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Availability of phosphate for phytoplankton and bacteria and of glucose for bacteria at different pCO_2 levels in a mesocosm study

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Abstract. Availability of phosphate for phytoplankton and bacteria and of glucose for bacteria at different pCO_2 levels were studied in a mesocosm experiment (PeECE III). Using nutrient-depleted SW Norwegian fjord waters, three different levels of pCO_2 (350 μatm: 1×CO_2; 700 μatm: 2×CO_2; 1050 μatm: 3×CO_2) were set up, and nitrate and phosphate were added at the start of the experiment in order to induce a phytoplankton bloom. Despite similar responses of total particulate P concentration and phosphate turnover time at the three different pCO_2 levels, the size distribution of particulate P and $^{33}PO_4$ uptake suggested that phosphate transferred to the >10 μm fraction was greater in the 3×CO_2 mesocosm during the first 6–10 days when phosphate concentration was high. During the period of phosphate depletion (after Day 12), specific phosphate affinity and specific alkaline phosphatase activity (APA) suggested a P-deficiency (i.e. suboptimal phosphate supply) rather than a P-limitation for the phytoplankton and bacterial community at the three different pCO_2 levels. Specific phosphate affinity and specific APA tended to be higher in the 3×CO_2 than in the 2×CO_2 and 1×CO_2 mesocosms during the phosphate depletion period, although no statistical differences were found. Glucose turnover time was correlated significantly and negatively with bacterial abundance and production but not with the bulk DOC concentration. This suggests that even though constituting a small fraction of the bulk DOC, glucose was an important component of labile DOC for bacteria. Specific glucose affinity of bacteria behaved similarly at the three different pCO_2 levels with measured specific glucose affinities being consistently much lower than the theoretical maximum predicted from the diffusion-limited model. This suggests that bacterial growth was not severely limited by the glucose availability. Hence, it seems that the lower availability of inorganic nutrients after the phytoplankton bloom reduced the bacterial capacity to consume labile DOC in the upper mixed layer of the stratified mesocosms.

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1 Introduction

Rising atmospheric CO_2 concentration changes seawater carbonate chemistry by lowering seawater pH, carbonate ion concentration and carbonate saturation state, and increasing the dissolved CO_2 concentration (reviewed by Riebesell, 2004). If global CO_2 emissions continue to rise on current trends (business as usual), the world oceans will suffer an estimated pH drop of about 0.5 units, which is equivalent to a 3 fold increase in the concentration of hydrogen ions, by the year 2100 (Wolf-Gladrow, et al., 1999; Caldeira and Wickett, 2003). While the magnitude of ocean acidification can be predicted with a high level of confidence, its impact on marine organisms, their activities, and biogeochemical role are largely unknown.

Studies dealing with biological responses to increasing CO_2 partial pressure (pCO_2) and related changes in carbonate chemistry range from a single-species level in laboratory cultures up to a semi-natural community level in outdoor mesocosms. Some of these studies show that increasing

pCO_2 can enhance primary production (Zondervan, et al., 2001; Leonardos and Geider, 2005), release of dissolved carbohydrates by phytoplankton (Engel, et al., 2004), and also modify phytoplankton species composition and succession (Tortell, et al., 2002). Such pCO_2 dependent changes in phytoplankton parameters further enhance growth rate and production as well as α- and β-glucosidase activity of heterotrophic bacteria, especially of particle-attached bacteria (Grossart, et al., 2006a). It should be noted, however, that other studies have reported that increased pCO_2 gives no significant increase in primary production (Tortell, et al., 2002; Sciandra, et al., 2003; Delille, et al., 2005). In addition, even within the same experiments (i.e., Tortell, et al., 2002; Engel, et al., 2004; Grossart, et al., 2006a), no significant increase in total phytoplankton biomass (Tortell, et al., 2002) and total bacterial biomass (Rochelle-Newall, et al., 2004; Grossart, et al., 2006a) at increasing pCO_2 levels have been detected. It thus seems that pCO_2 dependent changes in phytoplankton and bacterial parameters are not necessarily consistent.

The elemental composition (e.g. C, N, P) in living organisms is to a certain extent constrained by the necessity to maintain their metabolism (homeostasis) as compared to the rest of the material world (reviewed by Sterner and Elser, 2002). Changes in pCO_2 dependent carbon production by phytoplankton and bacteria (see above) may alter their nutrient demands. On a global scale, such pCO_2 dependent changes will greatly influence carbon and nutrient cycling in the ocean. Significant changes in the consumption ratio of various inorganic nutrients due to increasing pCO_2 levels have been found in one study (Tortell, et al., 2002) but not in another one (Engel, et al., 2005). In this context, changes in nutrient availability for phytoplankton and bacteria at different pCO_2 levels seem to be unclear and thus need to be investigated in greater detail.

