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High nitrate to phosphorus ratio attenuates negative effects of rising pCO_2 on net population carbon accumulation

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Abstract

The ongoing rise in atmospheric pCO_2 and the consequent increase in ocean acidification have direct effects on marine calcifying phytoplankton which potentially translates into altered carbon export. To date it remains unclear first, how nutrient ratio, in particular from coccolithophores preferred phosphate limitation, interacts with pCO_2 on particulate carbon accumulation. Second, how direct physiological responses on the cellular level translate into a net population response. In this study cultures of *Emiliania huxleyi* were full-factorially exposed to two different N:P ratios (Redfield and high N:P) and three different pCO_2 levels. Effects on net population particulate inorganic and organic carbon (PIC, POC) were measured after *E. huxleyi* cultures reached stationary phase. Thereby cell sizes and total cell abundance were taken into account. Corresponding to literature results show a significant negative cellular PIC and POC response which, however, was strongest under high N:P ratio. In contrast, net population PIC and POC accumulation was significantly attenuated under high N:P ratio. We suggest that less cellular nutrient accumulation allowed for higher cell abundances which compensated for the strong negative cellular PIC and POC response to pCO_2 on the population level. Moreover, the design of this study also allowed following natural alteration of carbon chemistry through changing DIC and alkalinity. Our results suggest that at high initial pCO_2 natural alteration of pCO_2 during the experimental runtime was regulated by algal biomass. In contrast, at low initial pCO_2 the PIC/POC ratio was responsible for changes in pCO_2.

Our results point to the fact that the physiological (i.e. cellular) PIC and POC response to ocean acidification cannot be linearly extrapolated to total population response and thus carbon export. It is therefore recommended to consider effects of nutrient limitation on cell physiology and translate these to net population carbon accumulation when predicting the influence of coccolithophores on both, the atmospheric pCO_2 feedback and their function in carbon export mechanisms.

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1 Introduction

At present earth faces an atmospheric CO_2 partial pressure of 398 µatm which already is approximately 100 µatm higher as at preindustrial conditions. This fraction, however, would be considerably larger if the surface oceans had not absorbed approximately 50 % of previous fossil fuel emissions (Sabine et al., 2004). This leads to an attenuation of global warming on the one hand but causes the effect known as ocean acidification on the other hand (Caldeira and Wickett, 2003). The ongoing increase in atmospheric pCO_2 results in decreasing surface ocean pH and $[CO_3^{2-}]$ and increasing $[HCO_3^-]$- and CO_2-concentrations. These variations in ocean carbonate chemistry have direct implications on physiological processes, like photosynthesis and calcification of many organisms (Turley et al., 2010). Especially calcifiers at the surface ocean such as coccolithophores, foraminifera and pteropods are threatened by malformation and/or dissolution (Fabry, 2008). Since about half of the pelagic calcification is accomplished by coccolithophores (Broecker and Clark, 2009) and the sinking of their calcareous coccoliths might play a crucial role in carbon export mechanisms (Klaas and Archer, 2002) the physiological response of coccolithophores to ocean acidification is of special interest. Therefore, coccolithophores are among the best examined organisms with respect to their response to ocean acidification. These mainly negative responses in calcification and photosynthesis of various coccolithophore species and species strains, however, were mostly measured per unit cell in the exponential growth phase and under constant carbonate specifications during the experimental runtime (e.g. Riebesell et al., 2000; Zondervan et al., 2001, 2002; Langer et al., 2006, 2009; Shi et al., 2009; Krug et al., 2011).

Research progress on the physiology of pelagic calcifiers caused by ocean acidification cannot be imagined without the previously mentioned studies. However, they do not consider three irrevocable points for drawing conclusions on the consequences of ocean acidification on net population carbon accumulation. Therefore, current extrapolations on the global carbon cycle are presumably overlooking important information.

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These three points being: (1) Variations in the cellular carbon content caused by nutrient limitations (Paasche, 1998; Riegman et al., 2000). (2) The number of cells in a population. Only the consideration of change in cell size under nutrient limitation, i.e. the increase in cell size under phosphate limitation (Riegman et al., 2000; Müller et al., 2008) and the decrease in cell size under nitrate limitation (Riegman et al., 2000; Sciandra et al., 2003; Müller et al., 2008), together with potentially changing number of cells in a population allows to draw conclusions on the implications of ocean acidification on net particulate carbon accumulation. But rather than nitrogen limitation, which is in general referred as to be the limiting resource of phytoplankton (Falkowski, 1997), the effects of phosphate limitation have to be considered as the prevailing limitation factor for *Emiliania huxleyi* (Egge and Heimdal, 1994; Tyrrell and Taylor, 1996). (3) Natural changes in the carbonate system by photosynthesis and calcification (Taylor et al., 1992; Robertson et al., 1993; Purdie and Finch, 1994). Thereby, photosynthesis decreases pCO_2 by consuming dissolved inorganic carbon (DIC) and calcification increases pCO_2 by reducing total alkalinity (TA).

