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High nitrate to phosphorus regime attenuates negative effects of rising pCO_2 on total population carbon accumulation

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Abstract. The ongoing rise in atmospheric pCO_2 and consequent increase in ocean acidification have direct effects on marine calcifying phytoplankton, which potentially alters carbon export. To date it remains unclear, firstly, how nutrient regime, in particular by coccolithophores preferred phosphate limitation, interacts with pCO_2 on particulate carbon accumulation; secondly, how direct physiological responses on the cellular level translate into total population response. In this study, cultures of *Emiliania huxleyi* were full-factorially exposed to two different N:P regimes and three different pCO_2 levels. Cellular biovolume and PIC and POC content significantly declined in response to pCO_2 in both nutrient regimes. Cellular PON content significantly increased in the Redfield treatment and decreased in the high N:P regime. Cell abundance significantly declined in the Redfield and remained constant in the high N:P regime. We hypothesise that in the high N:P regime severe phosphorous limitation could be compensated either by reduced inorganic phosphorous demand and/or by enzymatic uptake of organic phosphorous. In the Redfield regime we suggest that enzymatic phosphorous uptake to supplement enhanced phosphorous demand with pCO_2 was not possible and thus cell abundance declined. These hypothesised different physiological responses of *E. huxleyi* among the nutrient regimes significantly altered population carrying capacities along the pCO_2 gradient. This ultimately led to the attenuated total population response in POC and PIC content and biovolume to increased pCO_2 in the high N:P regime. 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 necessary to consider both effects of nutrient limitation on cell physiology and their consequences for population size when predicting the influence of coccolithophores on atmospheric pCO_2 feedback and their function in carbon export mechanisms.

1 Introduction

At present, earth faces an atmospheric CO_2 partial pressure of 398 µatm, which is approximately 100 µatm higher than pre-industrial 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 (Caldeira and Wickett, 2003). The ongoing increase in atmospheric pCO_2 results in decreasing surface ocean pH and CO_3^{2-} concentration and increasing HCO_3^- – and CO_2-concentrations. These variations in ocean carbonate chemistry have direct implications on physiological processes, such as photosynthesis and calcification of many organisms (Turley et al., 2010). Calcifiers in the surface ocean such as coccolithophores, foraminifera and pteropods, are particularly 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. As a result, coccolithophores are among the best examined organisms with respect to their response to ocean acidification. However, these mainly negative responses in calcification and photosynthesis of various coccolithophore species and species strains were usually measured per unit cell in the exponential growth phase (e.g. Riebesell et al., 2000; Zondervan et al., 2001, 2002; Langer et al., 2006, 2009; Shi et al., 2009; Krug et al., 2011).

Progress in research into the effects of ocean acidification on pelagic calcifier physiology could not have occurred without the previously mentioned studies. However, exclusive physiological studies do not consider two major points

necessary for drawing conclusions on the consequences of ocean acidification on total population carbon accumulation and draw down. These two points are: (1) variations in the cellular carbon content caused by nutrient limitation (Paasche, 1998; Riegman et al., 2000), and (2) physiological responses cannot directly be up scaled to the ecological level, as this requires taking the total number of cells in a population into account. Only the considerations of potential change in cell abundance accompanied by potential change in cell size under nutrient limitation allow conclusions to be drawn on the implications of ocean acidification on net particulate carbon accumulation. Besides total cell abundance, the increase in individual cell size under phosphorous 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) potentially determines total population carbon accumulation. However, rather than nitrogen limitation, which is in general referred to as the limiting resource of phytoplankton (Falkowski, 1997), the effects of phosphorous limitation have to be considered as the prevailing limiting factor for *Emiliania huxleyi* (Egge and Heimdal, 1994; Tyrrell and Taylor, 1996).

