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Primary production during nutrient-induced blooms at elevated CO_2 concentrations

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Abstract

Mesocosms experiments (PeECE II and PeECE III) were carried out in 9 transparent mesocosms. Prior to the experimental period, the seawater carbonate system was manipulated to achieve three different levels of CO_2. At the onset of the experimental period, nutrients were added to all mesocosms in order to initiate phytoplankton blooms. Rates of primary production were measured by in-situ incubations using ^{14}C-incorporation and oxygen production/consumption. Particulate primary production by ^{14}C was also size fractionated and compared with phytoplankton species composition. Nutrient supply increased the primary production rates, 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 returned to near-zero and ^{14}C-fixation rates relaxed back to lower than initial. We found a trend in the ^{14}C-based measurements towards higher cumulative primary production at higher pCO_2, consistent with recently published results for DIC removal (Riebesell et al., 2007). There where found differences to the size fractionated primary production response to CO_2 treatments. The plankton composition changes throughout the bloom, however, resulted in no significant response until the final phase of the experiment where phytoplankton growth became nutrient limited, and phytoplankton community changed from diatom to flagellate dominance. This opens for the two alternative hypotheses that such an effect is associated with mineral nutrient limited growth, and/or with 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 there or was exported vertically.

1 Introduction

In the upper photic zone where primary production is 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

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element, grossly described by the import-export and the regenerated cycles (Dugdale and Goering, 1967). Onto this set of nutrient cycles, the C-cycle is linked via more or less flexible stoichiometric relationship in the different trophic levels and their interactions. Relatively small alterations in either element cycles or stoichiometric C:nutrient coupling may have consequences for the ocean's C-cycle, particularly if the net result is a change the C:limiting element ratio of the exported material, and/or in the quality of this matter in a manner affecting the relative depths at which C and limiting elements are released from sinking to non-sinking forms (Thingstad, 1993).

Increased atmospheric CO_2 leads to both an increased pCO_2 and a lowered pH (Riebesell, 2004). 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 fixation of C 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 Jonston, 1991; Clark and Flynn, 2000) The RUBISCO enzyme has however a relatively low affinity for CO_2 (Raven and Jonston, 1991) and this has led to a discussion of the possibility that increased CO_2-levels may stimulate primary C-fixation (Riebesell, 2004). Should this occur without a proportional change in the cycle of limiting elements, the consequence is a change in the stoichiometric relationships in the microbial food web. Based on measurements of removal of inorganic-C and nitrate, the PeECE-experiments have shown such an effect (Riebesell et al., 2007).

The affinity for CO_2 differs among phytoplankton species (Rost et al. 2003), and some phytoplankton species are able to change their CO_2 supply by CO_2-concentrating mechanisms (CCM) (Raven, 1991). The efficiency and regulation of CCM differs among phytoplankton species and functional groups. Changes in CO_2 availability might therefore affect competition and succession of phytoplankton species (Burkhardt et al., 2001; Rost et al., 2003). A shift in dominance between *Phaeocystis* and diatoms has been observed in a natural plankton community where CO_2 has been manipulated (Tortell et al., 2002). Changes in phytoplankton composition may affect primary pro-

duction, and the effect of increased CO_2 on primary production has been investigated theoretically as well as experimentally. Some papers report small, if any, effect (Clark and Flynn, 2000; Tortell et al., 2002) whereas other papers document increased primary production with increasing CO_2 (Heine and Sand-Jensen, 1997; Schippers et al., 2004; Riebesell et al., 2007).

3 mesocosm experiments, in 2001, 2003 and 2005 have been carried out in the framework of the Pelagic Ecosystem CO_2 Enrichment study (PeECE). The aim of these experiments has been to study the effects of elevated levels of CO_2 on the planktonic community and on sea water chemistry (Delille et al., 2005; Engel et al., 2004, 2005; Rochell-Newall et al., 2004; Grossart et al., 2006). Blooms of the coccolithoporid *Emiliania huxleyi* and/or diatoms were initiated by nutrient addition, and the plankton community was monitored for about 3 weeks. Primary production has been measured during all PeECE experiments. No differences in primary production were observed in the 2001 experiment where CO_2 concentration in the mesocosms was manipulated to 180, 370 and 700 μatm (Delille et al., 2005). The same CO_2 concentrations were also used in the 2003 experiment, but elevated to 350, 700 and 1050 μatm CO_2 in 2005 (Schulz et al., 2007)

This paper reports primary production results from PeECE III, with a comparison of the corresponding data from(PeECE II conducted in 2003. ^{14}C and O_2 measurements were used for estimating production in both experiments, but different techniques were used for O_2-detection (Winkler titration and optodes in PeECE II and PeECE III, respectively). Primary production in size-fractions: 0.2–1, 1–5, 5–10 and >10 μm, was measured only during the 2005 experiment.

2 Materials and methods

2.1 Set up and sampling

The PeECE III mesocosm experiment was carried out at Marine Biological Station, University of Bergen, Norway between May 16 and June 10, 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. 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 in order to achieve an increase in growth and biomass of osmotropic organisms. For further details concerning the set-up of the experiment see Schulz et al. (2007). The PeECE II experiment was carried out between 4–24 May 2003, where CO_2 concentration in the nine mesocosms were adjusted to 190 μatm (glacial), 370 μatm (present) and 750 μatm (future) CO_2, in triplicates. While in 2005 low concentrations of silicate were available in the fjord water used to fill the mesocosm, allowing for an initial bloom of diatoms followed by coccolithophore dominance, silicate was added in excess in 2003. For further details see Grossart et al. (2006).