Nutrient availability (e.g. deficiency, limitation) is not necessarily readily examined, especially for natural communities of phytoplankton and bacteria. The specific affinity for a substrate is the slope of the specific uptake rate versus a substrate concentration curve, and is analogous to a specific clearance rate, the volume cleared for food (substrate) per unit biomass and unit time (Thingstad and Rassoulzadegan, 1999). Under P-depletion, bacteria and phytoplankton are known to produce alkaline phophatase (AP) which can split phosphate-monoester bonds of organic phosphorus complexes and release phosphate. The presence of AP activity (APA) can therefore be used as a convenient molecular indicator of P-deficiency (reviewed by Cembella, et al., 1984; Jansson, et al., 1988; Hoppe, 2003). A recent study suggests that the specific affinity for phosphate uptake and the specific APA are useful tools for examining phosphate availability in natural phytoplankton and bacterial communities in different P starved aquatic systems (Tanaka, et al., 2006). Similarly, the specific affinity for glucose is expected to be useful for examining the glucose availability for bacteria (e.g., Koch, 1971; Button, 1994).

The objective of the present study is to examine how the availability of phosphate for phytoplankton and bacteria and of glucose for bacteria, is affected by different pCO_2 levels during a mesocosm experiment. Particulate P concentrations, turnover times of phosphate and glucose, and APA were measured together with a variety of other parameters during the experiment. By combining these results with biomass measurements of phytoplankton and bacteria (Paulino, et al., 2007; Schulz, et al., 2007), we have analyzed the specific phosphate affinity, specific APA, and specific glucose affinity.

2 Materials and methods

2.1 Experimental setup and sampling

The mesocosm experiment was carried out at the Espegrend Marine Biological Station (University of Bergen, Norway) from 15 May to 9 June 2005 (see Riebesell, et al., 2007; Schulz, et al., 2007 for details). Briefly, nine mesocosms (polyethylene, ca. 25 m^3, 9.5 m water depth) were filled with unfiltered, nutrient-poor, post-bloom fjord water, and were covered by gas-tight tents (ETFE foil). Three different CO_2 concentrations, 350 μatm ($1\times CO_2$), 700 μatm ($2\times CO_2$), and 1050 μatm ($3\times CO_2$), were set up in triplicates by CO_2 aeration (see Engel, et al., 2005 for details). To induce the development of a phytoplankton bloom, nitrate and phosphate were added before the start of the experiment (Day –1) to obtain initial concentrations of 14 μmol L^{-1} NO_3 and 0.7 μmol L^{-1} PO_4. Depth-integrated water samples (0–5 m) were taken at 10h00 by using a tube sampler (5 m long, 10 cm diameter). Samples for dissolved and particulate nutrients, chlorophyll-*a* (Chl-*a*), and bacterial abundance and production were collected from all nine mesocosms (Paulino, et al., 2007; Schulz, et al., 2007; Allgaier, et al., 2008), while those for particulate P, turnover times of glucose and phosphate, and APA were taken from one mesocosm of each pCO_2 level (M2: $1\times CO_2$, M5: $2\times CO_2$, and M8: $3\times CO_2$) because of logistic constraints. During this study, no significant differences in the temporal changes of dissolved and particulate nutrients, biomass and production of phytoplankton and bacteria were found between the triplicate mesocosms of each pCO_2 level (ANCOVA test, $P>0.05$: Egge, et al., 2007; Paulino, et al., 2007; Schulz, et al., 2007; Allgaier, et al., 2008). Therefore, we assume that the three mesocosms (M2, M5, and M8) selected in this study were representative for each pCO_2 level.

2.2 Dissolved and particulate nutrients

Samples for dissolved and particulate nutrients were collected every day or every second day (see Riebesell, et al., 2007; Schulz, et al., 2007 for details). Concentrations of nitrate, nitrite, soluble reactive phosphorus (SRP), and silicate were measured with an autoanalyzer (AA II) (Hansen and

Koroleff, 1999). Concentrations of dissolved organic carbon (DOC) were measured with a Shimadzu TOC-V_{CSN} analyzer (Qian and Mopper, 1996). POC were collected on precombusted (450°C, 5 h) glass fiber filters (Whatman GF/F), fumed overnight with saturated HCl, dried, and measured with an elemental analyzer (EuroEA 3000, EuroVector).

Size-fractionated particulate P was measured within 2–6 days intervals. Samples were size-fractionated in triplicates on polycarbonate filters (47 mm diameter) with 10, 5, 1, and 0.2 μm pore sizes, respectively. After oxidization of particulate P, liberated P was measured spectrophotometrically (Koroleff, 1983). The mean coefficient of variation was 14% for the >10 μm fraction, 10% for the >5 μm fraction, 11% for the >1 μm and >0.2 μm fractions (n=24 for each fraction). Only the mean concentrations are given for simplicity.

2.3 Biomass of phytoplankton and bacteria, and bacterial production

Chla concentration and bacterial abundance were measured every day or every second day (see Paulino, et al., 2007; Schulz, et al., 2007 for details). Water samples for Chla measurements were filtered onto 25 mm Whatman GF/F filters. Chla was extracted in 100% acetone and then determined by a reverse-phase high-performance liquid chromatography (HPLC) (Barlow, et al., 1997). Samples for enumeration of bacteria were fixed with glutaraldehyde (0.5% final concentration), stained with SYBR Green I (Molecular Probes Inc., Eugene, OR), and counted by a flow cytometer (FACSCalibur, Becton Dickinson, Franklin Lakes, NJ) equipped with an air-cooled laser providing 15 mW at 488 nm and with standard filter set-up (Marie, et al., 1999).

