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Primary production during nutrient-induced blooms at elevated CO_2 concentrations

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Abstract. A CO_2 enrichment experiment (PeECE III) was carried out in 9 mesocosms in which the seawater carbonate system was manipulated to achieve three different levels of pCO_2. At the onset of the experimental period, nutrients were added to all mesocosms in order to initiate phytoplankton blooms. Primary production rates were measured by in-vitro incubations based on ^{14}C-incorporation and oxygen production/consumption. Size fractionated particulate primary production was also determined by ^{14}C incubation and is discussed in relation to phytoplankton composition. Primary production rates increased in response to nutrient addition and a net autotrophic phase with ^{14}C-fixation rates up to 4 times higher than initial was observed midway through the 24 days experiment before net community production (NCP) returned to near-zero and ^{14}C-fixation rates dropped below initial values. No clear heterotrophic phase was observed during the experiment. Based on the ^{14}C-measurements we found higher cumulative primary production at higher pCO_2 towards the end of the experiment. CO_2 related differences were also found in size fractionated primary production. The most noticeable responses to CO_2 treatments with respect to primary production rates occurred in the second half of the experiment when phytoplankton growth had become nutrient limited, and the phytoplankton community changed from diatom to flagellate dominance. This opens for two alternative hypotheses that the effects are either associated with mineral nutrient limited growth, and/or with a change in phytoplankton species composition. The lack of a clear net heterotrophic phase in the last part of the experiment supports the idea that a substantial part of production in the upper layer was not degraded locally, but either accumulated or exported vertically.

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1 Introduction

In the upper photic zone where primary production is usually limited by mineral nutrients (e.g. N, P or Fe), the microbial food web can be seen as a set of cycles of the limiting elements, grossly described by the import-export and regenerated nutrient cycles (Dugdale and Goering, 1967). Onto this set of nutrient cycles, the C-cycle is linked via a more or less flexible stoichiometric relationship in organisms at the different trophic levels and in their interactions. Relatively small alterations in either the element cycles or in the stoichiometric C:nutrient coupling may have consequences for the ocean's C-cycle. Increased atmospheric CO_2 leads to both an increased pCO_2 and a lowered pH (Wolf-Gladrow et al., 1999). It is an a priori possibility that both of these environmental changes may affect either the cycling of the limiting element, and/or its stoichiometric coupling to C. In either case, this would be expected to lead to changes in the rate of C fixation into organic material and in the processes producing and consuming oxygen.

CO_2 is often quoted as being a non-limiting factor for primary production in seawater (Raven and Johnston, 1991; Clark and Flynn, 2000). The fact that RUBISCO, the primary carboxylating enzyme in marine phytoplankton has a relatively low affinity for CO_2 (Raven and Johnston, 1991), however, has led to a discussion of a possible stimulating effect of increased CO_2 levels on primary C-fixation in some groups of phytoplankton (Riebesell, 2004). Should this occur without a proportional change in the cycle of limiting

Table 1. Experimental period and CO_2 and nutrient manipulation of PeECE I, II and III carried out in 2001, 2003 and 2005, respectively. Temperature range and average global radiation (Geophysical institute, University of Bergen) is given.

	2001	2003	2005
Experimental period	31 May–25 June	4 May–24 June	16 May–10 June
CO_2 concentration	180, 370, 700 μatm	190, 370, 700 μatm	375, 750, 1150 μatm
Initial nutrient supply	17 μM N, 0.5 μM P	9 μM N, 0.5 μM P, 12 μM Si	15 μM N, 0.6 μM P
Temperature range	10–13 °C	8–10 °C	9–11.5 °C
Average global radiation	17.46 MJ m^{-2}	11.45 MJ m^{-2}	12.81 MJ m^{-2}

elements, the consequence would be a change in the stoichiometric relationships in the microbial food web. Based on measurements of the removal of inorganic-C and nitrate, the PeECE-experiments have shown such an effect (Riebesell et al., 2007). A possible enhancement of organic carbon fixation at increased CO_2 has been attributed to the production of transparent exopolymer particles (Engel 2002; Riebesell et al. 2007).

The impact of increased CO_2 on primary production has been investigated theoretically as well as experimentally. Some studies report small, if any, effects (Clark and Flynn, 2000; Tortell et al., 2002), whereas others show increased rates of phytoplankton growth and/or primary production with increasing CO_2 (Riebesell et al. 1993; Heine and Sand-Jensen, 1997; Schippers et al., 2004). A change in community primary production may be rooted in a change in phytoplankton community composition. While the affinity for CO_2 differs among phytoplankton groups (Tortell, 2000), most species are able to regulate their carbon acquisition by CO_2concentrating mechanisms (CCM) (Raven, 1991). The efficiency and regulation of CCM, however, differs among phytoplankton species and functional groups (Giordano et al., 2005). Changes in CO_2 availability might therefore affect competition and succession of phytoplankton species (Burkhardt et al., 2001; Rost et al., 2003; Tortell et al., 2002).