2.2 ^{14}C Primary production

Primary production was measured using the ^{14}C method, according to Steemann-Nielsen (1952) and Gargas (1975). Plastic bottles (76 ml) (NUNC Easyflask) spiked with approximately 4 μCi were incubated in situ between 10:00 and 14:00 h. Dark uptake was measured bottles wrapped in aluminium foil. Bottles were incubated in the sea outside the mesocosms, at the irradiance level corresponding to middle dept of the upper layer of the mesocosms (see Schulz et al., 2007). The incubation depth was determined base 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 2005, triplicate incubation was made from all 9 mesocosm, while in 2003 only two mesocosms per treatment were analysed.

In addition to short time incubation, 24 h incubations were also conducted in 2005. For these incubations 118 mL glass bottles were used, and only one mesocosm per treatment was sampled, M2, M5 amd M8. These samples were either filtrated onto Sartorius membrane filters or onto Nuclepore filters of 0.2, 1, 5 and 10 μm. After filtration were all filters treated with fuming HCl in order to remove inorganic ^{14}C. Scintillation solution (Ecosint O) was then added, and the samples were stored overnight before counted in a Packard Tri Carb Liquid Scintillation Analyser, model 1900 A. Calculations were done according to Gargas (1975). Daily primary production, based on 4 h incubation was calculated as function of incoming irradiance during incubation period (4 h) and total irradiance for the 24 h. Calculation of primary production was done according to Gargas (1975).

2.3 Oxygen and Oxygen production

In 2003 dissolved oxygen was determined based on the Winkler method using an automated titration (Titrino, Metrohm) and potentiomentric endpoint detection. Samples were collected from the mesocosms at 9.00 using a Ruthner water sampler and transferred without air bubbles to 100 ml BOD bottles through a silicon tube. A total of 6 bottles were filled from each mesocosm. Two bottles were immediately fixed with 1 ml of manganese chloride solution and 1 ml of potassium iodate (0.003 M) and mixed. Another two bottles were placed in photoresistant plastic foil and incubated together with the remaining two bottles in the fjord at about 1m depth for 9 h. The samples were removed in the evening (~19.00), fixed immediately, mixed well and stored for more than 12 but less than 24 h in the dark. After dissolving the precipitated with sulfuric acid (5 M) the iodine was titrated with 0.02 N thiosulfate solution. Calculation of the oxygen concentration was performed after Dickson (1994).

In 2005, 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 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} in 2005 (Fig. 1). After addition of nutrients, a rapid production increase 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 increasing production with increasing CO_2. Although not statistically significant, this trend is visible from ca. Day 10 in the cumulative production (Fig. 1b). In the PeECE II experiment in 2003, average primary production ranged from 0.28 to 0.52 μmol C L^{-1} h^{-1}. (Table 2) Present CO_2 concentration (370 μatm) gave slightly higher production than past (190 μatm) and future (750 μatm), which also can be seen in gross production, leaving no consistent increasing or decreasing trend with increasing CO_2 (Table 2). For comparison, average primary production during PeECE III was somewhat higher, ranging from 0.57 to 0.62 μmol C L^{-1} h^{-1}.

In 2005, the highest gross production, as measured by oxygen incubation, was observed on day 6 in 1× and 2× CO_2, with 56 and 58 μmol O_2 $L^{-1}d^{-1}$ respectively, whereas the corresponding value, 58 μmol O_2L^{-1} d^{-1} in 3× CO_2 was observed a few days later (Fig. 2). For all treatments, maximum net community production was observed on day 6, and after Day 10 no net production was observed in the system in any of the treatments. With this method, CO_2 did not have any clear effects, neither on timing nor or scaling of production or respiration.

While there was no detectable variation in primary production with CO_2-level in the PeECE II (2003) experiment, there was a difference in phytoplankton community com-

position. In PeECE III (2005), we therefore decided to carry out fractionated primary production. The fractions 0.2–1, 1–5, 5–10 and >10 μm contained on average 29, 18, 12 and 41%, respectively, of total primary production. All fractions showed an increase in production at the onset of the experiment, but during the first week production was dominated by organisms in the fraction >10 μm. On day 6, 70% of the total production was found in this fraction, thereafter the contribution from the >10 μm fraction decreased rapidly (Fig. 3). A tendency of higher production, although not significant, was observed in 3×, followed by 1× and 2× (Fig. 4). Cumulative production indicated that the differences was more pronounced after day 6 (data not shown) A distinct, but much smaller peak was observed in the fraction 5–10 μm on day 10. Average production in the fraction 5–10 μm was 0.9, 0.8 and 0.6 μmol C L^{-1} d^{-1} in 3×, 2× and 1× CO_2, respectively (Fig. 4).

From day 6 onwards we observed a decreasing trend in production in the smallest fraction (0.2–1 μm). The production in fraction 1–5 μm increased somewhat in the same period (Fig. 3). This was particularly the case in 1× and 2× CO_2, and on the very last day of the experiment, production in 3× CO_2 was significantly lower than in the two other treatments (ANOVA, $P<0.05$). The largest differences between treatments were found in the smallest size fraction 0.2–1 μm, where average production for the whole experimental period was 1.3 μmol C L^{-1} d^{-1} in the 1× CO_2 treatment and increased with increasing CO_2 to 1.7 and 2.1 μmol C L^{-1} d^{-1} in 2× and 3× CO_2 respectively (Fig. 4).

