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Combined effects of inorganic carbon and light on *Phaeocystis globosa* Scherffel (Prymnesiophyceae)

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Abstract

Phaeocystis globosa (Prymnesiophyceae) is a globally dominating phytoplankton species. It plays an important role in both the global sulfur and carbon cycles, by the production of dimethylsulfide (DMS) and the drawdown of inorganic carbon. *Phaeocystis globosa* has a polymorphic life cycle and is considered to be a harmful algal bloom (HAB) forming species. All these aspects make this an interesting species to study the effects of increasing carbon dioxide (CO_2) concentrations, due to anthropogenic carbon emissions.

Here, the combined effects of three different dissolved carbon dioxide concentrations ($CO_{2(aq)}$) (low: 4 µmol kg^{-1}, intermediate: 6–10 µmol kg^{-1} and high $CO_{2(aq)}$: 21–24 µmol kg^{-1}) and two different light intensities (low light, suboptimal: 80 µmol photons m^{-2} s^{-1} and high light, light saturated: 240 µmol photons m^{-2} s^{-1}) are reported.

The experiments demonstrated that the specific growth rate of *P. globosa* in the high light cultures decreased with increasing $CO_{2(aq)}$ from 1.4 to 1.1 d^{-1} in the low and high CO_2 cultures respectively. Concurrently, the photosynthetic efficiency increased with increasing $CO_{2(aq)}$ from 0.56 to 0.66. The different light conditions affected photosynthetic efficiency and chlorophyll-*a* concentrations, both of which were lower in the high light cultures as compared to the low light cultures. These results suggest that in the future, inorganic carbon enriched oceans, *P. globosa* will become less competitive and feedback mechanisms to global change may decrease in strength.

1 Introduction

The genus of *Phaeocystis* (Prymnesiophyceae) has a global distribution and consists of several species, three of which are known to form large blooms of colonies (*P. antarctica, P. pouchetii* and *P. globosa*, Schoemann et al., 2005). While the first two species are found in Antarctic and Arctic waters respectively, *P. globosa* is found in temperate

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and tropical waters. Like the other two species, *P. globosa* is well known for its polymorphic life cycle, during which it alternately occurs as single cells, single flagellated cells and large, mucus producing, colonies (Schoemann et al., 2005). Blooms of *P. globosa* occur annually (April–June) amongst others in the Dutch coastal North Sea and the Wadden Sea. Here, these blooms are a cause of nuisance when the organic foam, which is formed due to partial degradation of the colonies, is blown on the beaches, thereby having negative impacts on tourism (Peperzak, 2003; Schoemann et al., 2005). Furthermore, blooms of *P. globosa* have been found to be responsible for mass mortality of caged fish in China in 1997 (Lu and Huang, 1999; Chen et al., 2002) and of shellfish (mussels) in the Oosterschelde estuary in 2001 (Peperzak and Poelman, 2008). These events of mass mortality are most likely due to the bacterial degradation of the large amounts of organic matter formed during a bloom, which can lead to local hypoxia and increased concentrations of sulfide and ammonia (Peperzak and Poelman, 2008). Therefore, this species is generally classified as a harmful algal bloom (HAB) species.

As a dominant bloom forming species, *P. globosa* is regarded as an important driver of the global oceanic carbon cycle, assimilating significant amounts of inorganic carbon as cell material and mucus. Measurements have shown that the surface water pCO_2 of the Wadden Sea was reduced to on average 270 µatm during April and May 2009, the spring bloom period, compared to an atmospheric concentration of approximately 380 µatm (L. Salt, personal communication, 2009). Most of the assimilated carbon is recycled in the surface waters through the microbial food web and never reaches deeper waters (Schoemann et al., 2005). An important actor in carbon recycling is viral lysis, which is a major loss factor for *P. globosa* and can terminate a bloom in a relatively short time span, thereby providing other microbes with substantial amounts of organic carbon (Brussaard et al., 2005).

Sequestration of inorganic carbon in subsurface waters by *P. globosa* is influenced by the environmental conditions under which a bloom develops (Mari et al., 2005). Carbon sequestration by *P. globosa* is reduced when the bloom develops under nitrogen

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limitation, as compared to phosphorus limitation (Mari et al., 2005). It is therefore important to consider the effects of nutrient conditions for predictions of bloom development, especially when predicting bloom development in the future ocean.