In this study we experimentally set out to test whether the effect of different initial CO_2 concentrations on net population carbon accumulation of *Emiliania huxleyi* is dependent on the degree of phosphate limitation. This hypothesis was tested by following population growth to the natural depletion of phosphate and monitoring the natural change in carbonate specification by the consumption of DIC and the reduction of TA. To the best of our knowledge this study allows for the first time to draw conclusions about effects of ocean acidification on net population carbon accumulation of single coccolithophore species.

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2 Methods

2.1 Experimental design

A batch culture experiment was designed in order to analyse the influence of nutrient limitation and pCO_2 and their interactionon net calcification population response of *Emiliania huxleyi*. Cultures were exposed to three different pCO_2-levels and two different NO_3^- to PO_4^{3-} ratios which resulted in a fully crossed two-factorial design. Each treatment was replicated four times, resulting in 24 experimental units.

The experiment took place in 2000 ml polycarbonate bottles randomly distributed across four climate cabinets. Before the onset of the experiment, 200 cells ml^{-1} were transferred into each experimental unit. Cells were acclimated to the respective experimental pCO_2 levels (see below), temperature (16 °C) and light (130 µmol photons $m^{-2}\,s^{-1}$ and 14h/10h light/dark cycle) conditions for six to eight generations. In order to limit sedimentation during the acclimation process and the subsequent experiment, bottles were carefully rotated three times a day each time with 15 rotations. The duration of the experiment was determined by the species capability to use up nutrients and reach the stationary phase. After being three days in the stationary phase cultures were sampled and prepared for analysis.

2.2 Treatments and medium preparation

E. huxleyi cells were freshly isolated from waters originated from the island Terceira (Azores, North Atlantic, 38°39′22″ N 27°14′08″ W) and have been in culture for not longer than five month.

Trace metals and vitamins according to a tenth of a common f/2-medium (Guillard, 1975) were added to 100 l of 1.4 µm pre-filtrated North Sea Water with a salinity of 32 psu. By the addition of Na_2CO_3, total alkalinity was elevated to 2700 µmol kg^{-1} to abate expected variations in the carbonate specification. After 0.2 µm sterile filtration three different CO_2 levels were established by aeration with enriched air, according to

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pCO_2 of 460, 1046 and 1280 µatm, respectively (Table 1).

Within each level of pCO_2 two different nutrient ratios were established. This led to initial nutrient concentrations of 17.3 µmol N kg^{-1}: 0.23 µmol P kg^{-1} ("high N:P") and 8.9 µmol N kg^{-1}: 0.54 µmol P kg^{-1} ("Redfield") (Table 2).

2.3 Sampling and response variables

At the end of the experiment samples were taken (Whatman GF/F filters 25 mm Ø) in order to determine the content of particulate organic nitrogen (PON), total particulate carbon (TPC) and particulate organic carbon (POC) For the latter, the particulate inorganic carbon (PIC) was removed by exposing filters containing TPC to fuming hydrochloric acid for 2 h. All filters were dried at 60 °C and analysed in an elemental analyser with a heat conductivity detector (Thermo Flash, 2000) according to Sharp (1974). The PIC content was calculated by the subtraction of POC from TPC. In order to determine the particulate carbon content per cell, the concentration per litre was divided by the cell abundance. The divisions of PIC by POC and POC by PON resulted in the PIC/POC- and C/N – ratio, respectively.

Cell abundance was measured every day with a Z2™ COULTER COUNTER® cell and particle counter. The decision to terminate a culture was based on the statistical significant fit to the growth model

$$n_t = a / \left(1 + \left((a-b)/b\right) \times e^{(-\mu \times t)}\right) \quad (1)$$

with n_t indicating the cell number after t days, a the maximum cell abundance, b the start cell number and µ the growth rate. The first day, the growth curve of a culture significantly fitted to the model, i.e. reached the stationary phase, was defined as the first of three days in the stationary phase, after which the cultures were terminated.

The growth rate was calculated by

$$\mu = \frac{\ln(n_2) - \ln(_1)}{t_2 - t_1} \quad (2)$$

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with n indicating the cell abundance and t the time from one day to the other. The presented values correspond to the mean of μ during the exponential phase. Additionally, cell size was measured with the Z2™ COULTER COUNTER®. The resulting diameter was used to calculate the biovolume following Hillebrand et al. (1999). Population biovolume was determined by multiplying cell abundance with cell biovolume. Additional samples for PO_4^{3-} and NO_3^{2-} were taken and filtered through GF/F filters. The filtrates were frozen in polyethylene bottles. Duplicate samples from each bottle were analysed colometrically with an accuracy of ±0.1 μmol (Hansen and Koroleff, 1999).