In this study we set out to test the hypothesis that the effect of different initial CO_2 concentrations on total population carbon accumulation of *E. huxleyi* depends on nutrient regime, in particular on the degree of phosphorous limitation. CO_2 concentrations were manipulated initially, instead of continuous supply of the corresponding pCO_2 to match natural conditions, where loss of DIC through primary production cannot be immediately balanced by atmospheric exchange. The hypothesis was tested by following population growth relative to the natural depletion of inorganic phosphate. To the best of our knowledge this study allows for conclusions to be drawn for the first time about effects of ocean acidification on total population carbon accumulation of single coccolithophore species in different nutrient regimes.

2 Methods

2.1 Experimental design

The strain of *E. huxleyi* originated from waters off Terceira Island (Azores, North Atlantic, 38°39′22″ N 27°14′08″ W) and prior to the experiment had been in culture for no longer than five months. *E. huxleyi* cultures were full-factorially exposed to three different pCO_2 levels and two different nitrogen to phosphorus regimes. Each treatment was replicated fourfold, resulting in 24 experimental units that comprised of 2 L polycarbonate bottles randomly distributed across four climate cabinets. At the onset of the experiment, 200 cells ml^{-1} were transferred into each experimental unit. Prior to the experiment, 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 14 h/10 h 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 three days of being in the stationary phase, cultures were terminated and samples prepared for analysis.

2.2 Treatments and medium preparation

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-filtered North Sea Water with a salinity of 32. Total alkalinity was elevated to 2700 µmol kg^{-1} by the addition of Na_2CO_3, to abate expected variations in the carbonate specification. After 0.2 µm sterile filtration, three different CO_2 levels were established by aeration with CO_2 enriched air, according to CO_2 concentrations of 460, 1046 and 1280 µatm, respectively (Table 1).

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

2.3 Sampling and response variables

At the end of the experiment samples were taken to determine total particulate carbon (TPC), particulate organic and inorganic carbon (POC, PIC), particulate nitrogen (PON), and biovolume on both the cellular and population level. POC:PON-ratio, cell abundance, change in cell size (Δ cell size) from onset to end of the experiment, as well as inorganic nitrogen and phosphorous were additionally determined. Samples for TPC, POC and PON were filtered (Whatman GF/F filters 25 mm Ø). To obtain POC, the particulate inorganic carbon (PIC) was removed from the TPC sample by exposing filters to fuming hydrochloric acid for 2 h. All filters were dried at 60 °C and analysed in an elemental analyser with a thermal 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 L was divided by the cell abundance. Cell abundance and size were measured daily with a Z2™ COULTER COUNTER® cell and particle counter. The decision to terminate a culture was based on a statistically significant fit to the growth model $n_t = a/(1+(a-b/b)\times e^{(-\mu\times t)})$, 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 that 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.

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

Table 2. Start and end concentrations of nitrate, ammonium, their sum, inorganic 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.	
Regime	pCO_2			$\sum$ N		N/P		
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.

The measured diameter was used to calculate cell biovolume following Hillebrand et al. (1999). Total population biovolume was determined by multiplying cell abundance with cell biovolume. Change in cell size was obtained by comparing average cell size at the beginning and at the end of the study. As biovolume highly correlated with TPC, and with PIC and POC on both the cellular and the population level (Pearson correlation cellular level: r_{TPC}= 0.97, r_{POC}= 0.93, r_{PIC}= 0.96, $p < 0.01$, N = 22; population level: r_{TPC}= 0.99, r_{POC}= 0.98, r_{PIC}= 0.97, $P < 0.01$, N = 22), biovolume was used to express total carbon content and to explain whether cell abundance and/or cell size change was responsible for the findings. Samples for inorganic nitrogen and phosphorus were taken and filtered through GF/F filters. The filtrates were frozen in polyethylene bottles. Duplicate samples from each bottle were analysed colorimetrically with an accuracy of $\pm$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 and 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 $\pm$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 transformed. A General Linear Model (GLM) was used to address our hypothesis and test the effects of pCO_2, nutrient regime and their interactions on POC, PIC and PON content per cell as well as per litre, and on individual cell biovolume and total population biovolume. The model was also used to test effects on total cell number, Δ cell size, final available inorganic N and on the ratio of POC:PON. In the model, nutrient regime was used as a categorical and pCO_2 as a continuous factor. Due to significant interactions between the factors of nutrient regime and pCO_2, separate regression analyses with pCO_2 as predictor were conducted for each nutrient regime for each response variable.

