386 <sup>+</sup>             | n.s.                            | 0.0092 <sup>+</sup>   | 0.0479 <sup>+</sup>   | 0.0114 <sup>+</sup>   |
| PN (μmol L <sup>-1</sup> )   | 0.0165 <sup>-</sup>        | n.s.                            | n.s.                            | n.s.                            | 0.0092 <sup>+</sup>   | 0.0479 <sup>+</sup>   | 0.0114 <sup>+</sup>   |
| C/P  | n.s.                       | n.s.                            | n.s.                            | n.s.                            | n.s.                  | n.s.                  | n.s.                  |
| PO <sub>4</sub> -TO time (d)   | < 0.0001 <sup>-</sup>      | < 0.0001 <sup>-</sup>           | n.s.                            | n.s.                            | n.s.                  | n.s.                  | n.s.                  |
| PO <sub>4</sub> total uptake (nmol L <sup>-1</sup> h <sup>-1</sup> ) | < 0.0001 <sup>-</sup>      | n.s.                            | n.s.                            | n.s.                            | 0.0188 <sup>+</sup>   | n.s.                  | 0.0188 <sup>+</sup>   |
| PO <sub>4</sub> uptake > 3μm (nmol L <sup>-1</sup> h <sup>-1</sup> ) | n.s.                       | n.s.                            | n.s.                            | n.s.                            | n.s.                  | n.s.                  | n.s.                  |
| PO <sub>4</sub> uptake < 3μm (nmol L <sup>-1</sup> h <sup>-1</sup> ) | n.s.                       | n.s.                            | n.s.                            | n.s.                            | n.s.                  | n.s.                  | n.s.                  |
| BBP total (μg CL <sup>-1</sup> h <sup>-1</sup> )                     | n.s.                       | 0.0188 <sup>+</sup>             | n.s.                            | n.s.                            | < 0.0001 <sup>+</sup> | < 0.0001 <sup>+</sup> | < 0.0001 <sup>+</sup> |
| BPP > 5μm (μg CL <sup>-1</sup> h <sup>-1</sup> )                     | n.s.                       | n.s.                            | n.s.                            | n.s.                            | < 0.0001 <sup>+</sup> | < 0.0001 <sup>+</sup> | 0.0001 <sup>+</sup>   |
| BPP 0.2–5μm (μg CL <sup>-1</sup> h <sup>-1</sup> )                   | n.s.                       | 0.0188 <sup>+</sup>             | n.s.                            | n.s.                            | < 0.0001 <sup>+</sup> | 0.0001 <sup>+</sup>   |                       |

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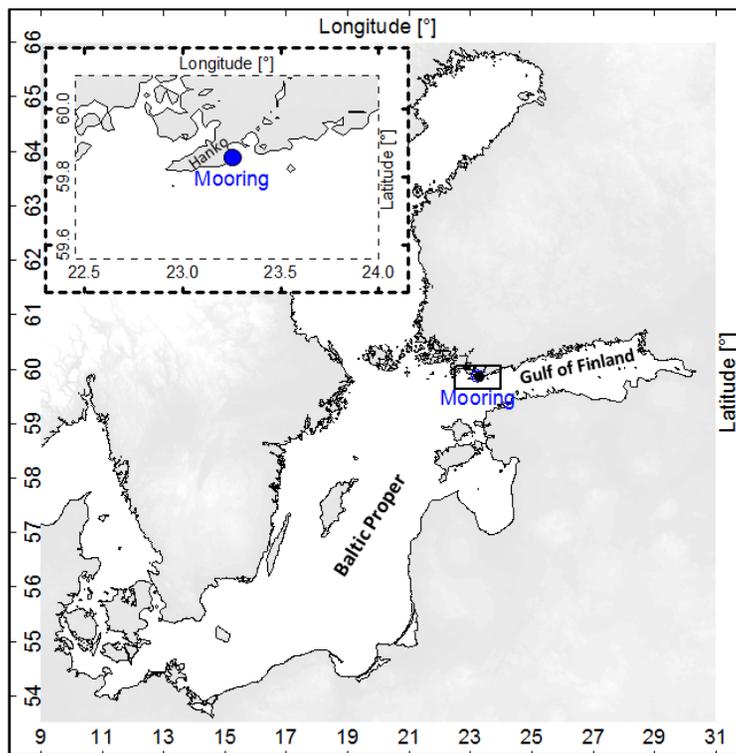
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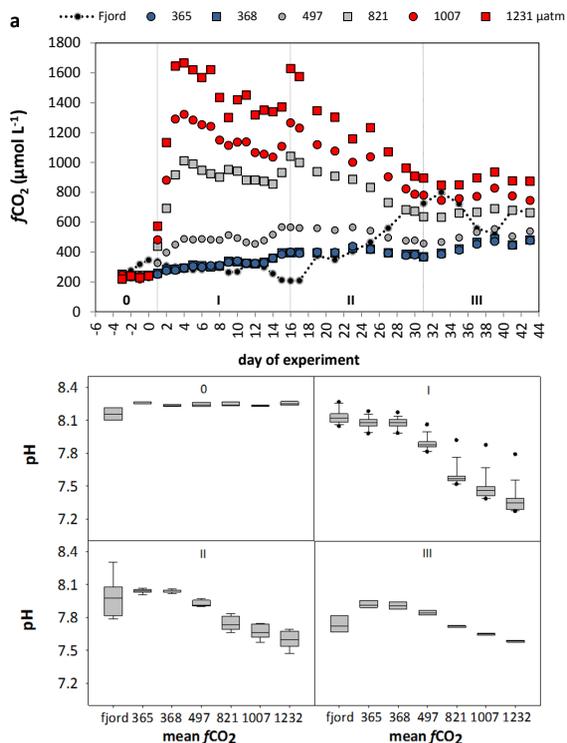
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**Figure 1.** The Baltic Sea and the location near the peninsula Hanko in the western Gulf of Finland where the mesocosms were deployed.