Samples for DIC and total alkalinity were taken at the beginning and at the end of the experiment. The DIC measurements were carried out photometrically in a Bran & Lübbe QUAATRO analyzer equipped with a XY-2 sampling unit (Stoll et al., 2001). For TA determination by potentiometric titration, duplicate samples (25 ml) were filtered (Whatman GF/F filters 0.2 μm) and titrated at 20 °C in an automated titration device (Metrohm Basic Titrino 794) with 0.05 M HCl-solution (Dickson, 1981; Dickson et al., 2003) and a precision of ±3 μmol kg^{-1}. Certified reference material (University of California (San Diego), Marine Physical Laboratory, A. G. Dickson) was used as a standard and measured every day before and after measuring the samples. The corresponding partial pressure of CO_2 and the residual parameters of the carbonate system were calculated with the equilibrium constants for carbonic acid by Mehrbach et al. (1973) refitted by Dickson and Millero (1987).

2.4 Statistics

Prior to statistical analyses data were tested for normality and homogeneity of variances. If data were not normally distributed and / or variances were not homogeneous data were log- or square root transformed. Addressing our hypothesis, effects of pCO_2, nutrient ratio and their interactions on POC, PIC and PON content per cell as well as per litre, on cell size, on growth rate (μ), on total biovolume and on the ratios of PIC and POC as well as of POC and PON were tested by calculating a General Linear Model (GLM). Nutrient ratio was used as a categorical and pCO_2 as a continuous factor.

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Due to significant interactions between the factors nutrient ratio and pCO_2 separate regression analyses with pCO_2 as predictor were conducted for each nutrient ratio for each response variable. In order to test for alterations of the initially manipulated pCO_2 over the course of the experiment t-tests between initial and end pCO_2 values were calculated. In order to test if ΔpCO_2 depends on TPC or on PIC/POC ratio multiple regressions with TPC and PIC/POC ratio and their interaction as factors were used to select the most important drivers within each level of pCO_2. Please note, that due to problems during the sampling procedure we omitted two replicates(460 µatm at "high N:P" and 1280 µatm at Redfield Ratio).

3 Results

3.1 Particulate matter

Particulate inorganic and organic carbon (PIC and POC) and organic nitrogen (PON) on the cellular as well as on the population level were significantly affected by the manipulated factors pCO_2 and nutrient ratio (Tables 3, 4). Overall PIC and POC content significantly decreased on both the cellular and the population level in response to increasing pCO_2 (Table 3, Figure 1a–d). In general cellular PON content significantly increased with pCO_2 (Table 3, Fig. 1e). Overall net population PON was not affected by pCO_2 (Table 3, Fig. 1f). Also nutrient ratio significantly affected POC, PIC and PON content on the cellular level (Table 3). For all three response variables cellular content was significantly higher for the "High N:P" treatments (Table 3, Fig. 1a, c, e). On the population level only PIC and POC but not PON was significantly affected by nutrient ratio. In contrast to the cellular content on the population level the "Redfield" treatments were significantly higher (Table 3, Fig. 1b, d, f).

In general the response of cellular PIC content to increasing pCO_2 significantly depended on the nutrient ratio. The negative response to pCO_2 in the "High N:P" treatment was more pronounced compared to Redfield conditions (Table 3, see significant

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interaction between pCO_2 and nutrient ratio, Table 4, Fig. 1a). Vice versa, on the population level the negative response to pCO_2 was significantly stronger for the Redfield treatments (Tables 3, 4, Fig. 1b). The dependence of the pCO_2 response on nutrient ratio occurred also for the POC fraction. As for PIC content the regression slope for the cellular POC content was significantly steeper in the "High N:P" treatment (Tables 3, 4, Fig. 1c). Vice versa on the population level only the Redfield cultures significantly decreased in response to pCO_2 whereas cultures growing under "high N:P" conditions were not affected by pCO_2 (Table 4, Fig. 1d). Although there was no significant interaction between pCO_2 and nutrient ratio (Table 3), the cellular PON responses revealed opposite trends for the two nutrient treatments. Thereby, cells growing under "High N:P" conditions significantly decreased their PON content in response to pCO_2, whereas cell growing under Redfield conditions increased in PON content (Table 4, Fig. 1e). Accordingly net population PON significantly increased only under Redfield conditions (Table 4, Fig. 1f).

Overall C/N ratio significantly decreased with pCO_2 and was not affected by nutrient ratio (Table 3). Despite the absence of a significant interaction term between pCO_2 and nutrient ratio C:N ratio significantly decreased only under Redfield conditions and showed no response under "high N:P"-ratio (Tables 3, 4, Fig. 2a).