Please note, that due to problems during the sampling procedure we omitted two replicates (460 µatm from high N:P and 1280 µatm from Redfield regime).

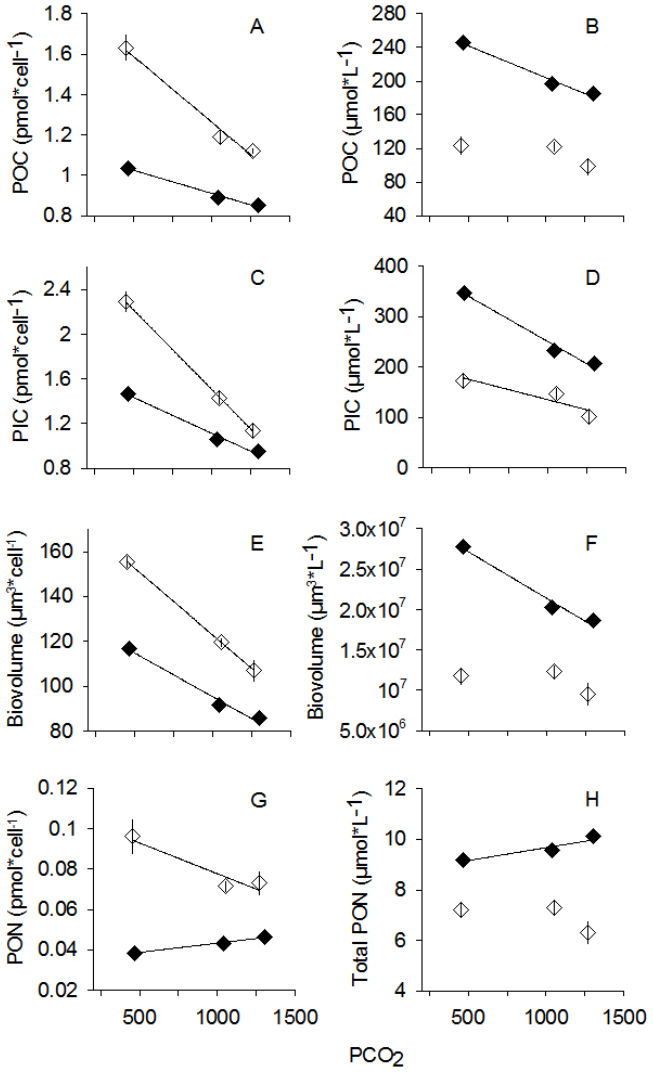

Fig. 1. POC **(A, B)**, PIC **(C, D)**, biovolume **(E, F)**, and PON **(G, H)** content per cell **(A, C, E, G)** and per litre **(B, D, F, H)**. Closed symbols indicate Redfield and open symbols refer to high N:P regime. Regression lines reflect a significant in increase or decrease in the separate regression models within each of the nutrient regimes.