Due to anthropogenic carbon dioxide (CO_2) emissions, atmospheric carbon dioxide concentrations are rising. Current predictions by the Intergovernmental Panel on Climate Change (IPCC), predict a rise of the atmospheric CO_2 concentration up to 800 µatm by the year 2100 AD (Meehl et al., 2007). A large part of this anthropogenic CO_2 is stored in the oceans, by the continuous exchange of CO_2 between the atmosphere and surface waters, and the CO_2 concentrations in the surface waters is therefore increasing (Sabine et al., 2004). This changes the carbonate chemistry of these waters, leading to an increase in aqueous carbon dioxide ($CO_{2(aq)}$) and bicarbonate ion (HCO_3^-) concentration, but a decrease in carbonate ion (CO_3^{2-}) concentrations (Hoogstraten et al., 2011). It is likely that these changes in the carbon chemistry will affect marine primary producers. To date, only few of the marine primary producers have been studied and the focus has been mainly on calcifying organisms, such as *Emiliania huxleyi* (Engel et al., 2005; Iglesias-Rodriguez et al., 2008), and diatoms, such as the temperate HAB species *Pseudonitzschia multiseries* (Sun et al., 2011) and the Southern Ocean diatom species *Proboscia alata* (Hoogstraten et al, 2011). Other than *Pseudonitzschia multiseries*, HAB species have not been intensively studied in this respect. However, due to the socio-economic relevance of these species, it is important to also maintain a focus on these species, in order to predict the occurrence of harmful algal blooms in the future. The consequences of environmental changes (increasing temperature and CO_2 concentration) on *P. globosa* and subsequently ecological and economic impacts are not completely clear yet. A study by Peperzak (2003) has shown that due to temperature increase, as an accompanying anthropogenic effect, blooms of *P. globosa* will most likely decrease. A more recent study by Wang et al. (2010a) focused on inorganic carbon effects, but did not include temperature, and showed that the colonial biomass of *P. globosa* will increase, while the biomass of solitary cells will decrease in the tested future high CO_2 scenario of 750 µatm.

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Here we report the results of a study on the combined effects of both inorganic carbon concentration and light intensity on *P. globosa* and give insight in possible future changes in the abundance of this species in the Dutch coastal North Sea and Wadden Sea. Solitary cells of *P. globosa* were cultured semi-continuously in two different light intensities (a suboptimal light condition classified as low light: LL 80 µmol photons m^{-2} s^{-1} and a saturating light condition of 240 µmol photons m^{-2} s^{-1}, classified as high light, or HL) and three different dissolved carbon dioxide ($CO_{2(aq)}$) concentrations (respectively 4, 10 and 21 µmol kg^{-1} for low, intermediate and high $CO_{2(aq)}$ in the high light cultures, and 4, 6 and 24 µmol kg^{-1} for low, intermediate and high $CO_{2(aq)}$ in the low light cultures). This corresponds to a pCO_2 range of 100 to 600 µatm, spanning a range of glacial pCO_2 under bloom conditions, to the predicted concentration in 50–60 yr from now (Meehl et al., 2007).

2 Materials and methods

2.1 Experimental setup

Prior to the experiments, a solitary cell strain of *Phaeocystis globosa* (strain Pg G"A") originating from the North Sea (T135, Terschelling, 135 km off the coast, Koeman, 2000) was cultured in aged seawater originating from the Bay of Biscay (as described by Timmermans et al., 2001). The seawater was filter sterilized with a Sartobran 105 filter (pre-filter 0.45 µm and end-filter 0.20 µm pore size), as described in Hoogstraten et al. (2011). Major nutrients were added to a final concentration of 60 µmol l^{-1} NO_3^-, 3.75 µmol l^{-1} PO_4^{3-} and 60 µmol l^{-1} Si. The stock culture of *P. globosa* was regularly diluted in order to keep the cells in exponential growth prior to the experiment.