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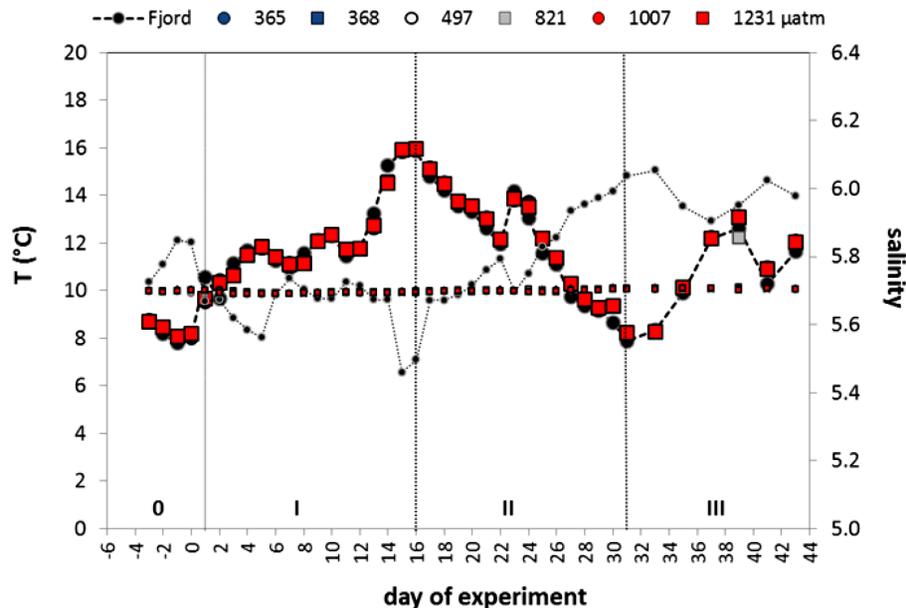
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**Figure 2.** (a)  $f\text{CO}_2$  values in the mesocosms and in the fjord throughout the experiment. Small black dots show the  $f\text{CO}_2$  in the ambient fjord water. Treatment of the mesocosms with CO<sub>2</sub> saturated fjord water at the beginning of the experiment (days 0–4) created different  $f\text{CO}_2$  levels in the mesocosms: blue symbols represents the untreated mesocosms, grey the intermediate, and red the high CO<sub>2</sub> treated mesocosms. The treatment was repeated at day 16. (b) Corresponding pH ranges in the mesocosms during the four phases. Despite decreasing trend over time, a gradient between the mesocosms was kept over the whole period.