Three mesocosm experiments, in 2001, 2003 and 2005, have been carried out in the framework of the Pelagic Ecosystem CO_2 Enrichment study (PeECE) with the aim to study the effects of elevated levels of CO_2 on the planktonic community (Delille et al., 2005; Engel et al., 2004, 2005; Rochell-Newall et al., 2004; Grossart et al., 2006). Although all PeECE mesocosm studies were carried out during the post-bloom period (May–June), blooms of different phytoplankton groups, e.g. *Emiliania huxleyi* and/or diatoms, were initiated by the addition of nutrients in different compositions. Differences in temperature and light conditions between the three experiments have likely contributed to the observed differences in phytoplankton composition and succession (Table 1). Primary production was measured during all PeECE experiments. No differences in primary production were observed in the 2001 and 2003 experiments where CO_2 concentration in the mesocosms was manipulated to 180, 370 and 700 μatm (Delille et al., 2005; Egge unpublished data). In the 2005 experiment (PeECE III), even higher CO_2 concentrations of up to 1050 μatm were used (Schulz et al., 2008). Here we report primary production results mainly from PeECE III, with a comparison to corresponding data from the previous experiments. ^{14}C-based particulate primary production, total and in size-fractions 0.2–1, 1–5, 5–10 and >10 μm, and O_2 measurements based on incubation in light and dark bottles were used for estimating (gross and net community) production.

2 Materials and methods

2.1 Set-up and sampling

The PeECE III mesocosm experiment was carried out at the Marine Biological Station, University of Bergen, Norway between 16 May and 10 June 2005 (see Table 1).

9 mesocosms (volume 27 m^3) made of polyethylene were filled with unfiltered, nutrient-poor post-bloom water from the fjord, and manipulated to achieve 3 different levels of CO_2 in triplicate mesocosms by aeration of the water column and the overlying atmosphere with CO_2-enriched air. The levels of CO_2 at the start of the experimental period were 350 μatm (1× CO_2), 700 μatm (2× CO_2) and 1050 μatm (3× CO_2). Nutrients, as nitrate and phosphate, were added to the mesocosms on day t_{-1}, the day before we start sampling, in order to achieve an increase in growth and biomass of osmotrophic organisms. For further details concerning the set-up of the experiment see Schulz et al. (2008).

2.2 ^{14}C Primary production

Primary production was measured using the ^{14}C method, according to Steemann Nielsen (1952) and Gargas (1975). Integrated water samples were collected 09:00 a.m., prior to the main sampling (Schulz et al., 2008), using a 5 m long ∼3 cm diameter tube. After mixing, the samples were filled into plastic bottles (76 ml) (NUNC Easyflask), spiked with approximately 4 μCi (Carbon 14 Central) and incubated in-vitro between 10:00 and 14:00 h. The concentration of ^{14}C in the bottle was recorded by removing a 25 μl aliquot from the incubation bottle prior to incubation and added to 600 μl 2 molar NaOH in a scintillation vial. Dark uptake of ^{14}C was measured in bottles wrapped in aluminium foil. Triplicate

bottles from each mesocosm were incubated in the sea outside the mesocosms, at the irradiance level corresponding to mid-depth of the upper layer of the mesocosms (see Schulz et al., 2008). The incubation depth was determined based on light profiles inside and outside the mesocosms. A Li-Cor Li 1000 datalogger with Li 190SA-Quantum sensor and Li-192SA Underwater Quantum Sensor was used both for profiling and logging. In addition to short term incubation, 24 h incubations were conducted 7 times during the experimental period in order to measure primary production in different fractions. For these incubations 118 mL glass bottles were used, and only one mesocosm per treatment was sampled, M2, M5 and M8. The samples were filtered onto Nuclepore filters with pore sizes of 0.2, 1, 5 and 10 μm. After filtration all filters were treated with fuming HCl in order to remove inorganic ^{14}C, a scintillation solution (Ecosint O) was added, and the samples were stored overnight before being counted in a Packard Tri Carb Liquid Scintillation Analyser, model 1900 A. Primary production rates were calculated according to Gargas (1975). For determination of total CO_2 concentration in the different mesocosms see Bellerby et al. (2008). Daily primary production, based on 4 h incubation, was calculated as a function of incoming irradiance during the incubation period (4 h) and total irradiance over 24 h according to the formula: Daily ^{14}C production = (^{14}C production during incubation period * 100)/Irradiance during incubation period (%).