3 Results

3.1 Particulate matter

Both on the cellular and on the population level, carbon related response variables (i.e. POC, PIC, biovolume) were significantly affected by the manipulated factors pCO_2 and nutrient regime and their interaction. Overall POC and PIC content, and biovolume significantly decreased in response to increasing pCO_2 (Fig. 1a–f; Table 3). Cellular yields of all three variables were significantly higher in the high N:P regime (Fig. 1a, c, e; Table 3) whereas total population yield of POC, PIC and biovolume were significantly higher in the Redfield regime (Fig. 1b, d, f; Table 3). The strength of the negative responses to pCO_2 depended significantly on nutrient regime. On the cellular level the decline of all three variables was more pronounced in the high N:P regime compared to Redfield treatments (Fig. 1a, c, e; Table 4). The relative decline of cellular POC and PIC content, and biovolume along the pCO_2 gradient was about 14, 29, and 3 %, respectively, higher in the high N:P compared to Redfield treatments. In contrast, on the population level the negative responses to pCO_2 were significantly attenuated in the high N:P regime, whereas in the Redfield regime, total population POC, PIC and biovolume significantly decreased (Fig. 1b, d, f; Table 4). In the high N:P regime, POC and biovolume (Fig. 1b and f) were not affected by pCO_2 (Table 4) and the response of PIC was significantly attenuated (Fig. 1d; Table 4). In fact the relative loss of PIC in the high N:P treatments was 10 % less compared to the Redfield regime.

PON per unit cell was significantly higher in the high N:P treatments compared to Redfield regime, whereas total population PON was significantly higher in the Redfield treatment (Fig. 1g, h; Table 3). The response of PON to pCO_2, however, significantly depended on nutrient regime on both the cellular and the population level. In the Redfield treatments both PON per unit cell and total population PON significantly increased in response to pCO_2 (Fig. 1g, h; Table 4). In contrast, in the high N:P regime cellular PON significantly decreased (Fig. 1g; Table 4) and total population PON was not affected in response to pCO_2 (Fig. 1h; Table 4).

Cell abundance was significantly higher in the Redfield regime compared to high N:P (Fig. 2a; Table 3). However, the response to pCO_2 depended on the nutrient regime. That is, cell abundance significantly decreased with pCO_2 in the Redfield treatments and was unchanged in the high N:P regime (Fig. 2a; Table 4).

Also, change in individual cell size (Δ cell size) was affected by the manipulated factors. In general all cells increased in size over the course of the experiment. Δ cell size, however, was significantly higher in the high N:P regime compared to Redfield (Fig. 2b; Table 3). Independent of nutrient regime, Δ cell size significantly decreased in response to pCO_2 (Fig. 2b; Tables 3 and 4).

At the end of the experiment considerable concentrations of nitrate were left in some treatment combinations whereas inorganic phosphate was depleted in all bottles (Table 2). Significantly more nitrate was left in the high N:P regime compared to the Redfield treatments (Fig. 2c; Tables 2 and 3). In the high N:P regime the remaining nitrate significantly increased along the pCO_2 gradient (Table 4). In the Redfield regime the regression revealed no significant increase, however the nitrate level remained sufficiently above the quantification limit of $0.1\,\mu mol \times L^{-1}$ only in the high pCO_2 treatment, i.e. nitrate was left over (Fig. 2c; Tables 2 and 4).

Particulate organic C:N ratio was also affected by the manipulated factors and their interaction. Overall particulate organic C:N was significantly higher in the Redfield regime (Fig. 2d; Table 3). The response to pCO_2 significantly

Table 3. Results of the General Linear Model with pCO_2 as continuous and nutrient regime as categorical factor. Response variables are POC, PIC, biovolume, and PON per cell and per litre; cell abundance, Δ cell size, inorganic nitrogen (N) end, and POC:PON.