Bacterial abundance and Chl-*a* were converted to C-biomass under the assumption that bacterial carbon content is 20 fg C cell^{-1} (Lee and Fuhrman, 1987) and C: Chl-*a* is 30 (w:w), respectively. The P-biomass of bacteria and phytoplankton was calculated from the C-biomass of bacteria using a C:P molar ratio of 50 (Fagerbakke, et al., 1996) and from the C-biomass of phytoplankton using a C:P molar ratio of 106 (Redfield, et al., 1963), respectively. Although C:P ratios are variable for both phytoplankton and bacteria (e.g. Fagerbakke, et al., 1996; Geider and La Roche, 2002), we applied the average C:P ratios for phytoplankton and bacteria. This is because a direct measurement of P biomass of osmotrophs was not done in this study (see Results and discussion for potential biases by these fixed ratios).

Bacterial production was measured on Day 0 and thereafter every second day between Days 6–24 (Allgaier, et al., 2008). Triplicates and a formalin-killed control were incubated with ^{14}C-leucine (Amersham, 1.15×10^{10} Bq mmol^{-1}) at a final concentration of 50 nmol L^{-1} (Simon and Azam, 1989) in the dark at in situ temperature for 1 h. After fixation with 2% formalin, samples were filtered onto 0.2 μm nitrocellulose filters (Sartorius) and extracted with ice-cold 5% trichloroacetic acid (TCA). Thereafter, filters were rinsed twice with ice-cold 5% TCA, dissolved with ethyl acetate, and radio-assayed. The amount of incorporated ^{14}C-leucine was converted to bacterial production 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).

2.4 Uptake of $^{33}PO_4$ and ^{14}C-glucose

Uptake rate of orthophosphate was measured every day or every second day using ^{33}P-orthophosphate (Thingstad, et al., 1993). Carrier-free ^{33}P-orthophosphate (Amersham, 370 MBq ml^{-1}) was added to samples at a final concentration of 125 pmol L^{-1}. Samples for the subtraction of the background and abiotic adsorption were fixed with 100% TCA before isotope addition. Samples were incubated under subdued (laboratory) illumination at in situ temperature. The incubation time varied between 5 min and 4 h: short enough to assure a linear relationship between the fraction of isotope adsorbed vs. the incubation time but it was long enough to reliably detect isotope uptake above background levels. Incubation was stopped by a cold chase of 100 mmol L^{-1} KH_2PO_4 (final conc. 1 mmol L^{-1}). Subsamples were filtered in parallel onto 25 mm polycarbonate filters with 10, 5, 1, and 0.2 μm pore sizes, which were placed on a Millipore 12 place manifold with Whatman (GF/C) glass fiber filters saturated with 100 mmol L^{-1} KH_2PO_4 as support. After filtration, filters were placed in polyethylene scintillation vials with Ultima Gold (Packard), and radio-assayed. After the radioactivities of the filter were corrected for those of the blank filter obtained from fixed samples, $T_{[PO4]}$ (h) was calculated as $T_{[PO4]}$=–t/ln(1-f) where f is the fraction (no dimension) of added isotope collected on the 0.2 μm filter after the incubation time (t:h).

Uptake rate of glucose, as an important labile DOC compound, was measured every day or every second day using ^{14}C-glucose (Hobbie and Crawford, 1969 modified by Havskum, et al., 2003). D-[U-^{14}C]-glucose (Amersham, 7.4 MBq ml^{-1}) was added to samples at a final concentration of 100 nmol L^{-1}. After 1 h of incubation under subdued (laboratory) illumination at in situ temperature, the sample was split into two. Particulate ^{14}C (>0.2 μm) uptake was measured on 10 ml samples filtered on 0.2 μm pore size cellulose nitrate filters, and ^{14}C-CO_2 was absorbed on 25 mm Whatman (GF/F) glass fiber filters with 250 μl phenetylamine fixed inside the cap of 20 ml polyethylene scintillation vials containing 10 ml. Filters were placed in polyethylene scintillation vials with Ultima Gold (Packard) and radio-assayed. Turnover time of glucose was calculated as the inverse of the fraction of added isotope consumed per hour. The measurement could not be done between Days 0–3 due to a technical problem.

The specific affinity for phosphate uptake was calculated by normalizing phosphate uptake rates (inverse of phosphate turnover times) to the summed P-biomass of phytoplankton

Table 1. Summary of temporal variations of inorganic nutrients and dominant phytoplankton groups (Schulz, et al., 2007) and phosphate turnover time (this study). Phytoplankton groups are based on HPLC pigment analysis. Dominant groups are shown for each phase of phosphate turnover time.