The experimental setup used is described in detail in Hoogstraten et al. (2011). In short, the setup was temperature controlled with thermostat baths (Lauda Ecoline StarEdition RE104), set to 15.0 °C (±0.1), to ensure constant temperature in the culture vessels. Light conditions in the culture vessels were saturating light (high light intensity

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or HL: 240 µmol photons m^{-2} s^{-1}) or suboptimal light (low light intensity or LL: 80 µmol photons m^{-2} s^{-1}) in a 16:8 h light:dark cycle. The culture vessels were aerated with recycled air from the medium supply vessels, thus reducing the amount of CO_2 enriched gas needed for the aeration. Three different natural gas mixes (HiQ-Line Linde gas, Germany) were used for the experiments: 190 µatm, 380 µatm and 750 µatm CO_2. The aeration resulted in three different $CO_{2(aq)}$ concentrations for each light condition (Table 1). Prior to the experiments, *Phaeocystis globosa* was grown in the experimental setup as a batch culture for 8 days in order to determine the different growth phases (lag phase, exponential phase and the senescent phase, Fig. 1a). After this, the cultures were diluted to low cell abundance (early in the exponential growth phase: 1.5×10^4 cells ml^{-1}) and cultured semi-continuously for 6 days (pre-experimental phase, cell abundance ranging between 1.3×10^4 and 5.6×10^4 cells ml^{-1}, Fig. 1b), during which the total culture volume was exchanged three times, in order to realize and maintain a stable environment and allowing the cells to adapt to the experimental conditions (as described by Riebesell et al., 2010). Dilutions were made according to cell abundance, which was determined as described below.

Following the pre-experimental dilution series, the experimental phase started. Again *P. globosa* was cultured semi-continuously, with cell abundance ranging between 1.5×10^4 to 5.3×10^4 cells ml^{-1} between dilutions. The experimental phase lasted for 6 days (Fig. 1c and d; Supplement, Table S1), during which several parameters were measured before and after each dilution: cell abundance, photosynthetic efficiency, inorganic carbon (dissolved inorganic carbon "DIC" and total alkalinity "A_T") and dissolved inorganic nutrients (nitrate and phosphate). In addition, before dilution the chlorophyll-*a* concentration, particulate organic carbon (POC) and particulate organic nitrogen (PON) were measured. All samples were taken during the light period of the cultures and at a fixed time every day in the light:dark cycle (3 h after onset of the light and 1 h after dilution in the afternoon).

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2.2 Inorganic carbon

Following the protocols described by Dickson and Goyet (1994) and Dickson et al. (2007), 250 ml samples were taken from the culture vessels, fixed with 50 µl $HgCl_2$, closed and stored in the dark at room temperature. Total alkalinity (A_T) and total dissolved inorganic carbon (DIC) were determined with a VINDTA 3C (**V**ersatile **IN**strument for the **D**etermination of **T**otal inorganic carbon and titration **A**lkalinity, Marianda, Germany). Certified reference materials (CRM, batch # 100) obtained from A. G. Dickson (Scripps Institution of Oceanography, USA), were included in every series of measurements in order to determine the quality of the data. The sample values were adjusted accordingly. The resulting sample values had a typical accuracy of 5.8 µmol kg^{-1} for both A_T and DIC. From the A_T and DIC the other parameters of the carbonate system (pH and pCO_2) were calculated as well as the concentration of 3 different carbon species: bicarbonate and carbonate ions and aqueous CO_2, using the CO_2sys Excel spreadsheet (Lewis and Wallace, 1998). The CO_2 dissociation constants of Mehrbach et al. (1973) refitted by Dickson and Millero (1987), the CO_2 solubility coefficient of Weiss (1974), the borate acidity constant of Dickson (1990b) and the SO_4^{2-} dissociation constant of Dickson (1990a) were used for the calculations. Salinity, phosphate and silicate concentrations (for the latter the concentration as added to the culture medium, 60 µmol l^{-1}, was used), were incorporated in the calculations.

As mentioned by Kim et al. (2006), particulate organic carbon may have significant effects on the measured A_T. Therefore, the measured A_T ($A_{T(meas)}$) was compared to the predicted A_T ($A_{T(pred)}$), which was calculated from the A_T of the supply vessels corrected for the nitrate uptake by the phytoplankton (Brewer and Goldman, 1976; Goldman and Brewer, 1980).