		Whole model				Contributing factors				
Response variable	Factor	df	$R2$	F	p	df	MS	F	p	Regression slope
Cellular level										
log POC		3, 18	0.95	130.51	<.001					
	Nutrients					1, 18	0.036	85.06	<.001	
	pCO_2					1, 18	0.059	140.96	<.001	−0.00015***
	Interaction					1, 18	0.006	14.33	< .01	
log PIC		3, 18	0.94	111.46	<.001					
	Nutrients					1, 18	0.042	47.56	<.001	
	pCO_2					1, 18	0.223	250.49	<.001	−0.003***
	Interaction					1, 18	0.012	12.93	<.01	
Biovolume		3, 18	0.95	143.58	<.001					
	Nutrients					1, 18	1445.10	63.35	<.001	
	pCO_2					1, 18	6049.53	265.18	<.001	−0.0494***
	Interaction					1, 18	248.48	10.89	<.01	
log PON		3, 18	0.92	84.50	<.001					
	Nutrients					1, 18	0.156	93.91	<.001	
	pCO_2					1, 18	0.002	1.37	0.26	−0.00003
	Interaction					1, 18	0.039	23.38	<.001	
Population level										
POC		3, 18	0.94	106.91	<.001					
	Nutrients					1, 18	12358	65.03	<.001	
	pCO_2					1, 18	6625.2	34.86	<.001	−0.0517***
	Interaction					1, 18	1547.2	8.14	<.05	
PIC		3, 18	0.93	98.41	<.001					
	Nutrients					1, 18	28124.4	58.299	<.001	
	pCO_2					1, 18	41676.8	86.392	<.001	−0.130***
	Interaction					1, 18	5922.1	12.276	<.01	
Biovolume		3, 18	0.93	95.35	<.001					
	Nutrients					1, 18	2.4E+14	77.35	<.001	
	pCO_2					1, 18	1.2E+14	37.92	<.001	−6904***
	Interaction					1, 18	5.5E+13	17.50	<.001	
PON		3, 18	0.84	36.55	<.001					
	Nutrients					1, 18	38.889	118,7	<.001	
	pCO_2					1, 18	0.009	0.02	.88	0.00006
	Interaction					1, 18	2.264	6.02	<.05	
Abundance		3, 18	0.97	223.27	<.001					
	Nutrients					1, 18	2.0E+10	129.02	<.001	
	pCO_2					1, 18	2.4E+07	0.16	.70	−3.1
	Interaction					1, 18	1.2E+09	8.26	<.05	
Other										
Δ Cell size		3, 18	0.85	42.28	<.001					
	Nutrients					1, 18	1770.42	16.71	<.001	
	pCO_2					1, 18	3460.57	32.67	<.001	−0.0374***
	Interaction					1, 18	43.78	0.41	.53	
log N end		3, 18	0.98	315.20	<.001					
	Nutrients					1, 18	0.449	93.39	<.001	
	pCO_2					1, 18	0.019	3.99	.06	0.000088
	Interaction					1, 18	0.002	0.42	.52	
POC : PON		3, 18	0.90	61.59	<.001					
	Nutrients					1, 18	112.83	62.96	<.001	
	pCO_2					1, 18	92.33	51.52	<.001	−0.0061***
	Interaction					1, 18	46.02	25.68	<.001	

Table 4. Results of separate regression analyses in the Redfield and high N:P regime with pCO_2 as predictor. Response variables are POC, PIC, biovolume, and PON per cell and per litre; cell abundance, Δ cell size, inorganic nitrogen (N) end, POC:PON.

Response variable	Nutrient regime	df	R^2	F	P	Regression slope
Cellular level						
log POC	Redfield	1, 10	0.81	43.22	<.001	−0.000105***
	High N:P	1, 10	0.91	97.20	<.001	−0.000204***
log PIC	Redfield	1, 10	0.95	173.03	<.001	−0.000232***
	High N:P	1, 10	0.92	116.85	<.001	−0.000368***
Biovolume	Redfield	1, 10	0.95	188.93	<.001	−0.0394***
	High N:P	1, 10	0.92	119.94	<.001	−0.0594***
PON	Redfield	1, 10	0.77	34.24	<.001	0.000009***
log PON	High N:P	1, 10	0.46	9.59	<.05	−0.000156*
Population level						
POC	Redfield	1, 10	0.94	95.40	<.001	−0.0767***
	High N:P	1, 10	0.15	2.83	.13	−0.0267
PIC	Redfield	1, 10	0.96	173.67	<.001	−0.1786***
	High N:P	1, 10	0.49	10.67	<.01	−0.0808**
Biovolume	Redfield	1, 10	0.93	134.76	<.001	−11593***
	High N:P	1, 10	0.02	1.18	.31	−2214
PON	Redfield	1, 10	0.41	7.82	<.05	0.001016***
	High N:P	1, 10	0.06	1.64	.23	−0.000896
Abundance	Redfield	1, 10	0.71	25.85	<.001	−25.7***
	High N:P	1, 10	0.06	1.65	.23	19.51
Other						
Δ Cell size	Redfield	1, 10	0.70	23.92	<.001	−0.03318***
	High N:P	1, 10	0.56	13.48	<.01	−0.0416**
log N end	Redfield	1, 10	−0.04	0.62	.45	0.000060
	High N:P	1, 10	0.39	7.43	<.05	0.000117*
POC:PON	Redfield	1, 10	0.93	129.66	<0.001	−0.0104
	High N:P	1, 10	0.05	1.53	0.25	−0.0018