4 Discussion

4.1 Effects of CO_2 on total primary production

Based on in situ measurements of dissolved inorganic carbon, determined significantly higher carbon consumption at elevated CO_2 (Riebesell et al., 2007; Bellerby et al., 2007). 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 treatments. Plotting our ^{14}C-data as cumulative production, we found (Fig. 1b) a somewhat smaller but comparable (22 μmol-C L^{-1}) difference in particulate primary production. The ^{14}C-based results thus support the increase in C-fixation with increased CO_2-level proposed by Riebesell et al. (2007). The standard deviation (±18 μmol-C L^{-1}) of the ^{14}C-based difference in particulate primary production is, however, insufficient for the effect to be statistically significant based on these measurements alone. Higher primary production as a result of elevated CO_2 has also been reported by others (Hein and Sand-Jensen, 1997; Schippers et al., 2004). On the other hand, Clark and Flynn (2000) found neither the rate nor the extent of primary productivity to be significantly limited by DIC concentration.

Riebesell et al. (2007) also reported a difference in in-situ O_2 concentrations supporting the hypothesis of a net stimulatory effect of elevated CO_2 levels on C-fixation. We could not detect a similar trend in our O_2 light/dark-bottle measurements. Our results also indicate a higher O_2 production, in all treatments, relative to drawdown of DIC (Riebesell et al., 2007). The reason for this apparent discrepancy between in situ measurements and incubated samples is unknown, but one may suspect disturbances of auto- and/or heterotrophic processes during the 24 h confinement in the 125 ml bottles used in O_2 incubation. We also have an unexplained low ratio (ca 1:4) of particulate ^{14}C-fixation to gross oxygen production in our 24 h incubations. Using a 15 h (sun rise to sun set) incubation, Gazeau et al. (2007) found a near 1:1 (mol C: mol O_2) ratio between ^{14}C incubations and gross production. Theoretically, significant production of ^{14}C-DOC (not measured in this study) could help to balance the carbon fixation:O_2-production stoichiometry in our measurements.

Production in the 0.2–1 μm size-fraction was relatively high (29% of total production), and there is a tendency of increasing production in this size-fraction with increasing CO_2. There was an increase in *Synechococcus* abundance in the last part of the experiment (Paulino et al., 2007). *Synechococcus* cells are in the size-range from 0.8 to 1.5 μm (Johnson and Sieburt, 1979), and parts of the population may therefore have

passed through 1 μm filters. The even smaller *Prochlorophytes* (<1 μm), known to contribute to primary production at lower latitudes (Li, 1994; John et al., 2007), are not common in Norwegian coastal waters and were not observed during the experiment. ^{14}C found in the 0.2–1 μm size-fraction may also be due to bacterial uptake of labelled carbon released from phytoplankton, an option suggested by Li et al. (1993). The deviating trends observed for bacterial production (Allgaier et al., 2007[1]) and ^{14}C uptake in this fraction do not lend immediate support for this. Nevertheless, bacterial activity could still explain the comparatively high rate of ^{14}C incorporation in the 0.2–1 μm size-fraction, if uptake of ^{14}C-labeled DOC was accomplished by only a fraction of the bacterial community. Thus, neither the contribution of phytoplankton nor of heterotrophic bacteria to the radiolabel found in the 0.2–1 μm size-fraction can be ruled out based on these results. We note, however, that the highest primary production in this fraction was measured on Day 6, coinciding with the minimum in abundance of small organisms potentially passing a 1 μm filter (Paulino et al., 2007). An alternative possibility would be that ^{14}C-labelled organic material released from phytoplankton passed the 1 μm filter but was (partly) retained on the 0.2 μm filters. An increasing production of TEP with increasing CO_2 concentration has previously been shown (Engel, 2002; Engel et al., 2004; Rochelle-Newall et al., 2004). The CO_2 effect may thus be linked to the mechanism of carbon overproduction under nutrient limited conditions (Engel, 2002) in accordance with the impression one gets from Fig. 1b where all treatments behave similarly until nutrients are depleted.

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. Statistically significant CO_2 treatment effects on the concentration of DOC, however, were not detected in any of the PeECE experiments (Rochelle-Newall et al., 2004; Grossart et al., 2006; Schulz et al., 2007), and the Nuclepore filters

[1] Allgaier, M., Riebesell, U., and Grossart, H. P.: Microbial response to enrichment in pCO_2 and subsequent changes in phytoplankton and nutrient dynamics, Biogeoscienes Discuss., in preparation, 2007.

used in this experiment are supposed to have low absorption of ^{14}C-DOC (Karl et al., 1998).

Daily measured concentration of DOC, however, does not reveal the pattern of production and consumption (turnover) of DOC. A higher bacterial production, indicating a higher DOC consumption, was observed in the treatment with highest CO_2 level in PeECE II, but this was not the case in PeECE III (Grossart et al., 2006; Allgaier et al., 2007[1]).

If the effect of increased 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. It has been suggested that the ability of bacteria to consume labile DOC is highly dependent on the state of growth rate limitation in the bacteria (Thingstad et al., 1997) with C-limited bacteria in principle consuming all accessible organic material, while mineral nutrient limitation of bacterial growth may lead to accumulation of the otherwise degradable organic material. 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 factor for bacterial growth in the photic zone (Tanaka et al., 2007). 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., 2007[1]), highlights the need to better understand the ecological mechanisms regulating bacterial growth rate limitation in order to understand the net effects of any increased C-fixation at high pCO_2.