For the PIC/POC ratio the general linear model did not reveal any significant main effects by the manipulated factors (Table 3). Nonetheless, separated regression analyses for Redfield and high N:P treatments with pCO_2 as predictor revealed a decrease in the PIC/POC ratio for both nutrient ratios (Table 4, Fig. 2b).

3.2 Cell growth, size and total population biovolume

Despite for growth rate the factorial model did not reveal any significant main or interaction effects of the manipulated factors (Table 3), separate regression analyses show that growth rate significantly increased with pCO_2 only in the "High N:P" scenario Treatments facing Redfield conditions were not affected by pCO_2 (Table 4, Fig. 3).

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All cells increased in size in the stationary phase compared to exponential phase (Fig. 4). For both nutrient treatments this increase (i.e. delta cell size), however, was significantly lower with increasing pCO_2(Tables 3, 4, Fig. 4). Also nutrient ratio significantly influenced increase in cell size in all cases and led to stronger increase at the "High N:P" treatments (Table 3, Fig. 4). Also final population biovolume was significantly influenced by the manipulated factors and their interaction (Table 3). pCO_2 significantly reduced final population biovolume for the "Redfield" treatments but not for the "High N:P" treatments (Table 4, Fig. 3).

3.3 Natural pCO_2 alterations

Depending on the start pCO_2 conditions the ΔpCO_2 of the medium was altered over the course of the experiment to different extent (Fig. 5). While both nutrient treatments with a start pCO_2 of 460 µatm faced no change ("Redfield": $t = -0.27; p = 0.8$ "High N:P": $t = -1.57; p = 0.26$) all higher initial pCO_2 treatments of both nutrient ratios reduced pCO_2 during the experiment, i.e. led to higher ΔpCO_2 ($t \geq 3.84$; $p \leq 0.03$).

4 Discussion

Although, nitrogen is in general referred to be the limiting resource for phytoplankton growth (Falkowski, 1997), *E. huxleyi* preferably grow and bloom in parts of the ocean with relative high N:P ratios (Egge and Heimdal, 1994; Tyrrell and Taylor, 1996). Especially, under consideration of future extent of p-limited areas (Ammerman et al., 2003), simulating two different phosphate limitation scenarios provide complementary results to earlier ocean acidification studies on coccolithophores (Riebesell et al., 2000; Zondervan et al., 2001, 2002; Sciandra et al., 2003; Langer et al., 2006; 2009; Shi et al., 2009; Krug et al., 2011). To the best of our knowledge, this study shows for the first time different sensitivities to ocean acidification caused by different nutrient ratios for net population carbon accumulation of calcifying phytoplankton (*E. huxleyi*). The

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here presented data reveal that the decline of net population calcification in response to pCO_2 is significantly attenuated at high N:P ratio because higher nutrient use efficiency at high N:P led to higher total cell abundance which in turn compensated for a decline in net population carbon accumulation.

4.1 Interaction between pCO_2 and N:P ratio

Our results show less cellular PON at high N:P ratio and thus suggest a change in cells nutrient composition, i.e. a decrease in both cellular nitrogen and phosphorous. Since only inorganic phosphate was depleted and nitrogen was still available, the results reveal that not increased cellular PON but rather particulate organic phosphate (POP) is likely to cause higher cell abundance at high N:P ratio. Higher resource use efficiency in terms of the limiting resource phosphorous, thus, is likely to be the reason for higher cell abundance. Higher cell abundance in the high N:P treatments in turn compensated for the negative effect of ocean acidification on net population POC, PIC and PON accumulation and decreasing total biovolume in relation to the Redfield treatments. Vice versa in the Redfield treatments decreasing cell volume together with the drop in cell abundance explains the amplified reduction in total biovolume, resulting in a strong decline in total POC and PIC accumulation.

In general the response of cellular POC and PIC accumulation to rising pCO_2 in this study confirmed results of earlier experiments (Langer et al., 2009). Likewise, the cellular responses to phosphate limitation in general coincide with previous studies. Caused by an oversupply of nitrogen and the production of biomass without the possibility to divide, cells exposed to phosphate limitation grew considerably larger in volume compared to their size in the exponential phase (Riegman et al., 2000; Müller et al., 2008). In this study, cells growing under Redfield ratio, however, depleted both nutrient sources which limited cell growth. In contrast, cells facing high N:P ratio had an oversupply of nitrogen and thus, got significant larger in volume. As a consequences of decreasing cellular PIC and POC the increase in cell size caused by phosphate limitation is attenuated by rising pCO_2. Thereby, it is not clear if pCO_2 decreases the

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maximum cell size or just the cell size growth rate. It cannot fully be excluded that cells of all pCO_2 treatments would reach the same size after being in the stationary phase for longer time. Growth rate, however, is not changing or even increasing with pCO_2. This makes a constant cell size growth rate and a changing maximum cell size more likely.