depended on nutrient regime, i.e. POC:PON steeply declined in the Redfield but not in the high N:P treatments (Fig. 2d; Table 4).

4 Discussion

The strong quantitative differences of *E. huxleyi* cultures in POC, PIC and PON content as well as biovolume can be explained by different absolute nutrient, in particular phosphorous, concentrations in the Redfield and in the high N:P regime. While cell size in all treatments increased over the course of the experiment, the significantly larger cells in the high N:P regime explain the higher absolute POC, PIC and PON content per cell. The reversed pattern on the population level, taking into account how many cells are in the culture, can be explained by the higher concentration of inorganic phosphorous in the Redfield regime allowing more cell divisions and thus higher cell abundance. This in turn translates into higher total POC, PIC, and PON accumulation and biovolume.

The fact that *E. huxleyi* cultures grown in different nutrient regimes responded significantly different to increasing pCO_2 is not as easy to explain. In particular, we can only speculate regarding the physiological mechanism behind this finding. We hypothesise, however, that the degree of organic phosphate acquisition among the two nutrient regimes had major consequences for population carrying capacity, i.e. led to constant cell abundance in the high N:P regime and to declining cell numbers in the Redfield treatments. This ultimately affected response variables on the population level. The data presented here show that cells in the high N:P regime had decreased cellular N-content although cell abundance was the same between pCO2 treatments. Constant cell abundance along the pCO_2 gradient was in turn responsible for the attenuated pCO_2 effect on all carbon related response variables on the population level. The available inorganic phosphorous and assuming that during maximum growth rate cells take up inorganic nutrients in a constant (Redfield) ratio (Klausmeier et al., 2008), could indirectly suggest that cells with lower amounts of PON also take up lower amounts of inorganic phosphorous, i.e. show higher resource use efficiency. This would counteract phosphorous limitation, and

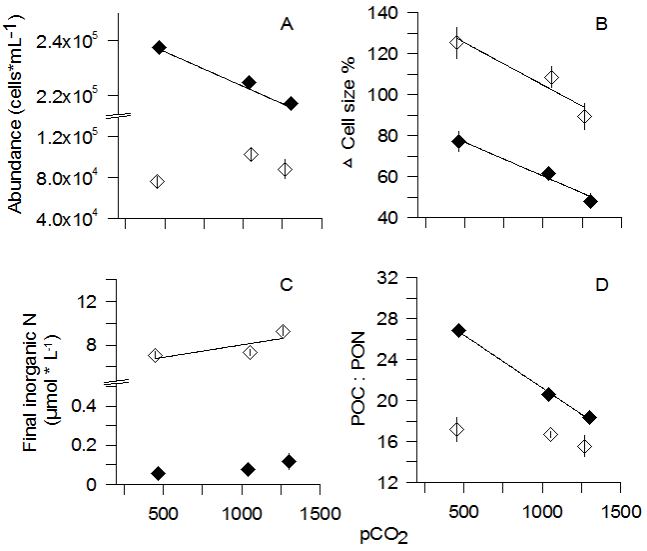

Fig. 2. Cell abundance **(A)**, Δ cell size **(B)**, final available inorganic N **(C)**, and ratio of particulate organic carbon to nitrogen **(D)**. Closed symbols indicate Redfield and open symbols refer to high N:P regime. Regression lines reflect a significant in increase or decrease in the separate regression models within each of the nutrient regimes.