4.2 Primary production related to osmotroph succession

All PeECE mesocosm studies were carried out in May–June, but nutrient additions, phytoplankton community composition, and other conditions were different (Table 2). By manipulating nutrient concentrations and stoichiometry, blooms of different phytoplankton groups, e.g. *Emiliania huxleyi* and/or diatoms were introduced. In 2001, nitrate and phosphate were added in a ratio of 34:1 (atomic) in order to induce blooms of the coccolitophorid *Emiliania huxleyi,* which towards the end of the experiment reached cell numbers ranging from 20 to 40×10^3 cells ml^{-1} (Rochelle-Newall et al., 2004). In 2003, nitrate and phosphate were initially supplied in a lower N:P ratio (18:1) with silicate in excess (N:Si=1:1.33) in order to initiate diatom blooms. Diatoms, but also *E. huxleyi* became dominant during this experiment and different species compositions were observed within different CO_2 treatment (Grossart et al., 2006).

As for the first PeECE experiment, the aim in PeECE III was to introduce *E. huxleyi* blooms. Only nitrate and phosphate in a 25:1 ratio were therefore added to the mesocosms. The initial water did, however, contain about 3 μmol L^{-1} silicate, favouring diatom growth (Schulz et al., 2007). Due to these start conditions the phytoplankton community became dominated by diatoms followed by a weak bloom of *E. huxleyi* and other nano- and pico-sized phytoplankton (Paulino et al., 2007). The nutrient environment during the experiment can be divided into 5 phases (Tanaka et al., 2007). During the first 6 days all nutrients were detectable and silicate was the first nutrient that was depleted (Phase I). Phosphate was depleted on day 11 (Phase II) and nitrate on day 15 (Phase III). Between day 16 and 20 all nutrients were depleted (Phase IV) before increased turnover times for phosphorus indicated regeneration of nutrients from day 21 (Phase V) (Tanaka et al., 2007).

The highest particulate primary production was observed in the first two phases. At the time of silicate depletion (Day 6), 70% of the total production was observed in the largest size fraction (>10 μm), and the same fraction used 50–70% of the $^{33}PO_4$-uptake (Tanaka et al., 2007). Pigment analysis showed that diatoms dominated among larger

algae during the two first phases (Schulz et al., 2007). As net production was hardly observed after Phase II, diatoms were probably the main contributors to the net primary production during the PeECE III experiment. Higher production was observed at 3× than at 1× and 2× CO_2 in the >10 μm fraction. The difference was not significant, and mainly observed after Day 6. Since photosynthetic carbon fixation rate of all diatoms tested so far are at or close to saturation at present CO_2 levels (Riebesell, 2004), we did not expect to see much effect in this group. In a field incubation experiment, Tortell et al. (2002) observed no change in primary production, but a relative shift in species composition between 150 and 750 ppm CO_2. In their experiment, diatoms became more abundant, *Phaeocystis* biomass decreased with increasing CO_2. Also the ratio between consumed nitrate and consumed silicate, N:Si, decereased with decreasing CO_2. In our experiment, neither consumption of nutrients, nor diatom abundance was affected by CO_2 levels (Schulz et al., 2007).

Most species of marine phytoplankton tested to date can use HCO_3^- in addition to CO_2 as a source for inorganic carbon (Tortell et al., 1997; Burkhard et al., 2001; Rost et al., 2003; Giordano et al., 2005). For the two diatoms *Thalassiosira weissflogii* and *Phaeodactylum tricornutum,* Burkhardt et al. (2001) showed that photosynthesis was unaffected by pCO_2 levels ranging from 36 to 1800 ppmv CO_2, but the uptake of HCO_3^- was more important as source of inorganic C at low CO_2 levels than at high. If this is a general trend among diatoms, it can also explain why primary production in our experiment was less affected by CO_2 levels in the period when diatoms dominated the phytoplankton community.

As in several previous mesocosms experiments, blooms of *E. huxleyi* occurred in mesocosms fertilized with nitrate and phosphate (Egge and Heimdal, 1994; Engel et al., 2005). In the PeECE III experiment, however, the maximum numbers were low compared with previous observations, and the highest cell numbers were observed during Phase II (Paulino et al., 2007). The size fraction 5–10 μm has previously been shown to represent *E. huxleyi* quite well -when the bloom is dominated by this species (Egge, 1994, Engel et al., 2007). Maximum primary production rates in the 5–10 μm

fraction occurred on Day 10, when *E. huxleyi* numbers ranged from 4.4 to 4.7×10^3 cells ml^{-1} (Paulino et al., 2007). Assuming *E. huxleyi* to be the dominant photoautotroph in this fraction, our measured production of 17–26 $\mu g\,C\,L^{-1}\,d^{-1}$ corresponds to 4–5 $pg\,C\,cell^{-1}\,d^{-1}$. This is in the same order of magnitude as reported from laboratory experiments (7–10 $pg\,C\,cell^{-1}\,d^{-1}$) (Skattebøl, 1995; Zondervan et al., 2001). The actual production of POC per *E. huxleyi* cell in the current experiment must have been considerably lower, as other species including other haptophyte species were more numerous and probably contributed more to the primary production in this fraction (Schulz et al., 2007; Paulino et al., 2007). Both increasing (Zondervan et al., 2001; Leonardos and Geider, 2005) and decreasing (Sciandra et al., 2003) production at elevated levels of CO_2 were reported for *E. huxleyi*. Average primary production in the fraction 5–10 μm showed a weak increase with increasing CO_2,. The same trend was observed both for *E. huxleyi* numbers and the other nano-sized phytoplankton from the flowcytometer counts (Paulino et al., 2007, Fig. 4). In the last part of the PeECE I experiment, 2001, *E. huxleyi* reached maximum cell numbers up to 10 times higher than the present experiment, but elevated CO_2 had no conspicuous effect on primary production (Delille et al., 2005; Engel et al., 2005). Sciandra et al. (2003) observed decreased production of POC in cultures of *E. huxleyi* at elevated levels of CO_2 when nitrate was depleted. Nitrate was also depleted when the bloom peaked in 2001, and could have reduced the stimulating effect of elevated CO_2 on *E. huxleyi* production observed in other studies.