2.3 Cell abundance and specific growth rate

Cell abundance in the cultures was determined with a Coulter Epics XL MCL flow cytometer (Beckman Coulter, Inc. Brea CA, USA). All cultures were analyzed in triplicates

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and average cell abundance was calculated for the determination of the specific growth rate, which was calculated by the linear regression of the natural logarithm of the cell abundance.

2.4 Photosynthetic parameters

2.4.1 Photosynthetic efficiency

Photosynthetic efficiency (F_V/F_M) was determined by the analysis of triplicate samples. Prior to the measurements, the samples were left in the dark for 5 min to allow for dark adaptation. A PAM fluorometer (Pulse Amplitude Modulated-CONTROL Universal Control Unit, WATER-mode, Walz, Germany) was used for the determination of F_0 (autofluorescence), F_M (maximum fluorescence) and F_V/F_M (photochemical quantum efficiency, where $F_V = F_M\text{-}F_0$).

2.4.2 Chlorophyll-*a*

Chlorophyll-*a* concentrations of the cultures were determined by filtration of 150 ml sample over a 0.2 µm pore size glass fiber filter (GF/F, diameter 25 mm). All samples were handled in low light conditions, preventing chlorophyll-*a* degradation. The filters were stored at −80 °C until further analysis. Prior to the analysis, chlorophyll-*a* was extracted in 10 ml 90 % acetone at −20 °C for 24 h. Following the protocol designed by Holm-Hansen et al. (1965), the fluorescence of the samples was measured with a SpectraMax M2 spectrofluorometer (with SoftMax Pro software, Molecular Devices) and the chlorophyll-*a* concentration was determined against a chlorophyll-*a* standard solution. The chlorophyll-*a* concentrations were normalized to the cell counts and expressed as pg cell^{-1}.

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2.5 Particulate organic carbon and nitrogen

Particulate organic carbon (POC) and nitrogen (PON) were determined by the filtration of 100 ml sample over a pre-combusted 0.2 µm pore size GF/F filter (diameter 25 mm). Filters were stored at −80 °C until further analysis. Prior to POC and PON analyses, the filters were freeze dried overnight. The POC and PON samples were analyzed with a Thermo-Interscience Flash EA1112 Series Elemental analyzer, after the protocol of Verardo et al. (1990). The results were corrected for blanks. The POC and PON contents on the filters were normalized to the cell counts and expressed in pmol cell^{-1}. For the determination of the POC:chl-*a* ratio, POC was also calculated as pg C cell^{-1}.

2.6 Nutrient concentrations

For the determination of the concentrations of nitrate and phosphate, the samples were filtered over a 0.2 µm filter (Acrodisc®, 32 mm syringe filter, Supor® membrane, Pall Co-operation, Newquay Cornwall UK) and stored at −20 °C in 6 ml Pony Vials (PerkinElmer Life and Analytical Sciences, Shelton, CT, USA) until further analysis. The vials and syringe were rinsed with MilliQ prior to filtration of the sample. The vials were rinsed three times with filtered sample before filling. Analysis of the nutrient samples was done on a TRAACS Auto Analyzer 800+ (Bran + Luebbe, Germany) with the use of spectrophotometric methods, as described by Grasshoff et al. (1983).

Nutrient uptake rates were calculated from the nutrient concentrations before and after the dilution and normalized to cell counts and expressed as fmol cell^{-1} d^{-1}.

2.7 Statistical analysis

All statistical analyses were done with SPSS 17.0 (SPSS Inc., Chicago, IL). Correlation analyses were done when [$CO_{2(aq)}$] was correlated to the different parameters. In order to distinguish between effects caused by light, [$CO_{2(aq)}$] or a combination of the two, the data were analyzed with analyses of covariance (ANCOVA, Field, 2007).

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3 Results

3.1 Dissolved inorganic carbon and total alkalinity

Both DIC and A_T (data available in Supplement, Table S2 and S3) remained constant throughout each of the experiments. The treatments resulted in 3 different $CO_{2(aq)}$ concentrations for each of the light conditions (Table 1), with less than 10 % variability per light condition throughout the experiments.