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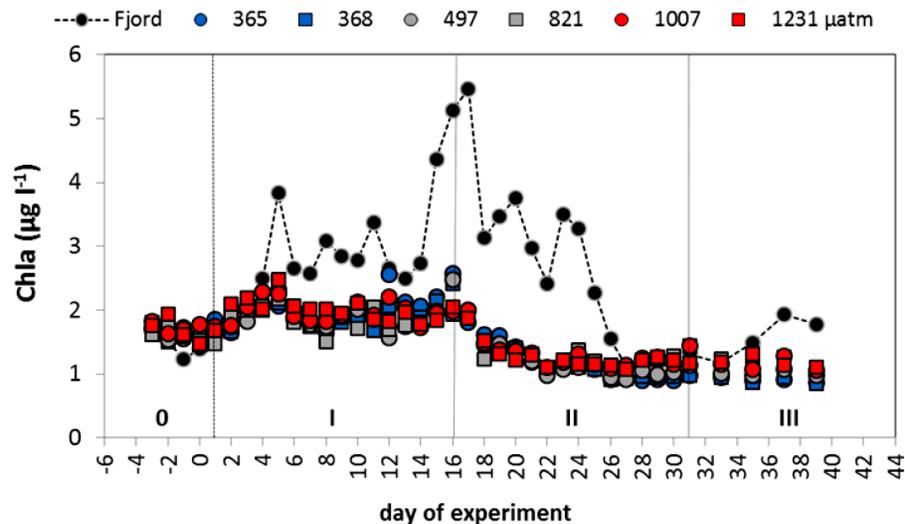
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**Figure 3.** Temperature and salinity averaged over the 17 m surface layer of the mesocosms and the fjord. The data were obtained from daily CTD casts. Large symbols represent temperature and the small symbols salinity. Fjord water is shown as black dots with broken line while blue symbols denote untreated, grey intermediate and red high  $f\text{CO}_2$  levels in the mesocosms. According to the temperature regime, the experimental period can be divided into four phases (phases 0, I, II and III).

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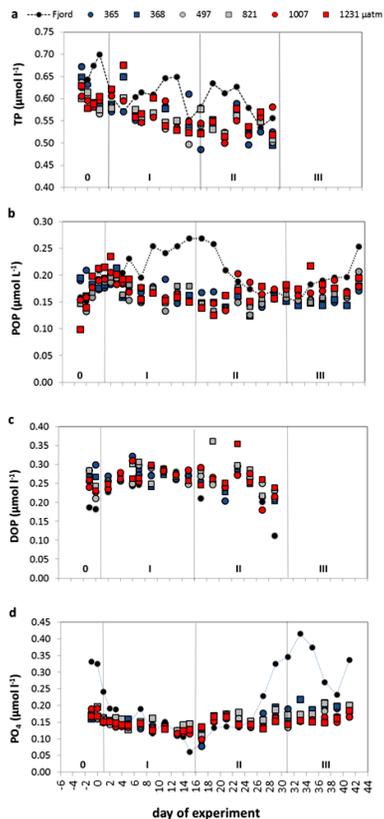


**Figure 4.** Chl *a* concentrations in fjord water and in the mesocosms with different  $f\text{CO}_2$  conditions. The development over time can be divided into three phases as well. Blue represent untreated, grey intermediate, and red highly treated  $f\text{CO}_2$  levels. Black dots are the Chl *a* concentrations in the fjord water.