Production in the 1–5 μm fraction increased during the experiment and contributed 18 % of the total production. *Synechococcus* was probably an important contributor to the primary production in this fraction, and this genus became numerous towards the end of this experiment reaching cell numbers between 3 and 4×10^5 cell ml^{-1} (Paulino et al., 2007). The development of primary production in this fraction mirrors the *Synechococcus* abundance, which increased markedly during the last week of the experiment. Cell numbers were higher in 1× CO_2than in the two treatments with elevated levels of CO_2 (Paulino et al., 2007). A similar trend was observed towards the end of the

experiment in 2001 where the highest cell numbers of *Synechoccocus* were observed in the treatment with lowest CO_2 (190 μatm) (Rochelle-Newall et al., 2004). Primary production in the 1–5 μm fraction was also highest in the 1× CO_2 treatment, but only on the very last day of the experiment. Analyses from FCM also showed that other picoautotrophs (1–2 μm cells) indicated the opposite response to elevated CO_2,with the highest cell numbers in the 3× CO_2. These opposing effects will reduce the total effect in this size fraction even though *Synechococcus* numbers were 5 fold higher than other small picoeucaryotes during the last days of the experiment.

5 Concluding remarks

With our techniques, we did not observe significant effects of elevated CO_2 on daily primary production during the PeECE experiments. The trend found in cumulative ^{14}C-based particulate primary production was, however, consistent with the overconsumption of DIC at high CO_2 reported by Riebesell et al. (2007) and Bellerby et al. (2007). Splitting the production into size-fractions gave more information, and when the dynamics of fractionated production was compared to species composition, the results indicate that in some groups or species primary production may be stimulated at elevated CO_2 levels. The 5–10 μm fraction showed a tendency towards increasing production with increasing CO_2 concentration, but as the production in this fraction was low, it did not contribute much to total production. Also, if two group of organism respond opposite to elevated CO_2 the effect on primary production will be reduced or eliminated, as suspected for *Synechococcus* and other picoautrophs the 1-5μm fraction.

The trend in ^{14}C-based particulate primary production was only visible when inorganic nutrients were depleted. This could be due to the fact that the diatoms dominating in the first part were not affected by pCO_2 or that pCO_2 only affects primary production in nutrient stressed phytoplankton. Nutrient limitation may affect phytoplankton as well as bacteria, and the net outcome on community production may therefore well be differ-

ent in different experiments where the conditions controlling bacterial carbon demand may differ.

Experiments with duration of a few weeks do not include all possibilities in a potentially adaptive plankton community, extrapolation to long time scales should therefore be done with caution.

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References

Bellerby, R. G. J., Schulz. K. G., Ribesell, U., Neil, C., Nondal, G., Johannessen, T., and Brown, K. R.: Marine ecosystem community carbon and nutrient uptake stoichiometry under varying ocean acidification during the PeECE III experiment, Biogeoscienes Discuss., accepted, 2007.

Burkhardt S., Amoroso, G., Riebesell, and U., and Sültemeyer, D.: CO_2 and HCO_3^- uptake in marine diatoms acclimated to different CO_2 concentrations, Limnol. Oceanogr., 46, 1378–1391, 2001.

Clark, D. R. and Flynn, K. J.: The relationship between the dissolved inorganic carbon concentration and growth rate in marine phytoplankton, Proc. R. Soc. Lond., 267, 953–959, 2000.

Delille, B., Harley, J., Zondervan, I., Jacquet, S., Chou, L., Wollast, R., Bellerby, R. G. J., Frankignoulle, M., Borges, A. V., Riebesell, U., and Gattuso, J. P.: Response of primary production and calcification to changes of pCO_2 during experimental blooms of the coccolithophorid Emiliania huxleyi, Global Biogeochem. Cy., 19, GB2023, doi:10.1029/2004GB002318, 2005.

Dickson, A. G.: Determination of dissolved oxygen in sea water by Winkler titration, WHP Operations and Methods, WHPO publication 90-1, 1994.

Dugdale, R. C. and Goering, J. J.: Uptake of new and regenerated forms of nitrogen in primary productivity, Limnol. Oceanogr., 12, 196–206, 1967.

Egge, J. K.: Nutrient control of phytoplankton growth: Effects of macronutrients composition (N, P, Si) on species succession, Dr. Scient thesis, University of Bergen, Norway, ISBN 82-7744-007-3, 1994

Egge, J. K. and Heimdal, B. R.: Blooms of phytoplankton including Emiliania huxleyi (Haptophyta), Effects of nutrient supply in different N:P ratios, Sarsia, 79, 333–348, 1994.

Engel, A.: Direct relationship between CO_2 uptake and transparent exopolymer particles production in natural phytoplankton, J. Plank. Res., 24, 49–53, 2002.