2.3 Oxygen production and consumption

Biological Oxygen Demand (BOD) bottles were incubated for 24 h and oxygen was measured using the OxyMini® optode system (World Precision Instruments). The instrument was two-point calibrated according to the manual and used with automatic temperature compensation. Oxygen concentration was determined individually in each BOD bottle both before and after incubation. 3 light and 3 dark bottles from each mesocosm were incubated at the same location as the ^{14}C bottles. NCP and community respiration were based on light and dark bottle incubations, respectively, and gross community production calculated by difference assuming respiration to be the same in light and dark bottles.

2.4 Transparent exopolymer particles (TEP)

The concentration of TEP was determined using the colorimetric approach by Passow and Alldredge (1995). Between 20 and 75 ml of sample water were filtered onto 0.4 μm polycarbonate filters (Nuclepore) and stained with Alcian Blue, a cationic copper phtalocyanine dye that specifically binds to carboxyl- and halfester sulphate reactive groups of acidic polysaccharides. Samples were stored in polypropylene tubes at −20°C until analysis. The concentration of TEP is given in units of μg X. eq. L^{-1}. To convert TEP into carbon units [μmol C L^{-1}] a conversion factor of f'=0.63 (Engel 2004) was used.

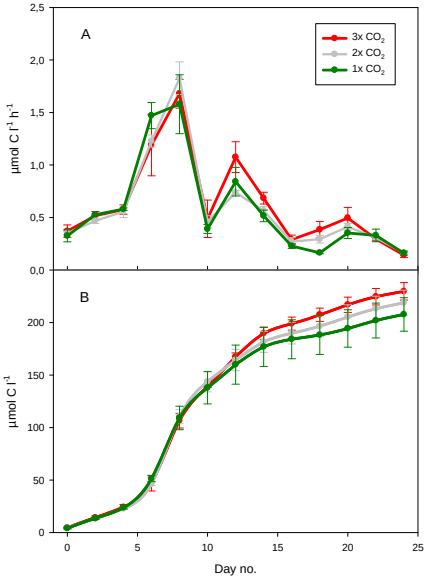

Fig. 1. Development of ^{14}C primary production based on 4 h incubations during the experiment(μmol C L^{-1} h^{-1}) **(A)** and cumulative production in μmol C $L^{-1}$for the 24 days experimental period **(B)**. Values are means ±SD of triplicate CO_2 treatments with 1× CO_2 (green), 2× CO_2 (grey) and 3× CO_2 (red).

2.5 Statistical analysis

In order to identify statistically significant differences between different treatments we used Student t-tests , Paired Two Sample for Means, according to Sokal and Rohlf (2001). The confidence level for all analysis was set at 95%.

3 Results

Initial particulate primary production rates, based on the ^{14}C method (4 h incubations), ranged from 0.33 to 0.37 μmol C L^{-1} h^{-1} (Fig. 1a). After the initial addition of nutrients, a rapid increase in production was observed in all treatments. Maximum rates were observed on day 8, ranging from 1.6 to 1.8 μmol C L^{-1} h^{-1}. Two weaker but distinct peaks were observed on day 12 and day 20 before the production rates decreased to levels lower than initial. In the second half of the experiment there was a tendency of higher production at elevated CO_2 levels. This trend is visible from ca. day 10 in the cumulative production, with a significant difference between 3× and 1× CO_2 (p<0.05) from day 20 onward (Fig. 1b).

The highest gross production, measured as oxygen production plus respiration, was observed on day 6 in 1× and 2× CO_2, with 56 and 58 μmol O_2 $L^{-1}d^{-1}$, respectively, whereas a similar maximum of 58 μmol O_2 L^{-1} d^{-1} was observed in 3× CO_2 a few days later (Fig. 2a). For all treatments, maximum NCP was observed on day 6, and after Day 14 no net production was found in the system in any of the treatments (Fig. 2c). When plotting cumulative O_2 production we