Phase	I	II	III	IV	V
Inorganic nutrients	Days 0–6 No obvious depletion	Days 7–9 Si depletion	Days 10–12 Si and phosphate depletion	Days 13–24 Si, phosphate, and nitrate depletion	Days 13–24 Si, phosphate, and nitrate depletion
Phosphate turnover time	Days 0–6 Long (>100 h)	Days 7–11 Decrease	Days 12–16 Short (<1 h)	Days 17–20 Increase	Days 21–24 Oscillation
Dominant phytoplankton	Diatoms and Prasinophytes	Diatoms and Prymnesiophytes	Prymnesiophytes and Prasinophytes	Prasinophytes, Dinoflagellates, and Diatoms	Dinoflagellates and Cyanobacteria

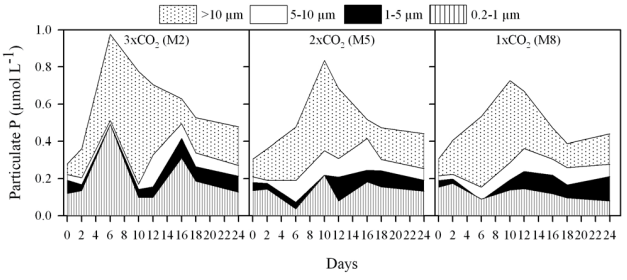

Fig. 1. Temporal development of particulate P concentration (μmol L^{-1}) for different size-fractions.

and bacteria (Tanaka, et al., 2006). Similarly, the specific affinity for glucose uptake was calculated by normalizing glucose uptake rates (inverse of glucose turnover times) to the bacterial C-biomass.

2.5 Alkaline phosphatase activity (APA)

APA was measured fluorometrically using 3-0-methylfluorescein-phosphate as substrate (Perry, 1972). Samples were collected every day or every second day for the period with low concentrations of SRP (Days 10–25: Schulz, et al., 2007). Fluorescence mixed in the samples (final concentration 0.1 μmol L^{-1}) was measured just after the addition of the substrate solution and at two or three subsequent times according to the fluorescence increase. After correcting fluorescence values of samples to those of autoclaved samples, APA (nmol-P L^{-1} h^{-1}) was calculated by using a linear regression of fluorescence values versus incubation time. As APA is derepressed when cellular P falls below a certain threshold level (e.g. Fitzgerald and Nelson, 1966, Rhee, 1973, Myklestad and Sakshaug, 1983) and biomass of phytoplankton and bacteria were variable with time and between mesocosms, biomass-specific APA (h^{-1}) was calculated by dividing APA by the estimated P biomass of osmotrophs (nmol-P L^{-1}). Measurements of APA and phytoplankton biomass were not always synchronized due to logistic constraint. To calculate specific APA, Chl-*a* values on Days 19, 21, and 23 (Data from Schulz, et al., 2007) and APA values on Day 24 were linearly interpolated.

2.6 Statistical analysis

The analysis of covariance (ANCOVA) was done using the *R* software (http://www.r-project.org/) with log-transformation of all data. Experimental day was used as the covariate and pCO_2 was used as the nominal predictor. Correlation analysis was used for a comparison of parameters (turnover times of phosphate and glucose, APA, concentrations of SRP and DOC, and bacterial abundance and production). As the temporal variations of these parameters were similar between the three mesocosms (see Results and discussion), all parameters were pooled for the correlation analysis.

3 Results and discussion

POC concentration and phytoplankton biomass increased from the start of the experiment, peaked around Day 10, and decreased onward in all mesocosms, while Si, SRP, and nitrate concentrations changed from replete to deplete during the phytoplankton bloom (Riebesell, et al., 2007; Schulz, et al., 2007). These nutrient dynamics can be summarized as follows: (1) no obvious nutrient depletions between Days 0–6, (2) only Si depleted between Days 7–9, (3) Si and phosphate depleted between Days 10–12, (4) Si, phosphate, and nitrate depleted from Day 13 onward (Table 1).

Total particulate P (>0.2 μm) ranged from 0.28 to 0.97 μmol L^{-1} (Fig. 1). The increase of total particulate P between Days 0–10 was driven by an increase of particulate P in the >10 μm fraction in all three mesocosms. This corresponded to an initial dominance of diatoms during the phytoplankton bloom (Riebesell, et al., 2007). The particulate P concentration in the >10 μm fraction peaked on Day 10 in all mesocosms, and was significantly higher in 3×CO_2 (0.61 μmol L^{-1}) than in 1×CO_2 (0.44 μmol L^{-1}) (t-test, $P<0.05$). The highest proportion of the >10 μm fraction in 2×CO_2 and 1×CO_2 was observed on Day 6 (60% and 71%, respectively). On Day 6 the particulate P concentration

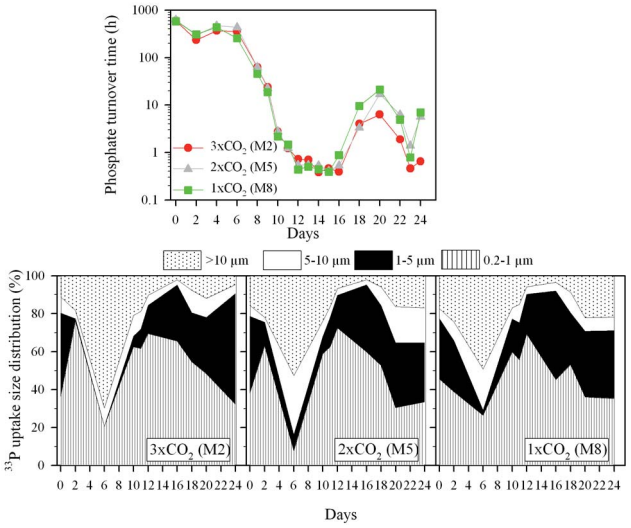

Fig. 2. Temporal changes of phosphate turnover time (h) (top) and size-fraction (%) of $^{33}PO_4$ uptake (bottom).