We are aware that nutrient concentration, i.e. higher phosphate concentrations in the Redfield treatment potentially confounds with nutrient ratio. For conclusion on the cellular level the absolute concentrations were insignificant, the nutrient ratio alone caused the observed differences in response to pCO_2. The absolute concentrations accounted for the significant differences in particulate matter between the two nutrient ratio treatments on a population level. However, for our conclusion only the slopes and not absolute carbon accumulation in response to pCO_2 were compared.

4.2 Natural variations in pCO_2

Loss of DIC by primary production of phytoplankton blooms leads to a decrease in pCO_2 which cannot immediately be balanced by atmospheric CO_2 (Taylor et al., 1992; Robertson et al., 1993; Purdie and Finch, 1994). Reduction of TA by calcification of coccolithophores in turn leads to an increase in pCO_2 and therewith counteracts the reduction of pCO_2 by photosynthesis (Purdie and Finch, 1994). Thus, the ratio of calcification and photosynthesis, approximately given by the PIC/POC ratio, determines whether pCO_2 in a bloom of coccolithophores is changing. Comparison of the different treatments at 460 and 1046 µatm pCO_2 (Fig. 5) shows that an insignificant difference in ΔpCO_2 is attended by an insignificant change in PIC/POC ratio and a significant change in TPC. At 1280 µatm pCO_2, however, a significant change in ΔpCO_2 is attended by a significant change in TPC ratio and an insignificant change in PIC/POC. This visualisation indicates that the ratio of PIC and POC balances variations in the pCO_2 at lower start pCO_2 values and thus the amount of total biomass is insignificant. At higher pCO_2 starting conditions the decrease in calcification is not able to compensate for changes caused by POC accumulation. Therewith, total biomass is

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the leading factor for pCO_2variations at higher pCO_2 conditions This effect is accelerated by a decreasing buffer capacity, i.e. increasing Revelle factor in the future oceans (for further reading: Revelle and Suess, 1957; Broecker et al., 1979; Egleston et al., 2010). Thereby, primary production will cause larger fluctuations in the surface pCO_2 (Riebesell et al., 2007). With rising atmospheric pCO_2 the reduction of DIC by photosynthesis leads to a stronger decline in the aqueous CO_2 concentration as the same level of calcification would lead to an increase.

5 Conclusion

The attenuated effect of ocean acidification on accumulation of total particulate matter by high N:P nutrient ratio points out that responses of cellular POC and PIC cannot easily be extrapolated to the population level. Considering these results, estimations on the future carbon cycle should not only account for pCO_2 and the response of coccolithophores to ocean acidification in exponential growth but also for the effects of future conditions on the carrying capacity of a population. Although the function of calcite as ballast for global ocean carbon export is not completely clear, for future predictions on carbon export in an acidified ocean our study reveals the necessity to consider nutrient ratios and their capability to attenuate negative responses of net population calcite accumulation.

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Table 1. Carbonate specifications at the beginning of the experiment. pCO_2 is given in µatm, all other parameters (with exception of pH and Ω) are given in µmol kg^{-1}.

pCO_2	TA	DIC	pH	$[CO_2]$	$[HCO_3^-]$	$[CO_3^{2-}]$	Ω
459 ± 7	2700 ± 2	2447 ± 3	8.15 ± 0.01	17 ± 0.3	2233 ± 5	197 ± 2	4.80
1046 ± 7	2700 ± 2	2595 ± 1	7.84 ± 0.00	39 ± 0.3	2452 ± 1	104 ± 1	2.54
1283 ± 18	2700 ± 2	2627 ± 2	7.75 ± 0.01	47 ± 0.7	2491 ± 3	88 ± 1	2.14

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Table 2. Start and end concentrations of nitrate, ammonium, their sum, phosphate and the ratio of total N at three different pCO_2. At the end the concentrations of ammonia and phosphate were always below detection limit (b.d.). Concentrations are given in µmol kg^{-1}.

		Start concentration					End conc.	
Ratio	pCO_2	NO_3^-	NH_4^+	$\sum$ N	PO_4^{3-}	N/P	NO_3^-	PO_4^{3-}
„Redfield“	460	3.88 ± 0.08	4.8 ± 0.2	8.68	0.5 ± 03	17	0.06	b.d.
	1046	3.88 ± 0.08	4.8 ± 0.2	8.68	0.5 ± 03	17	0.08	b.d.
	1280	3.86 ± 0.08	4.8 ± 0.2	8.66	0.5 ± 03	17	0.12	b.d.
„High N:P“	460	12.41 ± 0.20	4.8 ± 0.2	17.21	0.2 ± 01	75	7.98	b.d.
	1046	12.49 ± 0.23	4.8 ± 0.2	17.29	0.2 ± 03	75	8.23	b.d.
	1280	12.49 ± 0.23	4.8 ± 0.2	17.29	0.2 ± 01	75	10.29	b.d.