A comparison between the measured alkalinity ($A_{T(meas)}$) and the predicted alkalinity ($A_{T(pred)}$) showed some discrepancy between the two, with $A_{T(pred)}$ being slightly larger than $A_{T(meas)}$. No correlation between the POC concentration and the difference between the predicted and the measured alkalinity was found ($r^2 = 0.03$, $n = 34$) (see Fig. S1 in the Supplement).

When the data of the high light and the low light cultures were separated, it was observed that the difference between $A_{T(pred)}$ and $A_{T(meas)}$ slightly decreased with increasing POC concentrations in the high light cultures, ranging between −0.4 and 23.0 µmol kg^{-1} ($r^2 = 0.31$, $n = 16$). The average difference between $A_{T(pred)}$ and $A_{T(meas)}$ was 10.1 (±6.7) µmol kg^{-1}. In the low light cultures, this pattern was reversed and $\Delta A_{T(pred-meas)}$ showed a minimal increase with an increasing POC concentration. The range of the deviation was −1.9 to 18.6 µmol kg^{-1} ($r^2 = 0.10$, $n = 18$). In these low light cultures, the average difference between $A_{T(pred)}$ and $A_{T(meas)}$ was 6.2 (±5.9) µmol kg^{-1}.

3.2 Cell abundance and specific growth rate

The cell abundance of *P. globosa* in the cultures was kept constant between approximately 1.5×10^4 cells ml^{-1} after dilution and 5.0×10^4 cells ml^{-1} before dilution (Fig. 1c and d). Cells were dividing at least once a day (i.e. $\mu > 0.7\,d^{-1}$). Average specific growth rates in the high light cultures were significantly higher than in the low light

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cultures ($F_{(1.31)} = 5008$, $p = 0.033$), which is largely due to the high growth rates at low $CO_{2(aq)}$ concentrations in the high light cultures (Fig. 2). In the high light cultures growth rates were highest at low $CO_{2(aq)}$ (μ=1.4 d^{-1} ± 0.1) while growth rates significantly decreased with increasing $CO_{2(aq)}$ to μ= 1.1 d^{-1} (±0.1) in the high $CO_{2(aq)}$ treatment ($\tau = -0.667$, $p < 0.001$, $n = 18$) (Fig. 2). In the low light cultures the opposite trend was observed, growth rates increased with increasing [$CO_{2(aq)}$], ranging between $\mu = 1.0$ d^{-1} (±0.1) in the low $CO_{2(aq)}$ treatment to $\mu = 1.2$ d^{-1} (±0.1) in the high $CO_{2(aq)}$ treatment (Fig. 2). This increase was not significant ($\tau = 0.255$, $p = 0.140$, $n = 18$).

3.3 Photosynthetic efficiency and chlorophyll-*a*

The photosynthetic efficiency (F_V/F_M) was high throughout the experiment, indicative of healthy and fast growing cells. The F_V/F_M of the high light cultures ranged between 0.56 (±0.03) in the low $CO_{2(aq)}$ treatment and 0.66 (±0.02) in the high $CO_{2(aq)}$ treatment (Table 2). The F_V/F_M in the low light cultures was significantly higher than that of the high light cultures ($F_{(1.67)} = 81\,406$, $p < 0.001$, $n = 70$) and ranged between 0.71 (±0.02) in the low $CO_{2(aq)}$ treatment and 0.70 (± 0.02) in the high $CO_{2(aq)}$ treatment (Table 2). The [$CO_{2(aq)}$] also affected the F_V/F_M, but only in the high light conditions ($\tau = 0.475$, $p < 0.001$, $n = 34$). In contrast, in the low light cultures no effect of the $CO_{2(aq)}$ on the F_V/F_M was observed ($\tau = 0.016$, $p = 0.892$, $n = 36$).

Chlorophyll-*a* concentrations in the *P. globosa* cells varied significantly between the two light conditions with approximately 15 pg $cell^{-1}$ in the high light conditions as compared to higher concentrations, approximately 23 pg $cell^{-1}$ in the low light conditions ($F_{(1.33)} = 116246$, $p < 0.001$, $n = 36$, Table 2). In contrast, the ambient $CO_{2(aq)}$ concentrations did not affect the chlorophyll-*a* -concentration in *P. globosa* cells (high light: $\tau = -0.020$, $p = 0.910$, $n = 18$; low light: $\tau = 0.203$, $p = 0.240$, $n = 18$, Table 2).