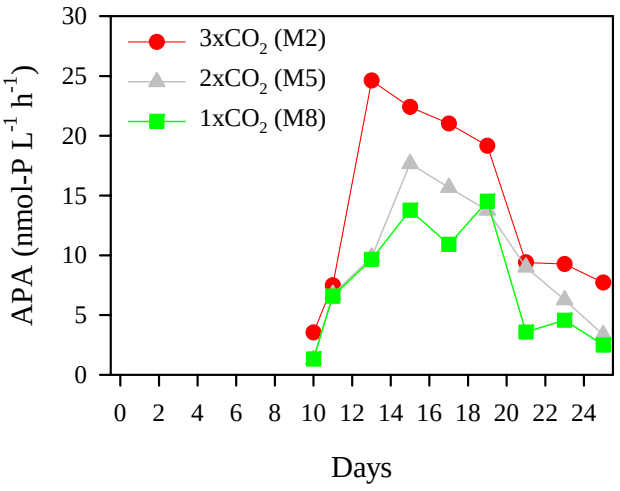

Fig. 3. Temporal changes of alkaline phosphatase activity (APA: nmol-P L^{-1} h^{-1}).

in the >10 μm fraction was significantly higher in 3×CO_2 (0.46 μmol L^{-1}) than in 2×CO_2 (0.29 μmol L^{-1}) (t-test, P<0.005), and marginally higher in 3×CO_2 than in 1×CO_2 (0.38 μmol L^{-1}) (t-test, P=0.053). This suggests that P transferred to the >10 μm fraction was greater in 3×CO_2 during this period. Since particulate P concentrations in the 0.2–1 μm fraction varied little between Days 0–10 except on Day 6 in 3×CO_2, the proportion of the 0.2–1 μm fraction to total particulate P decreased to 13–26% on Day 10. After Day 10, the proportion of the >10 μm fraction decreased to 33–43%, while that of the 1–10 μm fraction and of the 0.2–1 μm fraction increased to 30–45% and 25–50%, respectively, in all three mesocosms.

Temporal variations in the substrate turnover time reflect either those of substrate concentration or those of substrate flux through this pool or both. An overall significant positive correlation between phosphate turnover time and SRP concentration (r=0.926, P<0.0001, n=48) suggests that temporal variations of phosphate turnover time reflected those of phosphate concentration. When SRP concentrations were lower than 0.1 μmol L^{-1}, the relationship was still significant but with a reduced coefficient of correlation (r=0.646, P<0.0001, n=33). This suggests either an increased effect of background compounds such as acid labile DOP and arsenate (e.g., Murphy and Riley, 1962) or a reduced analytical precision with decrease of SRP concentrations. Phosphate turnover time varied by a factor of ca. 1500 with a range of 0.4 to 625 h (Fig. 2, top). During the first 6 days when SRP concentrations were high (ca. 0.4–0.8 μmol L^{-1}: Schulz, et al., 2007), phosphate turnover time was longest (in the order of 100 h). It rapidly decreased between Days 7–12 and remained shorter than 1 h between Days 12–16. Thereafter, turnover time increased between Days 17–20 and oscillated in a range of 0.5–21 h onward. Interestingly, these dynamics of phosphate turnover time corresponded approximately to those of the inorganic nutrients (Table 1). By using the five phases defined by the dynamics of phosphate turnover time, the succession of dominant phytoplankton groups based on HPLC pigment analysis (Schulz, et al., 2007) can be summarized as follows (Table 1): Diatoms and Prasinophytes in Phase I, Diatoms and Prymnesiophytes in Phase II, Prymnesiophytes and Prasinophytes in Phase III, Prasinophytes, Dinoflagellates, and Diatoms in Phase IV, and Dinoflagellates and Cyanobacteria in Phase V. These results suggest that temporal changes in the availability of inorganic nutrients influenced those of phytoplankton biomass and succession during the experiment.

When phosphate turnover time was shorter, more phosphate was taken up by the smaller fraction and vice versa (Fig. 2, bottom). During the initial period (Days 0–6) with turnover time >100 h, $^{33}PO_4$ uptake by the >10 μm fraction increased up to 70% in 3×CO_2, but only up to 50% in both 2×CO_2 and 1×CO_2. This also indicates that the concentration of particulate P in the >10 μm fraction was highest in 3×CO_2 during the phytoplankton bloom (Fig. 1). The mean uptake, however, was highest (47–53%) by the 0.2–1 μm fraction and smallest (8–11%) by the 5–10 μm fraction during the experiment.