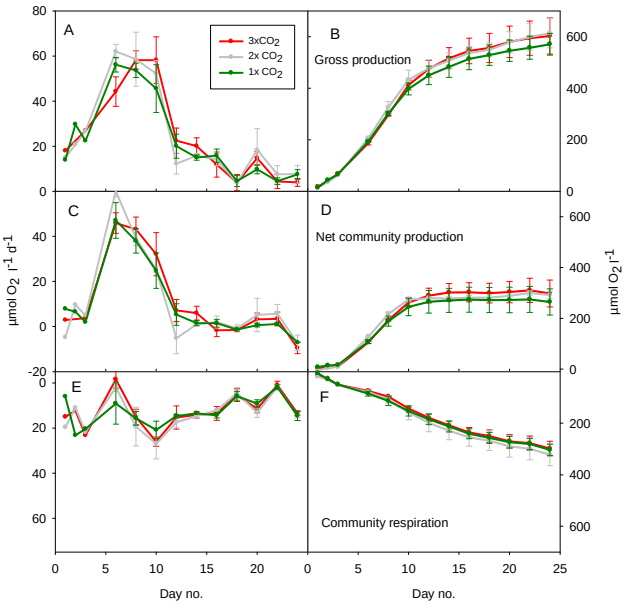

Fig. 2. Development of Gross- and Net community-production and Community respiration based on oxygen incubations. Daily production rates are given as μmol O_2 L^{-1} d^{-1} **(A, C, D)** and cumulative production and consumption for the 24 days experimental period as μmol O_2 L^{-1} **(B, D, E)**.Values are means ±SD of triplicate CO_2 treatments, colour code as in Fig. 1.

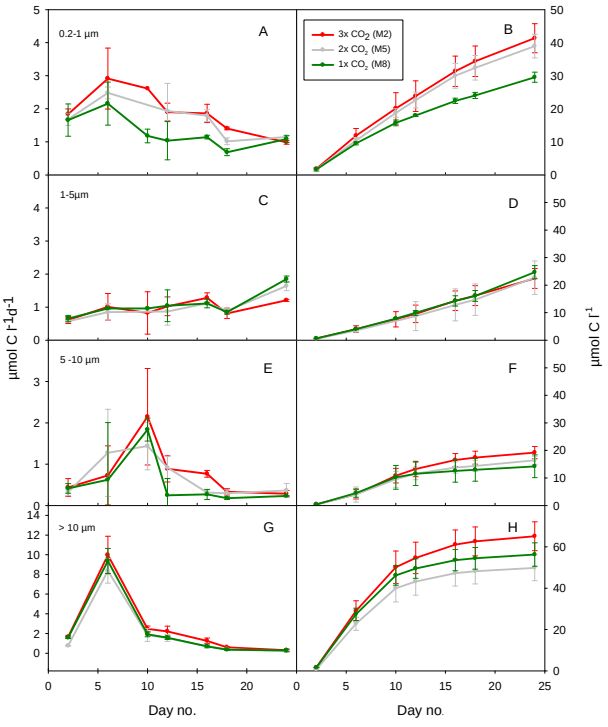

Fig. 3. ^{14}C primary production (μmol C L^{-1} d^{-1}), based on 24 h incubation during the experiment in the fractions 0.2-1 μm **(A)**, 1–5 μm **(C)**, 5–10 μm **(E)** and >10 μm **(F)**, and cumulative production (μmol C L^{-1}) in the same fractions (**B**, **D**, F, **G**). One mesocosm of each CO_2 treatment (M2, M5 and M8) was investigated. Values are means±SD of triplicate incubations in each mesocosm, and colour code as in Fig. 1.

observed a small tendency, although not statistically significant (p=0.2), of increased NCP at elevated CO_2 (Fig. 2d). Gross production and community respiration did not reveal any clear CO_2 effects, neither in terms of the timing nor the level of production/consumption (Fig. 2a, b, e, f).