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3.4 Particulate organic carbon and nitrogen

The POC content of *P. globosa* ranged between 0.9 pmol C cell^{-1} in the high light low $CO_{2(aq)}$ culture and 1.1 pmol C cell^{-1} in the low light high $CO_{2(aq)}$ culture (Fig. 3a). The cellular POC concentration was neither influenced by the different $CO_{2(aq)}$ treatments (high light: $\tau = 0.242$, $p = 0.161$, $n = 18$; low light: $\tau = 0.324$, $p = 0.070$, $n = 17$), nor by light ($F_{(1.33)} = 0.000$, $p = 1000$, $n = 36$, Fig. 3a).

The PON content of *P. globosa* ranged between 0.12 pmol N cell^{-1} in the low $CO_{2(aq)}$ high light culture and 0.13 pmol N cell^{-1} in the high $CO_{2(aq)}$ low light culture (Fig. 3b). Similar to the POC content of the phytoplankton cells, different $CO_{2(aq)}$ treatments did also not affect the cellular PON content (high light: $\tau = 0.216$, $p = 0.211$, $n = 18$; low light: τ=0.190, $p = 0.272$, $n = 18$), nor did the different light conditions ($F_{(1.33)} = 0.000$, $p = 1.000$, $n = 36$, Fig. 3b).

With little effect of both light or the $CO_{2(aq)}$ concentration on the POC and PON concentration of the cells, therefore the POC:PON ratio also remained virtually constant, ranging from 7.7 (±0.8) to 8.9 (±1.6) in the high light cultures and between 8.0 (±0.7) and 8.4 (±1.0) in the low light cultures (Fig. 3c).

The ratio values of organic carbon (POC) to chlorophyll-*a* (POC:chl-*a*) on the other hand, varied between 53.6 (±8.8) pg C pg chl-a^{-1} in the low [$CO_{2(aq)}$] low light treatment and 82.4 (± 11.4) pg C pg chl-a^{-1} in the intermediate [$CO_{2(aq)}$] high light treatment (Table 3).

There was no significant effect of the [$CO_{2(aq)}$] on the POC:chl-*a* ratio for both the high light ($\tau = 0.229$, $p = 0.185$, $n = 18$) and the low light cultures ($\tau = 0.124$, $p = 0.472$, $n = 18$). In the high light cultures POC:chl-*a* was significantly higher compared to the low light cultures ($F_{(1.33)} = 25.309$, $p < 0.001$, $n = 36$, Table 3).

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3.5 Nutrients

The removal of the dissolved nutrients between two consecutive dilutions was on average 0.15 (±0.07) µmol l^{-1} for phosphate and 2.34 (± 0.72) µmol l^{-1} for nitrate (Supplement, Table S4 and S5). The average removal between dilutions was therefore approximately 4 % of the initial nutrient stocks in the vessels. Nutrient removal was never more than 10 % of the total nutrient stocks and therefore never considered to result in nutrient limitation.

The rates of uptakes of phosphate by *P. globosa* were not significantly influenced by the different light treatments ($F_{(1.30)} = 0.036$, $p = 0.851$, $n = 32$) and ranged between 5.7 and 9.2 fmol cell^{-1} d^{-1} in the low light low $CO_{2(aq)}$ culture and the intermediate $CO_{2(aq)}$ high light culture, respectively (Table 4). Both in the high light ($\tau = 0.451$, $p = 0.025$, $n = 14$), and in the low light treatments ($\tau = 0.412$, $p = 0.017$, $n = 18$) a positive effect of $[CO_{2(aq)}]$ on phosphate uptake rates was detected, but the variability in the measurements was large.