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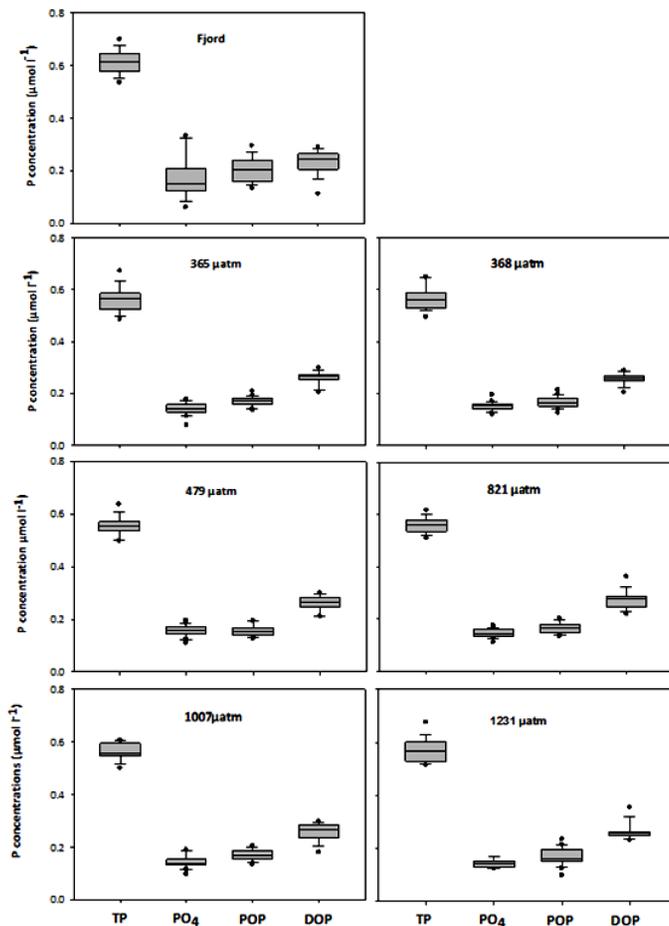


**Figure 5. (a–d)** Development of total phosphorus (TP) and the three measured P-fractions in fjord water (black dots with dotted line) and in the mesocosms over time. Blue represents untreated, grey intermediate and red high  $f\text{CO}_2$  treatment levels.

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**Figure 6.** Contribution of the individual P-fractions to TP in fjord water and in the respective mesocosms. The data are averaged for the period when TP measurements were done (day –3–day 29).

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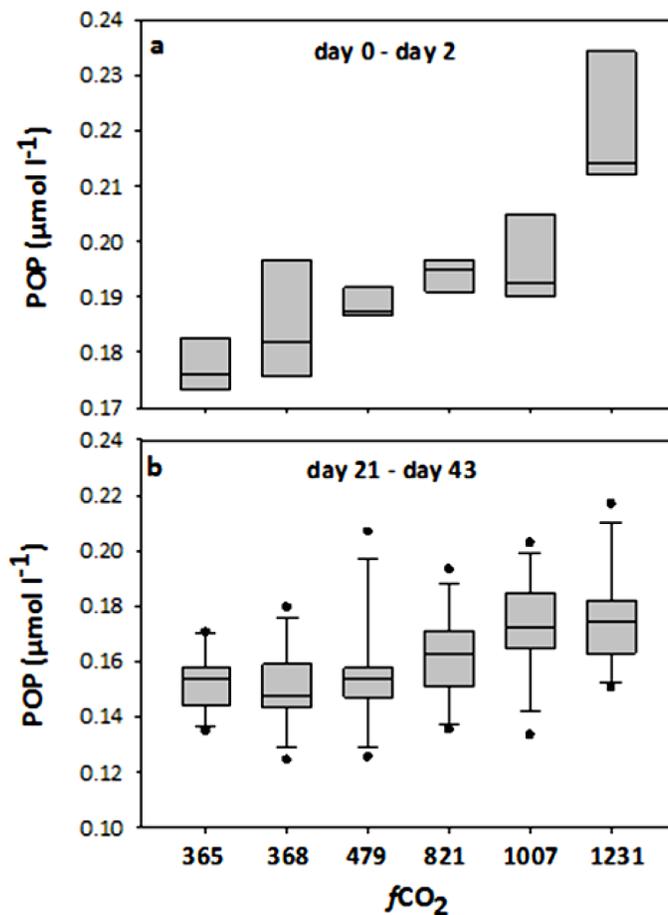
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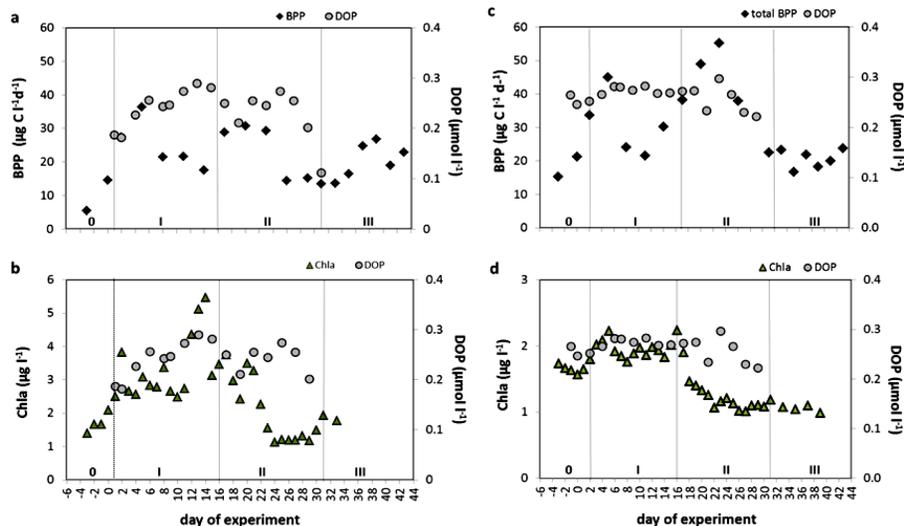