APA ranged from 1.3 to 24.6 nmol-P L^{-1} h^{-1} (Fig. 3). After SRP depletion around Day 10 (Schulz, et al., 2007), APA increased towards Days 13–15, and the fastest and highest increase in APA was observed in 3×CO_2. This suggests that the available phosphate pool in 3×CO_2 was smallest during this period. Thereafter, APA decreased in all three mesocosms. A significant correlation was found between APA and phosphate turnover time (r=–0.689, P<0.005, n=15), but not between APA and SRP (r=0.414, P>0.1, n=15). This can

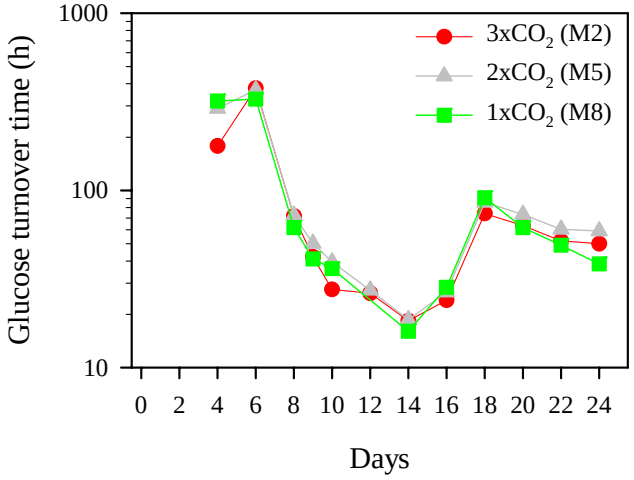

Fig. 4. Temporal changes of glucose turnover time (h).

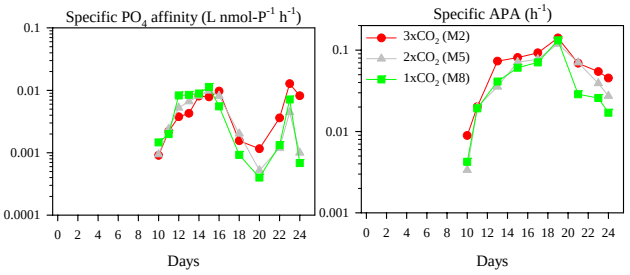

Fig. 5. Temporal changes of specific phosphate affinity (L nmol-P^{-1} h^{-1}) (left) and specific APA (h^{-1}) (right).

be explained by the fact that APA was measured only for the SRP depletion period, when the SRP concentration appeared to correspond to a lesser degree to the phosphate pool, as suggested by the reduced coefficient of correlation between phosphate turnover time and SRP concentration (see above).

The glucose turnover time was long (>100 h) between Days 4–6, and rapidly decreased to ca. 16 h on Day 14 (Fig. 4). Thereafter, it rapidly increased towards Day 18 (74–91 h) and fluctuated between 39–73 h onward. No significant correlation was found between glucose turnover time and bulk DOC concentration (r=–0.282, P>0.1, n=26), suggesting the glucose pool being a small fraction in the bulk DOC pool. Glucose is the most common monomer of neutral sugar polymers and hence it may account for a substantial fraction of the labile DOC pool. In this study, the phytoplankton bloom resulted in an increase in bulk DOC of 25–30 μmol L^{-1} (Schulz, et al., 2007), which to a large degree can be composed of glucose-rich exudates (Grossart, et al., 2006b). Glucose turnover time was significantly and negatively correlated with bacterial abundance (r=–0.645, P<0.0001, n=26) and bacterial production (r=–0.889, P<0.0001, n=20). This suggests that glucose was an important component of labile DOC for bacteria. Since the ^{14}C-glucose concentration (100 nmol L^{-1}) might not always have been at tracer level in this experiment, this could to some extent have caused an overestimation of glucose turnover time. Unfortunately, we did not measure glucose concentration in this study.

With regard to temporal changes of the parameters presented in Figs. 1–4, no significant time-effects caused by the different pCO_2 treatments were detected during the experiment (ANCOVA, P>0.05).

Specific phosphate affinity, which was calculated for the period of low SRP concentrations (Days 10–24: Table 1), ranged from 4.0×10^{-4} to 1.3×10^{-2} L nmol-P^{-1} h^{-1} in all three mesocosms (Fig. 5). As the temporal variations of phosphate turnover time were much greater than those of phytoplankton and bacterial biomass (Paulino, et al., 2007; Schulz, et al., 2007; Fig. 2, top), temporal variations of specific phosphate affinity mirrored those of phosphate turnover time. After SRP depletion, specific phosphate affinity increased towards Days 12–16, decreased between Days 16–20, and showed short-term oscillations and tended to be higher in $3\times CO_2$ than in $2\times CO_2$ and $1\times CO_2$ between Days 20–24. Specific APA ranged from 0.003 to 0.141 h^{-1} and peaked with a delay of 4–6 days (Day 19) compared to APA (Figs. 3 and 5). This was because the temporal changes of the summed biomass of phytoplankton and bacteria were greater than those of the APA between Days 13–19 (Paulino, et al., 2007; Schulz, et al., 2007). However, no significant time-effects caused by the different pCO_2 treatments were detected in specific phosphate affinity and specific APA between Days 10–24 or 25 (ANCOVA, P>0.05).