The rates of nitrate uptake showed similar responses to light as the rates of phosphate uptake (Table 4). There was no significant effect of the different light treatments on nitrate uptake rates ($F_{(1.30)} = 0.908$, $p = 0.348$, $n = 32$). In the high light cultures, the $[CO_{2(aq)}]$ did not have a significant effects on nitrate uptake rates ($\tau = 0.231$, $p = 0.250$, $n = 14$). In the low light cultures on the other hand, nitrate uptake rates significantly increased with increasing $[CO_{2(aq)}]$ ($\tau = 0.595$, $p = 0.001$, $n = 18$), ranging between 96.2 fmol cell^{-1} d^{-1} in the low $CO_{2(aq)}$ culture to 142.3 fmol cell^{-1} d^{-1} in the high $CO_{2(aq)}$ culture. The ratio of uptake of nitrate to phosphate (N:P), hence cellular N:P content was highest in the low light cultures, ranging between 17.0 and 21.6 (Table 4). There was no significant effect of the two different light intensities on the N:P ratio ($F_{(1.30)} = 1.062$, $p = 0.311$, $n = 32$). Furthermore $[CO_{2(aq)}]$ did not significantly affect the N:P ratio either (high light: $\tau = -0.385$, $p = 0.055$, $n = 14$; low light: $\tau = 0.098$, $p = 0.570$, $n = 18$).

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4 Discussion

The increasing atmospheric concentrations of carbon dioxide due to anthropogenic CO_2 emissions lead to changes the inorganic carbon chemistry of seawater. These changes may affect marine phytoplankton and here we report on the findings of carbon perturbation experiments with *Phaeocystis globosa*. The massive blooms of this species strongly affect the marine carbon cycle, by the sequestration and recycling of carbon. Moreover, during such a massive bloom $CO_{2(aq)}$ concentrations in the ambient seawater may strongly decrease and may as a feedback mechanism, thus affect the physiology and growth of *P. globosa*. We combined three different inorganic carbon treatments with two different light intensities on *P. globosa* in our experiments. In practice, even with constant aeration it proved to be very difficult to reach the desired level of carbon in the culture medium. This was mainly due to the photosynthesis by the phytoplankton, which could be concluded from the larger variability of the DIC concentrations (ca. 1 %) compared to the A_T (ca. 0.1 %). However, due to the daily dilution approach, this variability still was minor and resulted in a stable inorganic carbon chemistry in the cultures, with a maximum variability in the $CO_{2(aq)}$ concentrations of only 10 % within each culture. The slight discrepancy between the measured and the predicted A_T was within the measurement accuracy and the results showed that there was no systematic effect of the POC concentration and changes therein on the measured A_T. At most we found a weak trend, but this trend was opposite to the results reported by Kim et al. (2006).

The results of our experiments showed that *P. globosa* is a highly adaptive species which was able to maintain high growth rates in a large range of environmental conditions. Despite the substantial differences in $CO_{2(aq)}$ concentrations and the two different light treatments (saturating and suboptimal), cells in all cultures were growing fast, dividing more than once per day. The growth rates in the low light cultures were not influenced by the different $CO_{2(aq)}$ conditions. The cells in high light cultures grew comparatively faster, and here the growth rate was negatively influenced by higher

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[$CO_{2(aq)}$]. These results corroborate with the observations from Wang et al. (2010a) and Chen and Gao (2011), suggesting that this results is consistent among different strains of *P. globosa*.

Although F_V/F_M is generally considered a good measure for cell fitness, the results of the growth rates and F_V/F_M measurements did contradict each other. Increasing $CO_{2(aq)}$ concentrations lead to a minor, but significant increase in the photosynthetic efficiency in the high light cultures, while growth rates decreased. This suggests that the cells in this culture allocated the energy to growth instead of photosynthesis; however, the differences were too small to draw a firm conclusion.

Contrary to the findings by Wang et al. (2010a), cellular POC and PON concentrations were not affect by the different $CO_{2(aq)}$ treatments. While Wang et al. (2010a) studied both solitary and colonial cells, we only examined the effects of variations in light and inorganic carbon on solitary cells. The different results suggest that the elevated POC contents of the cells might be more likely present in the mucus layer of the colonial cells than in the solitary cells (see also Schoemann et al., 2005 and Rousseau et al., 2007 and references therein). The ratios of POC:PON that were measured in the present study were higher than the ratio's that have been previously measured in cultures or field samples of solitary *P. globosa* cells, but comparable to colonial stoichiometry (Schoemann et al., 2005 and references therein).