We also measured primary production during PeECE II (2003), but no consistent responses to CO_2 were observed, neither in ^{14}C (Egge, unpublished data) nor in O_2 production (Engel, unpublished data), although there was a difference in phytoplankton community composition (Grossart et al., 2006). In PeECE III (2005), we therefore decided to carry out fractionated primary production in one mesocosm of each treatment 1× (M8), 2× (M5) and 3× CO_2 (M2). On average, the size fractions 0.2–1, 1–5, 5–10 and >10 μm accounted for 29, 18, 12 and 41% of total primary production, respectively. All fractions showed an increase in production after the onset of the experiment, but during the first week organisms in the >10 μm fraction dominated primary production (Fig. 3g, h). On day 6, 70% of the total production was observed in this fraction, thereafter the contribution of the >10 μm fraction decreased rapidly. Cumulative production was highest in 3× CO_2, followed by 1× and 2× CO_2 in this fraction. The difference between 3× and 2× CO_2 was statistically significant (p<0.05) during the last week, whereas differences were not obtained between 3× and 1× or 2× and 1× CO_2. A distinct, but much smaller peak was observed in the fraction 5–10 μm on day 10 (Fig. 3e, f). Over the experimental period, cumulative production in this fraction amounted to 19, 16 and 14 μmol C L^{-1} in 3×, 2× and 1× CO_2, respectively. The difference between treatments was small, and not statistically significant between 3× and 1× CO_2 (p=0.07). The production in fraction 1–5 μm was generally low and similar for all treatments, except for the very last day of the experiment when production in 3× CO_2 treatments was significantly lower (p<0.05) (Fig. 3c, d). The largest CO_2-related differences between treatments were found in the smallest fraction 0.2–1 μm. Here, production rates showed a decreasing trend from day 6 onwards in all treatments, but were distinctly higher at elevated CO_2 throughout the experiment. In addition, the cumulative production increased from 30 μmol C L^{-1} at 1× CO_2 to 39 and 43 μmol C L^{-1} at 2× and 3× CO_2, respectively. The difference between 3× and 1× CO_2 was statistically significant from day 12 onward (p<0.05).

TEP concentration increased after day 6 in all treatments, reached highest values of 400–450 μg X eq. L^{-1} between days 11 and 13 (Fig. 4a), and declined thereafter. Net production of TEP, calculated as daily changes of TEP concentration, was observed between days 7 and 11 in all treatments and accounted for at most 5 μmol C L^{-1} d^{-1} (Fig. 4b). Net production occurred also occasionally during

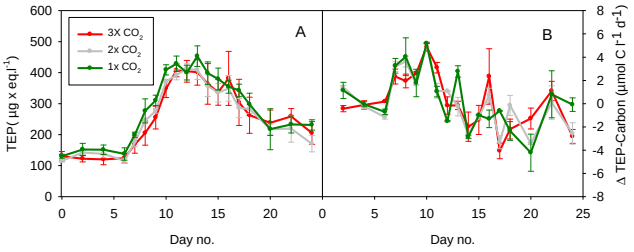

Fig. 4. Development of TEP concentration during the experiment. Mean TEP concentration ±SD of triplicate CO_2 treatments given as μg Xanthan equivalents X eq L^{-1} **(A)**, and estimated daily changes of TEP-carbon (μmol C L^{-1} d^{-1}) **(B)**.

the post-bloom period in individual mesocosms. No significant effect of the CO_2 treatment on TEP concentration was observed in the mesocosms (p=0.3).

4 Discussion

In addition to the CO_2 manipulation, all mesocosms in PeECE III were supplied with inorganic nitrate and phosphate. The fjord water initially contained about 3 μmol Si L^{-1}, and hence the addition of nutrients resulted in a rapid increase in primary production and a correspondingly enhanced algal biomass dominated by diatoms. A modest bloom of *E. huxleyi* and other nano- and pico-sized phytoplankton succeeded the diatoms (Paulino et al., 2008; Schulz et al., 2008). Over the 24 days experimental period, we recorded a trend of increasing primary production at elevated CO_2, although differences were not always statistically significant.

In situ measurements of dissolved inorganic carbon (DIC), during the current experiment, showed a significantly higher DIC consumption at elevated CO_2 (Riebesell et al., 2007; Bellerby et al., 2008). Over the course of the experiment excess DIC drawdown accumulated to approximately 40 μmol kg^{-1} higher carbon consumption at 3× CO_2 relative to 1× CO_2. Plotting our ^{14}C-data as cumulative production, we found a somewhat smaller but comparable difference of 22+/−18 μmol C L^{-1} in particulate primary production (Fig. 1b). Our 4 h incubated ^{14}C-based in vitro results thus show the same trend of increasing C-fixation with rising pCO_2-as reported by Riebesell et al. (2007), but differ in terms of absolute numbers. Production of DO^{14}C was not measured this study, and could probably explain at least part of the discrepancy between DIC drawdown and particular ^{14}C production. High productions of DO^{14}C, up to 50% compared to PO14C, was observed by Karl et al. (1998). In addition, respiration of ^{14}C organic products and excretion to the outside or recycling inside the cell, can take place during the incubation period (Williams and Lefèvre, 2008). As suggested in Gargas (1975) correction for respiration of ^{14}C of 6% of production is included in the calculation, but can be much higher (Williams and Lefèvre, 2008). Using oxygen probes, Pringault et al. (2007) measured light respiration up to 640 % higher than in dark. We acknowledge that these processes potentially influenced our measurements, but do not have data to quantify such effects. Consistent with the observed CO_2 treatment effect on DIC drawdown, Riebesell et al. (2007) reported changes in *in situ* O_2 concentrations to deviate between CO_2 treatments during the course of the experiment. Although we observed small differences in NCP with the O_2 in vitro technique, these differences were not statistically significant. The in vitro technique thus would suggest a change in the photosynthetic quotient not found in the in vivo measurements.