Engel, A., Delille, B., Jacquet, S., Riebesell, U., Rochelle-Newall, E., Terbrüggen, A., and Zondervan, I.: Tarnsparant exopolymer particles and dissolved organic carbon production by Emiliania huxleyi exposed to different CO_2 concentrations: a mesocosm experiment, Aquat. Microb. Ecol., 34, 93–104, 2004.

Engel, A., Zondervan, I., Aerts, K., Baufort, L., Benthien, A. Chou, L., Delille, B., Gattuso, J. P., Harley, J., Heeman, C., Hoffmann, L., Jacquet, S., Nejstgaard, J., Pizay, M. D., Rochelle-Newall, E., Schneider, U., Terbrueggen, A., and Riebesell, U.: Testing the direct effect of CO_2 concentration on a bloom of the coccolithophorid Emiliania huxley*i* in mesocosm experiment, Limnol. Oceanogr., 50, 493–507, 2005.

Engel, A., Schulz, K., Riebesell, U., Bellerby, R., Delille, B., and Schhartau, M.: Effects of CO_2 on particle size and phytoplankton abundance during a mesocosm bloom experiment (PeECE II), Biogeoscienes Discuss., 4, 4101–4133, 2007.

Gargas, E.: A manual for phytoplankton primary production studies in the Baltic, The Baltic Marine Biologists, Publication No. 2, The Danish Agency of Environmental Protection, Hørsholm, 1975.

Grossart, H. P., Allgaier, M., Passow, U., and Riebesell, U.: Testing the effect of CO_2 concentration on the dynamics of marine heterotrophic bacterioplankton, Limnol. Oceanogr., 51, 1–11, 2006.

Gazeau, F., Middelburg, J. J., Loijens, M., Vanderborght J.-H., Pizay, M.-D., and Gattuso, J.-P.: Planktonic primary production in estuaries: comparison of ^{14}C, O_2 and ^{18}O methods, Aquat. Microb. Ecol., 46, 95–106, 2007.

Giordano, M., Beardall, J., and Raven, J. A.: CO_2 concentrating mecanisms in algae: Mechanisms, environmental modulation, and evolution, Ann. Rev. Plant. Biol., 56, 99–131, 2005.

Hein, M. and Sand-Jensen, K.: CO_2 increases oceanic primary production, Nature, 338, 526–

527, 1997.
John, D. E., Wang, Z. A., Liu, X., Bryne, R. H., Corredor, J. E., López, J. M., Cabrea, A., Bronk, D. A., Tabita, F. R., and Paul, J. H.: Phytoplankton carbon fixation gene (RuBisCO) transcripts and air-sea CO_2 flux in the Mississippi River plume, The ISME Journal, 517–531, 2007.
Johnson, P. W. and Sieburth, J. M.: Chroococcoid cyanobacteria in the sea: a ubiquitous and diverse phototrophic biomass, Limnol. Oceangr., 24, 928–935, 1979.
Karl, D. M., Hebel, D. V., and Björmann, K.: The role of dissolved organic matter release in the productivety of the oligitropic North Pacific Ocean, Limnol. Oceaogr., 43, 1270–1286, 1998.
Leonardos, N. and Geider, R. J.: Elevated atmospheric carbon dioxide increases organic carbon fixation by Emiliania huxleyi (Haptophyta), under nutrient-limited high-light conditions, J. Phycol., 41, 1196–1203, 2005.
Li, W. K. W.: Primary production of prochlorophytes, cyanobacteria, and eukaryotic ultraplankton: Measurements from flow cytometer sorting, Limnol. Oceanogr., 39, 169–175, 1994.
Li, W. K. W., Irwin, B. D., and Dickie, P. M.: Variation related to biomass and productivety of phytoplankton and bacteria, Limnol. Oceanogr., 38, 483–494, 1993.
Paulino, A. I., Egge, J. K., and Larsen, A.: Effects of increased atmospheric CO_2 on small and intermediate sized osmotrophs during a nutrient induced phytoplankton bloom, Biogeoscienes Discuss., accpeted, 2007.
Raven, J. A.: Physiology of inorganic C acquisition and implications for resource use efficiency by marine phytoplankton: relation to increased CO_2 and temperature, Plant. Cell. Environ., 14, 779–774, 1991.
Raven, J. A. and Jonston, A. M.: Mechanisms of inorganic-carbon acquisition in marine phytoplankton and their implications for the use of other recourses, Limnol. Oceanogr., 36, 1701–1714, 1991.
Riebesell, U.: Effects of CO_2 enrichment on marine phytoplankton, J. Oceanogr., 60, 719–729, 2004.
Riebesell, U., Wolf-Gladrow, D. A., and Smetacek, V.: Carbon dioxide limitataion of marine phytoplankton growth rates, Nature, 361, 249–251, 1993.
Riebesell, U., Schulz, K. G., Bellerby, R. G. J., Botros, M., Fritsche, P., Meyerhöfer, M., Neil, C., Nondal, G., Oschies, A., Wohlers, J. and Zöllner, E.: Enhanced biological carbon consumption in high CO_2 ocean, Nature, 450, 545–548, doi:10.1038/nature06267, 2007.
Rochelle-Newall, E., Delille, B., Frankignoulle, M., Gattuso, J. P., Jacquet, S., Riebesell, U.,