thus could explain why cell abundance was unaffected by pCO_2. However, inorganic phosphate was depleted in all pCO_2 levels which means that it was either sufficient, or alternatively, phosphorous was available from other sources such as organic phosphate. *E. huxleyi* is known to be superior to other algal species in extremely phosphorous limited conditions (Egge and Heimdal, B. R., 1994; Tyrrell and Taylor, A. H., 1996), firstly, because of its extremely high affinity for inorganic phosphorous uptake, and secondly, due to its possession of several alkaline phosphatase (APase) enzyme systems making organic phosphorous available (Riegman et al., 2000). Indeed, it could be shown that under phosphorous limitation, the gene of one important APase in this respect was expressed and transcript levels increased by two to six orders of magnitude in a number of *E. huxleyi* strains from different geographical regions (Xu et al., 2010). Moreover, relevant APases were induced and enzyme activity rapidly increased with phosphorous limitation (Riegman et al., 2000; Xu et al., 2010). APase activity can enhance population growth by up to 90 % through organic phosphate uptake depending on the concentration of available organic phosphate (Riegman et al., 2000). It was suggested that the reported high transcript level potentially allows for rapid syntheses of large amounts of proteins (Xu et al., 2010). Protein biosynthesis naturally requires sufficient inorganic nitrogen, which in this experiment was available in the high N:P but not in the Redfield regime. Moreover, in chemostat experiments, organic phosphate uptake rate correlated negatively with growth rate and positively with organic phosphate concentration (Riegman et al., 2000). This means that APase activity becomes increasingly important during stationary phase (i.e. during plankton bloom in nature) when more organic phosphate is available through dissolution of dead cells, which again is relevant to the design of this study. Xu et al. (2010) were even able to show that APase activity was initially detected during late stationary phase and further increased with time.

In contrast to the high N:P treatments, cells in the Redfield regime were co-limited by nitrogen and phosphorous (i.e. both nutrients were depleted) in the lower CO_2 treatments. Contrary to the high N:P regime, cells increasingly incorporated more nitrogen with increasing pCO_2, which is also reflected in the declining C:N ratio. Assuming that consequently cells also take up more inorganic phosphorous led to phosphorous limitation at high pCO_2 and explained why cell abundances decreased with increasing pCO_2. Additionally, low amounts of leftover nitrate in the high pCO_2 treatments indicate that inorganic phosphorous became limiting. We suggest that due to inavailability of excess nitrogen in the Redfield regime, enzymatic uptake of organic phosphorous was not possible and thus could not outweigh inorganic phosphorous limitation to reach carrying capacity of 240 000 cells $\times$ mL^{-1} as in the low pCO_2 treatment. Indeed it could be shown that the responsible gene for the relevant APase was not expressed in nitrogen limited cells (Xu et al., 2010). Again, decreasing cell numbers with increasing pCO_2 explain why all carbon related response variables on the population level more steeply declined in comparison to the high N:P regime.