Using 24 h incubations for both O_2 and ^{14}C we found a NCP:^{14}C-fixation ratio of 1:1.3, corresponding well with previous reports of 1:1 (Marra et al., 2002), while our corresponding gross O_2 production: ^{14}C-fixation was high (ca. 4:1). In comparison, Gazeau et al. (2007) found a gross O_2-production:^{14}C-fixation ratio of 1:1 ratio when incubating samples for 15 h , from sunrise to sunset. According to Lizon and Lagadeuc (1998), 24 h-^{14}C incubations should approach net primary production, while 40 min incubations come close to gross primary production. Moreover, Lizon and Lagadeuc (1998) showed that increased incubation time from 4 h to 24 h may reduce production by as much as 40%, fitting well with our observed reduction (34–42%) in ^{14}C-based production estimates when increasing incubating times from 4 to 24 h.

In all treatments our in vitro measurements gave higher values for net O_2 production than what was obtained from in situ measurements. Differences between production based on bottle incubation and geochemical approaches, e.g. drawdown of DIC, have also been reported by others. In the Scheldt estuary, estimates of NCP in bottle incubation were 2-fold higher than those obtained from DIC budgets (Gazeau et al., 2005). In a net heterotrophic system, direct measurements of primary production and respiration were 4-fold higher than obtained from the geochemical approach (Wang et al., 2005). One reason for the discrepancy between the two methodologies in our experiment may therefore be disturbances of auto- and/or heterotrophic processes during the 24 h confinement in the 125 ml bottles used for the O_2 incubation. Other explanations may be the reduction of gas exchange in the production bottles, or the fact that they were incubated outside the mesocosm. Although the incubations were carried out at a light intensity corresponding to mid-depth of the mixed layer inside the mesocosms, the light conditions experienced by a plankton community inside a bottle at a fixed depth obviously differ from those in a mixed water column (e.g. inside the mesocosm).

Lack of statistical significance may reflect either the lack of measurement precision or the absence of an effect. With stronger temperature variation during the temperature-sensitive optode measurement of O_2 and generally more handling steps, there seems to be a higher potential for measurement errors in the in vitro compared to the in situ technique.

We therefore find it difficult to conclude whether the failure of our in vitro based O_2-measurement to confirm the in situ effect is rooted in a real disturbance of biological processes in the bottles, or just in a lower precision in the measurements.

In our study, inorganic nutrient availability changed along with phytoplankton succession and 5 different phases can be identified (Tanaka et al., 2008): During the first 6 days (Phase I days 0–6) all nutrients were detectable. Silicate was the first nutrient to become depleted (day 6), followed by phosphate depletion on day 10 (Phase II days 6–10), and nitrate depletion on day 13 (Phase III days 10–13). The last two phases were characterized by nutrient depletion and increasing (Phase IV) or oscillating (Phase V) phosphate turnover times. The highest particulate primary production rates were observed during the first two phases. At the time of silicate depletion (day 6), 70% of the total production occurred in the largest size fraction ($>10\,\mu$m), and the same fraction was responsible for 50–70% of the $^{33}PO_4$-uptake (Tanaka et al., 2008). Pigment analysis showed that diatoms dominated among larger algae during the first two phases (Schulz et al., 2008). Since NCP was close to zero from day 14, diatoms can be considered the main contributors to the net primary production in this study. Although cumulative production in the $>10\,\mu$m fraction was significantly higher at $3\times$ than at $2\times$ CO_2, there was no clear trend of primary production with CO_2 concentration as production was not different between $3\times$ and $1\times$ CO_2. Tortell et al. (2002) observed increased Si consumption as well as relatively more diatoms compared to other taxa at elevated CO_2, but in our experiment neither silicate drawdown nor pigment analyses indicated that CO_2 significantly influenced diatom growth (Schulz et al., 2008). Moreover, differences in particulate production due to CO_2 treatment were more evident towards the end of the experiment, after the peak in diatom abundance and after the strongest drawdown in pCO_2. Our results therefore do not lend support for a CO_2 effect on diatom primary production. This is in accordance with observations that photosynthetic carbon fixation rates of most diatoms tested so far are at or close to saturation at present CO_2 levels, with only a few species having responded positively to elevated CO_2 (Riebesell, 2004; Kim et al., 2006; Sobrino et al., 2008).