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Table 3. Results of a two factorial general linear model for the response variables POC, PIC and PON per cell and per litre, PIC/POC ratio, C/N ratio, growth rate, Δ size and total biovolume.

	Factor	df	R^2	Slope	MS	F	p		df	R^2	Slope	MS	F	p
sqrt POC cell^{-1}	Whole model	3,18	0.95		0.001	123	<0.001	POC litre^{-1}	3,18	0.94		190	107	<0.001
	Nutrients	1,18			0.066	95.0	<0.001		1,18			12468	65.6	<0.001
	pCO_2	1,18		-210^{-4}	0.095	136	<0.001		1,18		−0.05	6572	34.6	<0.001
	Inter.	1,18			0.02	21.6	<0.001		1,18			1585	8.3	<0.01
PIC cell^{-1}	Whole model	3,18	0.95		0.01	137	<0.001	PIC litre^{-1}	3,18	0.93		480	99	<0.001
	Nutrients	1,18			0.86	95	<0.001		1,18			28153	58.7	<0.001
	pCO_2	1,18		−0.001	2.62	291	<0.001		1,18		−0.13	41764	87.1	<0.001
	Inter.	1,18			0.36	40	<0.001		1,18			5943	12.4	<0.001
PON cell^{-1}	Whole model	3,18	0.95		2.04	139	<0.001	sqrt PON litre^{-1}	3,18	0.01		161511	1.17	0.33
	Nutrients	1,18			65.2	32	<0.001		1,18			11443	0.07	0.79
	pCO_2	1,18		0.01	183	90	<0.001		1,18		−0.12	77452	0.48	0.49
	Inter.	1,18			0.02	0.01	0.93		1,18			119043	0.74	0.40
Sqrt PIC/POC	Whole model	3,18	−0.01		122413	0.85	0.47	C/N ratio	3,18	0.2		115	4.4	<0.01
	Nutrients	1,18			202092	1.65	0.21		1,18			105	0.92	0.34
	pCO_2	1,18		−0.086	3565	0.29	0.59		1,18		−0.01	517	4.51	0.04
	Inter.	1,18			103255	0.84	0.36		1,18			0.65	0.006	0.94
μ	Whole model	3,18	0.10		0.009	1.77	0.12	Δ Size	3,18	0.93		24.4	94	<0.001
	Nutrients	1,18			0.04	4.1	0.06		1,18			1159	47.5	<0.001
	pCO_2	1,18		710^{-5}	0.01	1.3	0.26		1,18		−0.04	3129	128	<0.001
	Inter.	1,18			0.04	4.1	0.06		1,18			151	6.2	0.02
Total biovolume	Whole model	3,18	0.93		3×10^{-6}	95	<0.001							
	Nutrients	1,18			2×10^{-4}	77	<0.001							
	pCO_2	1,18		-710^{-6}	10^{-4}	38	<0.001							
	Inter.	1,18			5×10^{-5}	17.5	<0.001							

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Table 4. Results of regression analyses with pCO_2 as predictor on the response variables POC, PIC and PON per cell and per litre, PIC/POC ratio, C/N ratio, growth rate (μ), Δ size and total biovolume.

	Factor	R^2	$F_{1,10}$	p	Slope	SE		R^2	$F_{1,10}$	p	Slope	SE
POC cell^{-1}	*Redfield*	0.81	42.3	<0.001	-10^{-4}	2×10^{-5}	POC litre^{-1}	0.9	95	<0.001	−0.08	0.008
	High N:P	0.9	92	<0.001	-3×10^{-4}	3×10^{-5}		0.15	2.8	0.13	−0.03	0.02
POC cell^{-1}	*Redfield*	0.97	137	<0.001	-6×10^{-4}	6×10^{-5}	PIC litre^{-1}	0.95	174	<0.001	−0.18	0.014
	High N:P	0.94	168	<0.001	−0.001	10^{-1}		0.49	10.7	<0.01	−0.08	0.02
PON cell^{-1}	*Redfield*	0.77	34.2	<0.001	9×10^{-6}	2×10^{-6}	PON litre^{-1}	0.41	7.82	0.02	0.001	4×10^{-4}
	High N:P	0.46	9.5	0.01	-3×10^{-5}	10^{-5}		0.06	1.64	> 0.2	-9×10^{-4}	7×10^{-4}
PIC/POC	*Redfield*	0.93	143	<0.001	-4×10^{-4}	3×10^{-5}	C/N	0.93	130	<0.001	−0.01	9×10^{-4}
	High N:P	0.71	26	<0.001	-5×10^{-4}	9×10^{-5}		0.05	1.5	> 0.2	−0.002	0.001
μ	*Redfield*	−0.07	0.32	0.58	-5×10^{-5}	5×10^{-9}	Δ Size	0.89	82	<0.001	−0.03	0.003
	High N:P	0.35	6.4	0.03	2×10^{-4}	8×10^{-5}		0.86	61	<0.001	−0.04	0.006
biovolume	*Redfield*	0.93	135	<0.001	-10^{-5}	10^{-6}						
	High N:P	0.02	6×10^{-6}	0.31	-2×10^{-6}	6×10^{-6}						