Terbruggen, A., and Zondervan, I.: Chromophoric dissolved organic matter in experimental mesocosm maintained under different pCO_2 levels, Mar. Ecol. Prog. Ser., 272, 25–31, 2004.
Rost, B., Riebesell, U., Burkhardt, S., and Sültemeyer, D.: Carbon acquisition of bloom-forming, Limnol. Oceanogr., 48, 55–67, 2003.
Sciandra, A., Harley, J., Lefèvre, D., Lemée, R., Rimmelin, P., Denis, M., and Gattuso, J. P.: Response of coccolithophorid Emiliania huxleyi to elevated partial pressure of CO_2 under nitrogen limitation, Mar. Ecol. Prog. Ser., 261, 111–122, 2003.
Schippers, P., Lürling, M., and Scheffer, M.: Increase of atmospheric CO_2 promotes phytoplankton productivity, Ecol. Lett., 446–451, 2004.
Schulz, K. G., Riebesell, U., Bellerby, R., et al.: Built-up and decline of organic matter during PeECE III, Bigeoscienes Discuss., accepted, 2007.
Skattebøl, S.: Coccolitdannelse I lys og I mørke hos Emiliania huxleyi (Lohmann) Hat et Mohler. Laboratorie- og feltundersøkelser, Cand. Scient thesis, University of Oslo, Norway, p. 72, 1995.
Steeman Nielsen, E.: The use of radioactive (^{14}C) for measuring organic production in the sea, J. Cons. Perm. Int. Expl. Mer., 18, 117–140, 1952.
Tanaka, T., Thingstad, T. F., Løvdal, T., Grossart, H.-P., Larsen, A., Schulz, K., and Riebesell, U.: Availability of phosphate for phytoplankton and bacteria and of labile organic carbon for bacteria at different pCO_2 levels in mesocosm study, Biogeoscienes, Discuss., 4, 3937–3960, 2007.
Thingstad, T. F.; Microbial processes and the biological carbon pump, in: Towards a model of ocean biogeochemical processes, edited by: Evans, G. T. and Fasham, M. J. R., NATO ASI Series Vol I 10, Springer Verlag Heidelberg, 193–208, 1993.
Thingstad, T. F., Hagstrom, A., and Rassoulzadegan, F.: Accumulation of degradable DOC in surface waters: Is it caused by a malfunctioning microbial loop?, Limnol. Oceanogr., 42, 398–404, 1997.
Tortell, P. D., Reinfelder, J. R., and Morel, F. M. M.: Active uptake of bicarbonate by diatoms, Nature, 390, 243–244, 1997.
Tortell, P. D., DiTullino, G. R., Sigman, D. M., and Morel, F. M. M.: CO_2 effects on taxonomic composition and nutrient utilization in an Equatorial Pasific phytoplankton assemblage, Mar. Ecol. Prog. Ser., 236, 37–43, 2002.
Winkler, L. W.: Die Bestimmung des in Wasser gelösten Sauerstoffes. Berichte der Deutschen Chemischen Gesellschaft, 21, 2843–2855, 1888.

Zondervan, I., Zeebe, R. E., Rost, B., and Riebesell, U.: Decreasing marine biogenic calsification: a negative feedback on rising atmospheric pCO_2, Global Biogeochem. Cy., 15, 507–516, 2001.

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

	2001	2003	2005
Experimental period	31.05–25.06.	04.05–24.05.	16.05.–10.06.
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}

Table 2. Average primary producton (μmol C L^{-1} h^{-1}), gross production (μmol O_2 L^{-1} d^{-1}) net production (μmol O_2 L^{-1} d^{-1}) and net community respiration (μmol O_2 L^{-1} d^{-1}) for the PeECE II experiment, 2003.

CO_2 treatment	Primary production	Gross production	Net production	Community respiration
	μmol C L^{-1} h^{-1}	μmol O_2 $L^{-1}d^{-1}$	μmol O_2 $L^{-1}d^{-1}$	μmol O_2 $L^{-1}d^{-1}$
Past (190 ppmV)	0.28	18.37	−1.33	5.42
Past (190 ppmV)	0.40	16.02	1.95	4.26
Present (370 ppmV)	0.45	20.30	1.33	4.49
Present (370 ppmV)	0.52	20.77	3.94	3.89
Future (700 ppmV)	0.43	16.89	1.44	3.63
Future (700 ppmV)	0.44	18.41	0.49	4.05

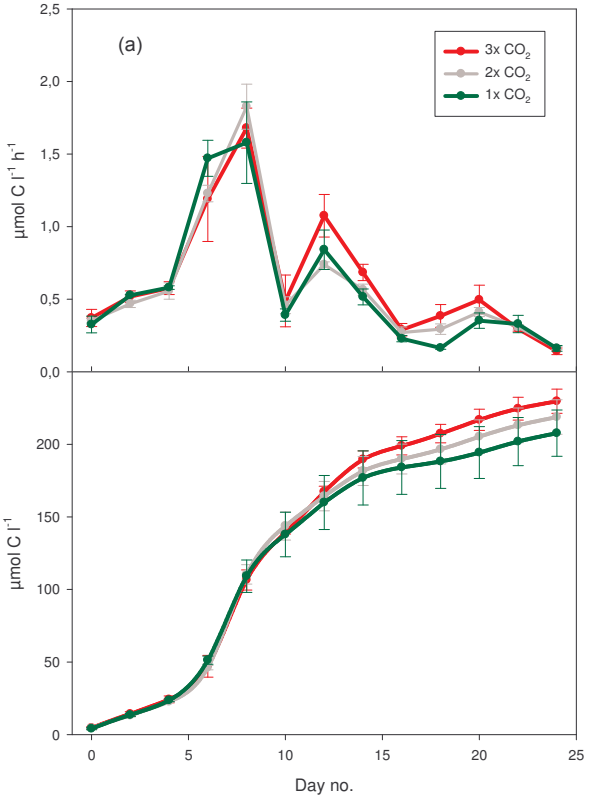

Fig. 1. Development of ^{14}C primary production through PeECE III; daily variation in μmol C l^{-1} h^{-1} **(a)** and cumulative production in μmol C $l^{-1}$for the 24 days experimental period **(b)**. Values are means ±SD of triplicate CO_2 treatments with 1× CO_2 (green), 2× CO_2 (grey) and 3× CO_2 (red).