As in several previous mesocosm experiments (Egge and Heimdal, 1994; Engel et al., 2005), an initial pulse of nitrate and phosphate induced an *E. huxleyi* bloom – although with relatively low maximum numbers (observed in Phase II) (Paulino et al., 2008). The 5–10 μm size fraction has been shown to represent *E. huxleyi* quite well in blooms dominated by this species (Egge, 1994; Engel et al., 2008). Consistent with this, maximum primary production rates in the 5–10 μm fraction were measured when *E. huxleyi* cell numbers were at their maximum in PeECE III (day 10, 4.4 to 4.7×10^3 cells ml^{-1}, Paulino et al., 2008). A tendency, although not significant, of increased cumulative primary production at elevated pCO_2 (pP=0.07) in the 5–10 μm fraction is in accordance with previous reports of CO_2 sensitivity in organic matter production of *E. huxleyi* (Zondervan et al., 2001; Leonardos and Geider, 2005). Elevated CO_2 did not have any effect on primary production when *E. huxleyi* dominated the phytoplankton community in PeECE I, however (Delille et al., 2005; Engel et al., 2005). This discrepancy may be explained by differences in the nutrient environment or phytoplankton composition during blooms in the two experiments. In the present experiment, both nitrate and phosphate were available when *E. huxleyi* peaked, while nitrate was depleted when the *E. huxleyi* reached maximum numbers in PeECE I (Engel et al., 2005; Schulz et al., 2008). Sciandra et al. (2003) observed a decreased production of POC in cultures of *E. huxleyi* at elevated CO_2 when nitrate was depleted, which may explain why no effect of increased CO_2 on primary production was observed in PeECE I. While *E. huxleyi* was dominating during PeECE I, other nano-sized species were as numerous as *E. huxleyi* in PeECE III. These phytoplankton taxa were affected by increased pCO_2 as well (Paulino et al., 2008), and their contribution to primary production were probably comparable to *E. huxleyi*. We did not observe any changes in primary production between size fractions to indicate a shift in community composition from diatoms to nano-phytoplankton at high CO_2 as was demonstrated by Hare et al. (2007).

Primary production in the 1–5 μm fraction was low (18% of total) but increased during the experiment, particularly during the last week. This development mirrors the abundance of *Synechococcus* which increased markedly during the last week of the experiment reaching cell numbers between 3 and 4×10^5 cells ml^{-1} (Paulino et al., 2008), suggesting that *Synechococcus* was an important contributor to primary production in this size fraction.

A rather high primary production (29% of total production), increasing with rising CO_2, was observed in the 0.2–1 μm size-fraction. A similarly high share of both primary production and chlorophyll in this size fraction has been reported from the northeast Atlantic Ocean (May–June) (Savidge et al., 1995). Børsheim et al. (2005) showed, however, that approximately half of the picocyanobacteria may pass through 1 μm filters and may thus contribute distinctly to the production of organic carbon in this size fraction. In our study, maximum production in the 0.2–1μm size fraction occurred on day 6, when all picophototrophs (*Synechococcus*, picoeukaryotes) were at a minimum (Paulino et al., 2008). Therefore we do not consider it likely these populations contributed significantly to the production in this fraction. ^{14}C found in the 0.2–1 μm size-fraction could also have been due to bacterial uptake of labelled carbon released from phytoplankton in the light bottles (Li et al., 1993; Børsheim et al, 2005), which however is not supported by the deviating trends observed for bacterial production (Allgaier et al., 2008) and ^{14}C uptake in this fraction. Another explanation may be that ^{14}C-labelled organic material released from phytoplankton aggregated into transparent exopolymer particles (TEP) which originate from dissolved carbohydrates

and extend from 0.4 μm to several 100 μm during continued aggregation. Newly formed TEP should be included in the ^{14}C-labeled material of the 0.2–1μm fraction that was observed after day 6. As both diatoms and *E. huxleyi* have been shown to produce TEP (Passow 2002; Engel et al., 2004), and both populations had their maximum in the first half of our experiment (Paulino et al., 2008; Schulz et al., 2008), production observed in the smallest fraction could have had its origin in the larger size fractions and be linked to the mechanism of carbon overproduction under nutrient limited conditions (Engel 2002). TEP have proven to increase at elevated CO_2 concentrations (Engel 2002; Engel et al., 2004). However, TEP concentration did not reveal a corresponding effect of CO_2 in the present experiment and declined during the 2nd half of the experiment, indicating that TEP dynamics after the diatom peak were mainly driven by loss processes, as also reported for excess organic carbon (Riebesell et al., 2007). Similarly, the apparent difference between primary production, determined in bottle enclosures and changes in suspended TEP concentrations in the mesocosms may be explained by differences in loss processes: high loss through rapid sinking of TEP in the mesocosms versus accumulation of TEP in the incubation bottles. The absence of a heterotrophic phase during the course of the experiment provides further evidence for vertical transport of primary produced organic matter.