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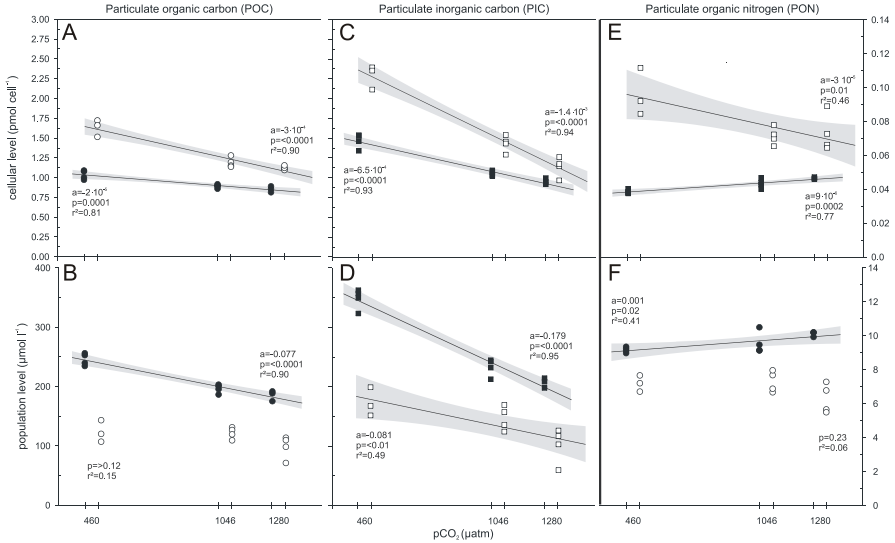

Fig. 1. POC **(A, B)**, PIC **(C, D)** and PON **(E, F)** content per cell **(A, C, E)** and per litre **(B, D, F)**. Closed and open symbols indicate "Redfield" treatments and "Higher N:P" treatments, respectively. Lines reflect the regression with *a* being the slope and r^2 the adjusted coefficient of determination. The gray shading reflects the 95 % confidence interval with *p* being the significance.

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High nitrate to phosphorus ratio attenuates negative effects

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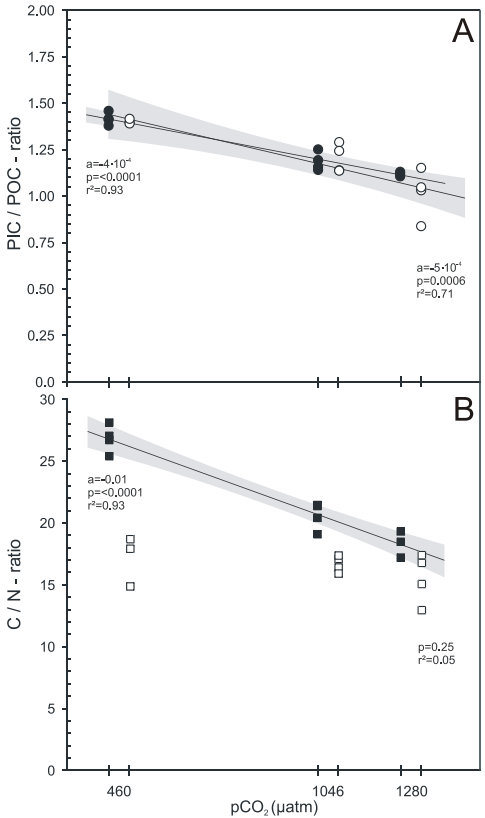

Fig. 2. Ratio of calcification to photosynthesis **(A)** and the ratio of carbon to nitrogen content in the cells **(B)**. Closed and open symbols indicate "Redfield" treatments and "Higher N:P" treatments, respectively. Lines reflect the regression with *a* being the slope and r^2 the adjusted coefficient of determination. The gray shading reflects the 95 % confidence interval with *p* being the significance.