**Figure 7.** POP concentration in the mesocosms during the initial phase from day 0 to day 2 (a) and from day 23 until the end (b) of experiment.

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**Figure 8.** Development of DOP in relation to bacterial production (BPP) and phytoplankton biomass (Chl *a*) in the fjord (**a**, **b**) and in the mesocosms (**c**, **d**). For mesocosms, mean values averaged over all treatments are given.

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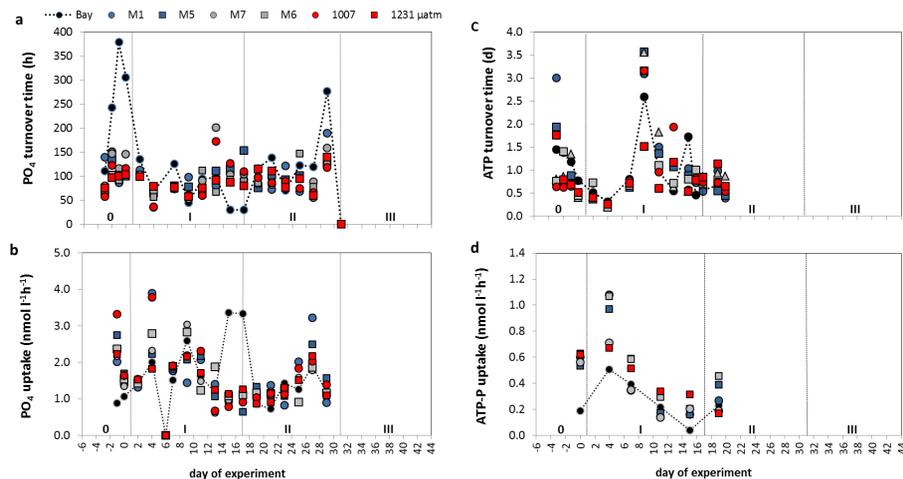
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**Figure 9.** Turnover times of PO<sub>4</sub> (a) and ATP (c) in fjord water and in the mesocosms as well as the respective uptake rates (b, d).

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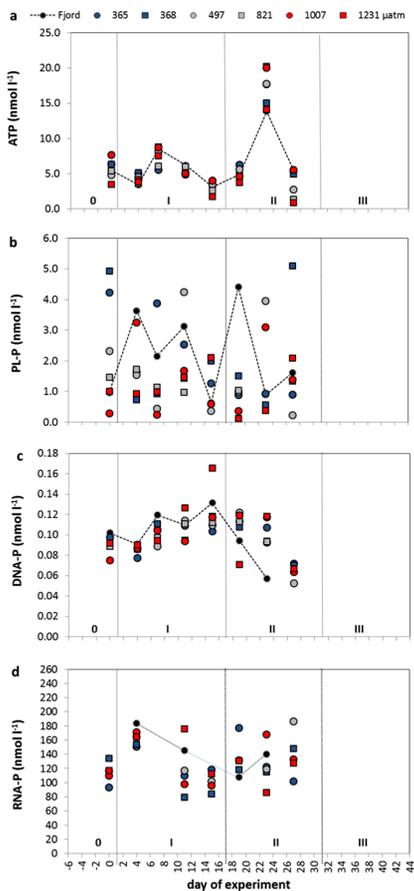
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**Figure 10.** Development of DOP compounds in the mesocosms and in the fjord from day 0 to day 27.

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