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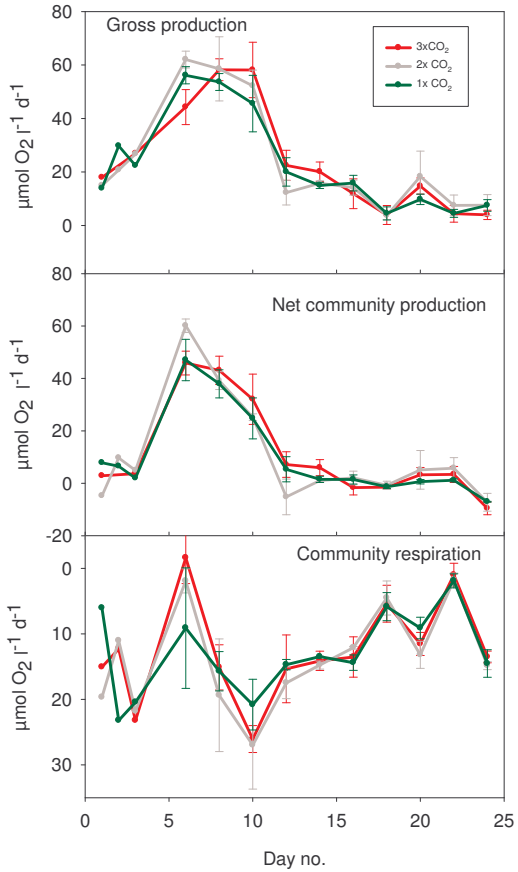

Fig. 2. Gross- and Net community-production and Community respiration given as μmol O_2 L^{-1} d^{-1} based on oxygen incubations. Values are means ±SD of triplicate CO_2 treatments, colour code as in Fig. 1.

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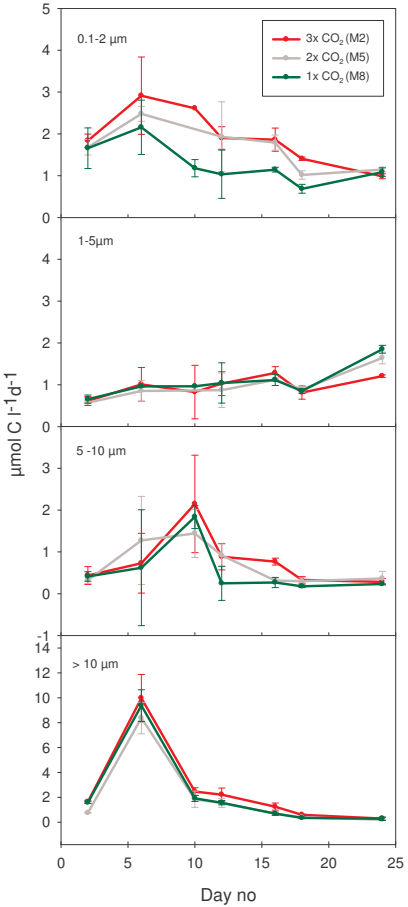

Fig. 3. Average ^{14}C primary production (μmol C l^{-1} d^{-1}), in the fractions 0.2–1, 1–5, 5–10 and >10 μm. One mesocosm of each CO_2 treatment (M2, M5 and M8) is investigated. Values are means±SD of triplicate incubations in each mesocosm, and colour code as in Fig. 1.

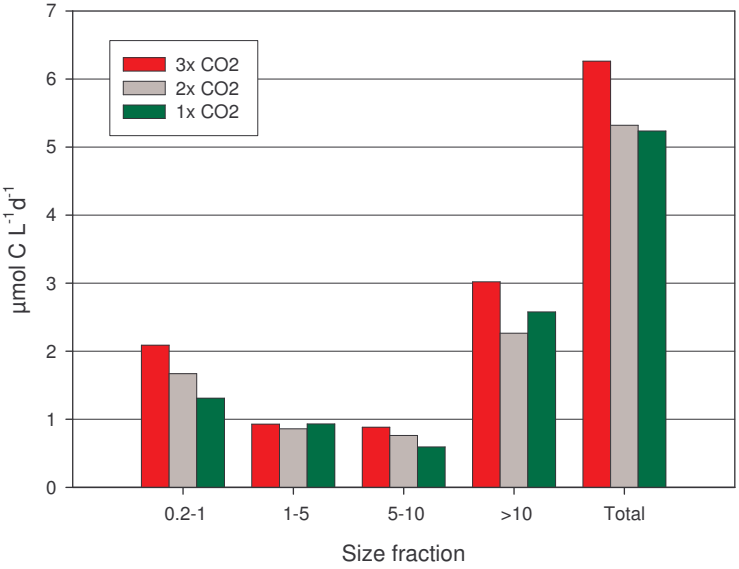

Fig. 4. Average ^{14}C primary production (μmol C L^{-1} d^{-1}) for the 24 days experimental period in the fractions 0.2–1, 1–5, 5–10, >10 μm and total. Colour code as in Fig. 1.

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