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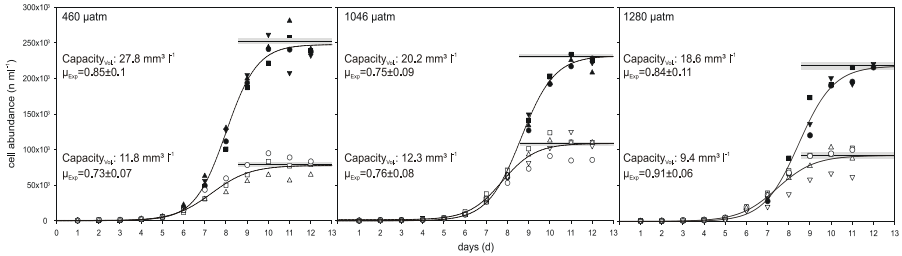

Fig. 3. Cell abundance at three different pCO_2 level. Closed symbols Indicate the four replicates of the Redfield ratio, open symbols that of the high N:P ratio. The total carrying capacity is given as the biovolume in µm^3 per liter. The growth rate (µ) is calculated only for the exponential phase.

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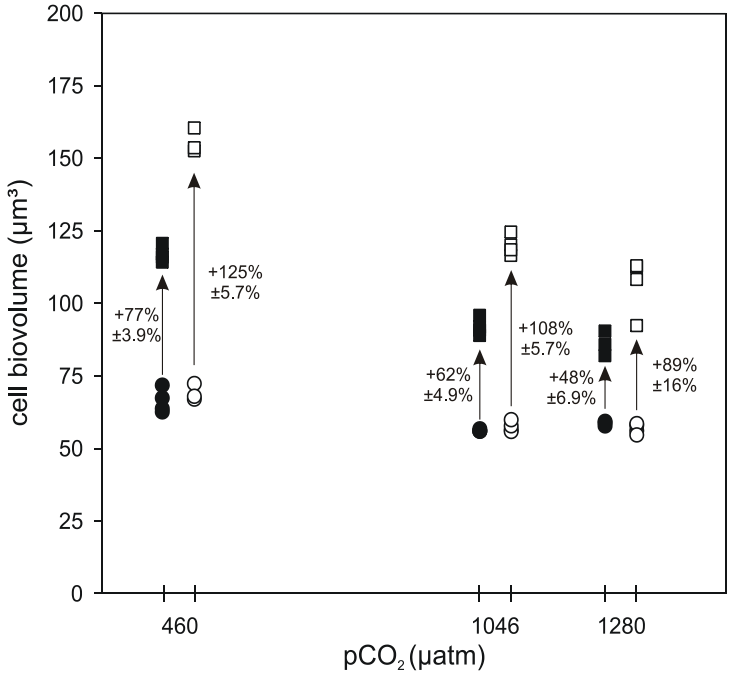

Fig. 4. Single cell biovolume at three different pCO_2 level. Circles indicate the cell volume in the exponential phase, squares the volume in the stationary phase. Closed and open symbols indicate "Redfield" treatments and "Higher N:P" treatments, respectively. Numbers express the percental increase plus/minus the standard deviation.

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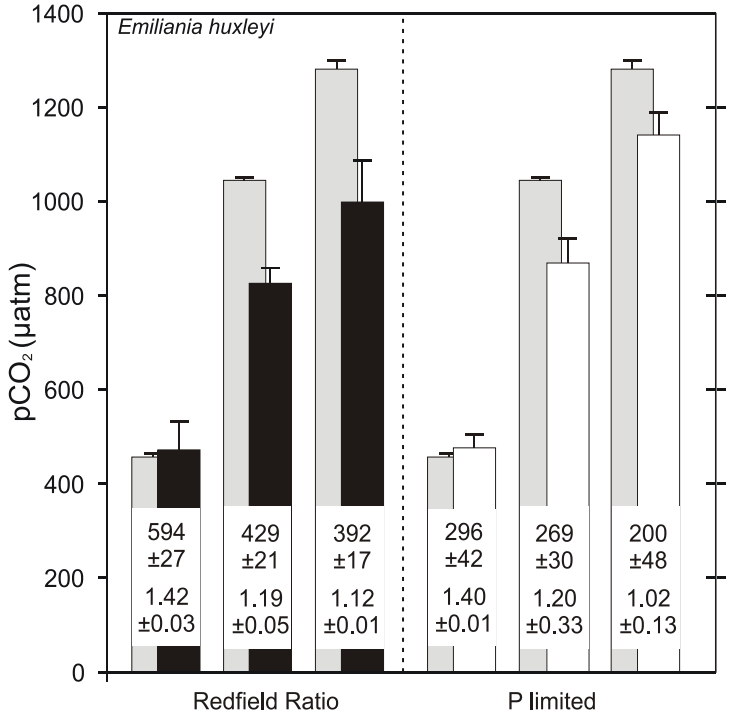

Fig. 5. Start pCO_2 values of the experiment (gray bars) and the associated end values for the Redfield ratio (black) treatments and for those treatments facing a high N:P ratio (white). Written values reflect the TPC in µmol per litre and the PIC/POC ratio, both with its standard deviation.

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