We do not know which and how physiological mechanisms such as protein synthesis and nutrient uptake rates are affected by pCO_2 stress. Little is also known in which direction particulate organic matter content of coccolithophores change with pCO_2 stress (Hutchins et al., 2009). However, our results that leftover nitrogen increased with increasing pCO_2 in both nutrient regimes might point to the fact that pCO_2 stress leads to higher phosphorous requirements. We suggest that in the high N:P regime, the enhanced demand for phosphorous could be outweighed by enzymatic uptake of phosphorous. This was potentially triggered through severe phosphorous limitation and fuelled by the availability of excess nitrogen as explained above. In the Redfield regime this alternative phosphorous source could not be used because of the lack of nitrogen for protein biosynthesis. These hypothesised different physiological responses of *E. huxleyi* among the nutrient regimes had major consequences for population carrying capacities, which ultimately affected response variables on the population level. Translated to nature, this result could mean that effects of ocean acidification on total *E. huxleyi* performance are less severe in phosphorous limited regions where this species successfully occupies its ecological niche. Moreover, in the case that other phytoplankton species, especially other coccolithophores, are not able to increase their phosphorous allocation like *E. huxleyi*,

could even mean that *E. huxleyi* might have a competitive advantage in a future more acidified ocean. Whereas Riegman et al. (2000) were able to show that *E. huxleyi* is superior in terms of phosphorous uptake compared to a number of phytoplankton species which are not closely related, this question remains to be answered for other coccolithophores.

Unfortunately, as nitrogen concentrations in the two nutrient regimes differed considerably, the design of this experiment does not allow us to disentangle whether only the elemental ratio between nitrogen and phosphorous, or the absolute concentration of excess nitrogen was responsible for the observed different responses of *E. huxleyi*. As mentioned above, we suggest that phosphorous limitation might have triggered the mechanism of the hypothesised enzymatic phosphorous uptake, which was fuelled by available nitrogen to synthesise the APase. Especially, under consideration of future extent of phosphorous limited areas (Ammerman et al., 2003), simulating two different phosphorous 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). However, more thorough experimental work is needed to adequately address the mechanistic explanation of our findings hypothesized here. In particular, an experimental design with varying degrees of phosphorous limitation but constant absolute concentration of nitrogen is required. Moreover, thorough measurements of particulate organic as well as dissolved organic phosphorous (POP, DOP), which unfortunately were lost during analyses in this study, is needed to answer whether, (i) cells with higher or lower PON content also contain more or less POP, and (ii) severe phosphorous limited cultures indeed show less concentration of DOP due to enzymatic uptake. The ideal case would be to also measure gene expression and/or enzyme activity of the relevant APases.

In general, the negative response of cellular POC and PIC content of *E. huxleyi* to rising pCO_2 in this study confirmed results of earlier experiments (e.g. Langer et al., 2006, 2009; Riebesell et al., 2000; Shi et al., 2009; Zondervan et al., 2001). Likewise, the cellular responses to phosphorous limitation in terms of increasing cell size coincide with previous studies (Riegman et al., 2000; Müller et al., 2008). Caused by an oversupply of nitrogen and the production of biomass without the possibility to divide, cells exposed to strong phosphorous limitation grew considerably larger in volume compared to their size at the onset of the experiment. The significant decrease of cell size with pCO_2 in all treatments is likely due to decreasing cellular PIC and POC in response to pCO_2.

5 Conclusions

To the best of our knowledge, this study shows for the first time different sensitivities to ocean acidification caused by different nutrient regimes for total population carbon accumulation of calcifying phytoplankton (*E. huxleyi*). The attenuated effect of ocean acidification on accumulation of total particulate matter in the high N:P nutrient regime points out that responses of cellular POC and PIC cannot linearly be extrapolated to the population level. This is due to the proposed different physiological responses regarding enzymatic organic phosphate acquisition in the two nutrient regimes, which ultimately altered the population response along the pCO_2 gradient. Considering these results, estimations on the future carbon cycle should not only account for pCO_2 and the response of coccolithophores to ocean acidification per unit cell during exponential growth, but also for the effects of future conditions on the carrying capacity (i.e. at bloom peak) of a phytoplankton population. Although the function of calcite as ballast for global ocean carbon export is not completely clear, our study reveals the necessity to consider nutrient ratios, and their capability to attenuate negative responses of population calcite accumulation for future predictions on carbon export in an acidified ocean.

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