Tanaka et al. (2006) recently propose that a specific phosphate affinity >0.02 L nmol-P^{-1} h^{-1} and/or a specific APA >0.2 h^{-1} indicate P limitation, i.e. the growth rate of the existing organisms is reduced due to the reduced P availability. They also suggest that a specific phosphate affinity in the order of 0.001 L nmol-P^{-1} h^{-1} and/or a specific APA in the order of 0.01 h^{-1} indicate a situation that is less strict than limitation, i.e. P deficiency or suboptimal P supply for the phytoplankton and bacterial communities. According to this, the specific phosphate affinity and specific APA suggested a P-deficiency of the phytoplankton and bacterial communities in all three mesocosms between Days 11–24, except for $2\times CO_2$ and $1\times CO_2$ on Days 20 and 24 (Fig. 5). We are aware of the fact that the P biomass estimate, which was used to determine specific phosphate affinity and specific APA, includes elements of uncertainty (see Materials and methods). However, the estimated P biomass never exceeded the chemically measured particulate P (>0.2 μm) (range: 14–60%, n=36). The ratios of POC to particulate P were similar for all mesocosms and slightly higher (mean±SD: 129±28, n=38) than the Redfield ratio of 106 (see Schulz, et al., 2007 for POC data). If the specific phosphate affinity is recalculated by correcting the assumed C:P ratios by the POC to particulate P ratios, only at one occasion ($3\times CO_2$ on Day 23) we

found P-limitation whereas all other data points indicated P-deficiency. We did not measure the active phytoplankton and bacteria in terms of phosphate uptake in this study, and an overestimation of active fraction in the phytoplankton and bacteria community (here assumed to be 100%) would lead to underestimation of the specific phosphate affinity. The osmotroph biomass was dominated by bacteria between the Phases III-V (mean±SD:67±12%, n=30), and thus effect of the active phytoplankton fraction on the specific phosphate affinity would not be significant. Under the assumption that the fraction of active bacteria was similar at the different pCO_2, an active fraction down to 54% indicated P-deficiency at all data points between Days 10–24. By decreasing the active fraction to 5%, P-limitation occurred at 12 occasions out of 36. We therefore believe that the uncertainties in the P biomass estimation and the active fraction of bacteria do not significantly change our conclusions concerning phosphate availability: A P-deficiency (i.e. suboptimal phosphate supply) rather than a P-limitation for the phytoplankton and bacterial community. During the P-deficient period, viral abundance was high in all three mesocosms (Larsen, et al., 2007), suggesting viral lysis of bacterial and phytoplankton cells to be one of the major sources for the increase in DOM. However, DOP concentrations increased gradually and slightly throughout the experiment (Schulz, et al., 2007). This can be explained by the fact that DOP produced via viral lysis is rather labile, and thus was rapidly degraded by DOP hydrolyzing enzymes such as APA (Berman, 1969) and 5'-nucleotidase (Ammerman and Azam, 1985). Both enzymes are essential for phosphate uptake from organic compounds via osmotrophs when the phosphorus demand exceeds the available phosphate pool.

Specific glucose affinity varied similarly in the three mesocosms with a range of 1.2×10^{-6} to 1.1×10^{-5} L nmol-C^{-1} h^{-1} (Fig. 6). It increased towards Day 9 and gradually decreased onward. Differences in temporal variations between turnover time and specific affinity were due to the large temporal variations in bacterial biomass (Paulino, et al., 2007; Fig. 4). As discussed above, the significant negative correlation between glucose turnover time and bacterial abundance/production suggests that glucose was an important component of labile DOC for bacteria, although glucose being a small fraction of the bulk DOC. A significant negative correlation between specific glucose affinity and bulk DOC concentration (r=–0.625, P<0.001, n=26) suggests that temporal changes of bulk DOC concentration were largely related to those of the glucose pool available for bacteria. This can be explained by increased production of labile DOC through phytoplankton DIC over-consumption (Toggweiler, 1993; Riebesell, et al., 2007), which to a large degree can be composed of glucose-rich exudates (Grossart, et al., 2006b). In order to examine the substrate availability for osmotrophs, the experimentally determined specific affinity (α^e) can be compared with the theoretical maximum (α^{max}). A theoretical expression for the maximum specific affinity

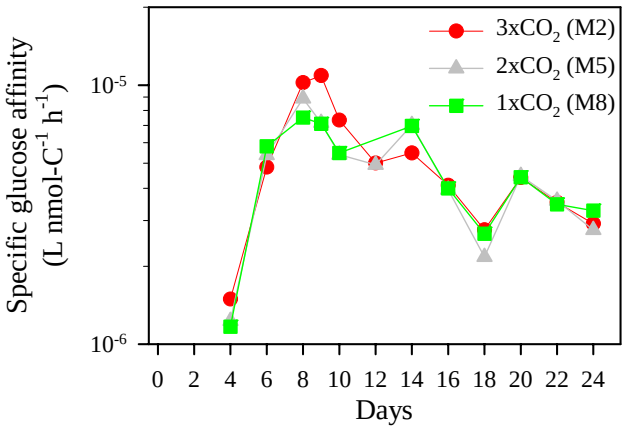

Fig. 6. Temporal changes of specific glucose affinity (L nmol-C^{-1} h^{-1}).