An increase in semi-labile DOC during the senescent phase of the *E. huxleyi*-bloom was evident in PeECE I (Joassin et al., 2007), but statistically significant CO_2 treatment effects on the concentration of DOC were not detected in any of the PeECE experiments (Rochelle-Newall et al., 2004; Grossart et al., 2006; Schulz et al., 2008) or in a mesocosm experiment with similar CO_2 treatments (Kim et al., 2006). Higher bacterial production, possibly indicating more available DOC, was observed at the highest CO_2 level in PeECE II, but the present experiment did not reveal similar results (Grossart et al., 2006; Allgaier et al., 2008). As our ^{14}C-based measurements did not include DO^{14}C, allowing for the possibility of a conversion of the over-consumption of DIC into DOC by e.g. excretion or leakage from phytoplankton cells, we are presently not in the position to draw a firm conclusion on this matter.

Experiments with duration of a few weeks do not include all possible responses of a potentially adaptive plankton community. Extrapolation to longer time scales should therefore be done with caution. It should also be noted that in large scale experiments, which are generally bound to a small number of replicates, there is a risk of erroneously accepting the hypothesis of "no treatment effect" when perturbations are small and variance is large (Brett and Goldman, 1996; Carpenter, 1996). Still, our results demonstrate a small, but statistically significant effect of elevated CO_2 on daily primary production. The trend found in cumulative ^{14}C-based particulate primary production was consistent with the over-consumption of DIC at elevated CO_2 reported by Riebesell et al. (2007) and Bellerby et al. (2008). Size-fractionated primary production measurements combined with data on phytoplankton composition further indicated that in some groups or species primary production may be stimulated at elevated CO_2 levels. However, in contrast to the CO_2 effect on DIC drawdown, which became evident already during the bloom development, the effect of pCO_2 on ^{14}C-based total particulate primary production was visible only after inorganic nutrients had been depleted and statistically significant only on the very last days of the experiment. The key to explaining this discrepancy may be in the early occurrence of silicate depletion, which may have caused the comparatively early production of TEP by diatoms, leading to the sinking of diatom biomass and TEP at a time when the phytoplankton bloom was still building up (see Schulz et al. 2008). Whereas a CO_2 treatment effect on primary production during this phase would still leave a signal in the time-integrating DIC drawdown, the short-term ^{14}C and O_2 primary production measurements in bottle enclosures would have difficulties detecting such an effect or may miss it completely. This may also explain why in bottle incubations a CO_2 treatment effect is detected in the second half of the experiment, at a time when TEP concentrations were much lower and TEP loss due to sinking was probably low.

If the effect of rising pCO_2 is an increase in the production of organic C under conditions of mineral nutrient limited phytoplankton growth, this will only have a feedback effect on atmospheric CO_2 if the extra material is not respired by bacteria in the photic zone. Excess organic matter may be unavailable to bacterial consumption for several reasons. It may be physically protected inside phytoplankton cells, or it may be in chemical forms resistant to bacterial enzymatic attack. It has also been suggested that degradation of otherwise labile DOC may be prevented by mineral nutrient limitation of bacterial growth (Thingstad et al., 1997). A net effect on C-sequestration may therefore depend not only on the physiological responses in phytoplankton, but also vary with ecological status and limiting factors for bacterial growth in the photic zone (Tanaka et al. 2008). The lack of any net heterotrophic phase in PeECE III shows that organic material produced during net autotrophy was not degraded by bacteria in the upper layer, but either accumulated or was exported vertically. This supports the interpretation of Riebesell et al. (2007) of a high export of organic material through the pycnocline in this experiment. This accumulation/export, combined with the observation of a CO_2 effect on bacterial production in PeECE II (Grossart et al. 2006), but not in PeECE III (Allgaier et al., 2008), highlights the need to better understand the whole microbial community, including ecological mechanisms regulating bacterial growth rate limitation, in order to understand the net effects of any increased C-fixation at high pCO_2.

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