for a spherical cell of radius r can be derived (Thingstad, et al., 2005):

$$\alpha^{max} = \frac{4\pi Dr}{m} \quad (1)$$

where D is the diffusion constant for the substrate molecules, and m is the amount of the limiting element required to produce a new cell. For carbon, the amount required for a cell is determined by both cell quota and respiration. Taking the respiration coefficient (R) into account for cell quota (Q) in unit of C, m is described as $\frac{Q}{1-R}$. If we assume that bacterial cells have a density of 1.2 g cm^{-3}, 50% dry weight of wet weight, 50% carbon of dry weight, a cell radius of 0.25 μm, and a respiration coefficient of 0.5 and that the diffusion constant for glucose is 6×10^{-6} cm^2 s^{-1} (e.g. Koch, 1971), the maximum specific affinity given by the diffusion limitation of substrate transport is calculated to be 2.1×10^{-3} L nmol-C^{-1} h^{-1}. This theoretical maximum is 2–3 orders of magnitude higher than the specific glucose affinities in all mesocosms (mostly in the order of 10^{-6} L nmol-C^{-1} h^{-1}, Fig. 6). This suggests that bacterial growth was not severely limited by the glucose availability during the study period. An overestimation of the active bacteria (here assumed to be 100%) would lead the specific glucose affinity being underestimated. If the active fraction were 1%, the specific glucose affinity might have been close to the theoretical maximum. However, if only this active fraction contributed to bacterial carbon production, generation time of the active bacteria must have been as short as 1 min between Days 10–24 (data not shown). Such a generation time seems to be too short. Thus, we believe that the uncertainties in the active fraction of bacteria do not significantly change our conclusions concerning the glucose availability for bacteria.

Riebesell et al. (2007) report that enhanced DIC over-consumption was observed at higher pCO_2 levels towards the peak of phytoplankton bloom (Day 12), but the ratio of

POC to PON remained very close to the Redfield ratio at the three pCO_2 levels. This deviation between DIC/NO_3 drawdown and POC/PON build-up was attributed to an enhanced DOC production at higher pCO_2 levels and a higher loss of organic C from the upper mixed layer at higher pCO_2 levels due to an enhanced export of POC including carbon-rich transparent exopolymer particles (TEP) originated from DOC (Riebesell, et al., 2007). This difference was mainly due to an enhanced organic ^{14}C production in the 0.2–1 μm fraction in higher pCO_2 levels, a fraction overlapping TEP size (Egge, et al., 2007), although the contribution of picophytoplankton to total phytoplankton biomass was relatively small in all mesocosms (Paulino, et al., 2007; Riebesell, et al., 2007). Small and intermediate sized phytoplankton groups increased their importance relative to diatoms towards the end of the experiment (Paulino, et al., 2007; Schulz, et al., 2007). This size shift of the phytoplankton community should have reduced vertical sinking flux, but the loss of organic C from the upper mixed layer continued towards the end of the experiment and was consistently higher at higher pCO_2 levels (Riebesell, et al., 2007). These results indicate that nutrient depletion (e.g. P-deficiency, Fig. 5) reduced the bacterial capacity to degrade organic C despite increased concentrations of DOC at higher pCO_2 levels, suggesting that DOC or small C-rich organic particles, such as TEP, formed sinking particles.

In summary, none of the parameters measured in the current study showed statistically significant time-effects among the three mesocosms, and thus were apparently independent of pCO_2. This may be partly due to the lack of parallel measurements (one mesocosm for each pCO_2 level). Such small differences could be explained by the variance between "parallel treatments" rather than "different treatments" (Martínez-Martínez, et al., 2006). This implies that the plankton food webs can buffer to a large extent the effects of increased pCO_2 and the related changes in carbon chemistry during a short time frame (ca. 1 month). Such small differences were however in contrast to clear differences in DIC/NO_3 uptake (Bellerby, et al., 2007; Riebesell, et al., 2007) and the emission and/or uptake of volatile organic compounds (Sinha, et al., 2007) between the different pCO_2 levels in the same experiment. In the other accompanying studies, effects of increased pCO_2 levels were, if found, relatively small and appeared towards the end of the experiment (Egge, et al., 2007; Paulino, et al., 2007; Schulz, et al., 2007; Allgaier, et al., 2008). The specific phosphate affinity suggested a P-deficiency in the three mesocosms with a comparatively lower phosphate availability in the $3\times CO_2$ mesocosm towards the end of the experiment. On the other hand, the specific glucose affinity suggested no bacterial growth limitation by the glucose availability in the three mesocosms. These results suggested that a higher loss of organic carbon from the surface mixed layer with increasing pCO_2 was likely because a smaller pool of available inorganic nutrients limited the bacterial capacity to degrade labile DOC in the upper mixed layer of the stratified mesocosms.

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