Furthermore, cellular PON production rates matched nitrate uptake rates, suggesting that indeed all the NO_3^- removed from the culture medium was assimilated and converted in structural biomass by *P. globosa*. Based on this observation the assumption was made that the same was true for phosphate and the ratios of C:P (carbon:phosphate) were determined (data not shown). The C:P ratios ranged between 126 and 188, with the highest ratios in the low light cultures. These ratios are comparable to the large range of C:P ratios found for colonial cells by Jahnke (1989).

Since light is an important trigger for *P. globosa* blooms in the Dutch coastal North Sea (Peperzak et al., 1998), two different light conditions were incorporated in the experimental setup. Field data has shown that *P. globosa* outcompetes diatoms at

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the light saturating conditions used in our experiments (240 µmol photons m^{-2} s^{-1}), while the suboptimal light conditions in our experiments (80 µmol photons m^{-2} s^{-1}) did sustain high growth rates, but not enough to outcompete diatoms (Meyer et al., 2000). Furthermore, the optimal light condition for cell division was reported to be 180 µmol photons m^{-2} s^{-1} (Meyer et al., 2000). The results of our experiments showed only a moderate effect of low light on the photosynthetic efficiency, but the cells responded by increasing the chlorophyll-*a* concentration by as much as 50 %. The light conditions did not affect the cellular carbon content (POC, Fig. 3) and as a result of the elevated chlorophyll-*a* concentration, the POC:chl-*a* ratio was also affected, becoming significantly larger at high light conditions, and comparable to ratios measured for an Antarctic *Phaeocystis* sp. at 110 µmol photons m^{-2} s^{-1} (Stefels and Van Leeuwe, 1998).

Combined effects of light and inorganic carbon were only observed for specific growth rates and photosynthetic efficiency. The decreased growth rate with increasing $CO_{2(aq)}$ concentrations suggest that the primary production by *P. globosa* will decrease in the future ocean. Furthermore, the increasing atmospheric CO_2 concentrations are accompanied by increasing temperature as discussed by Peperzak (2003) and results of previous studies with *P. globosa* on temperature effects on this species have shown that increasing temperatures also lead to a decreased growth rate (Peperzak, 2003) and decreased colony formation (Wang et al., 2010b). This suggests that the combination of increasing CO_2 and temperature will most likely amplify the effects of either one of these stress factors. Because *P. globosa* is a strong DMS producer, a decrease in *P. globosa* presence would cause a decrease in DMS production. Since DMS acts as cloud condensation nuclei (CCN), thereby increasing cloud albedo and counteracting global warming (Charlson et al., 1987; Clarke et al., 1998), a decrease in *P. globosa* presence would lead to a decrease in the strength of the negative feedback to global warming, hence increasing temperatures further.

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Supplementary material related to this article is available online at: http://www.biogeosciences-discuss.net/8/12353/2011/bgd-8-12353-2011-supplement.pdf.

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Table 1. Average (± standard deviation) concentrations of total alkalinity (A_T, $n = 12$) and dissolved inorganic carbon (DIC), both in µmol kg^{-1}, in the culture vessels with *P. globosa*. Based on these measured A_T and DIC concentrations, (data in Supplement, Table S2 and S3), the pCO_2 (µatm) and $CO_{2(aq)}$ (µmol kg^{-1}) were calculated (see Materials and methods).

	A_T measured (µmol kg^{-1})	A_T predicted (µmol kg^{-1})	DIC (µmol kg^{-1})	pCO_2 (µatm)	$CO_{2(aq)}$ (µmol kg^{-1})
High Light					
Low $CO_{2(aq)}$ ($n = 11$)	2476.3 (±3.9)	2480.7 (±1.8)	1921.7 (±15.7)	109.6 (±6.8)	4.1 (±0.3)
Intermediate $CO_{2(aq)}$ ($n = 10$)	2476.2 (±3.9)	2487.2 (±1.8)	2130.9 (±15.7)	259.0 (±6.8)	9.7 (±0.3)
High $CO_{2(aq)}$ ($n = 11$)	2477.1 (±5.8)	2492.4 (±1.4)	2284.1 (±19.1)	549.4 (±52.1)	20.5 (±1.9)
Low Light					
Low $CO_{2(aq)}$ ($n = 11$)	2475.8 (±3.9)	2477.7 (±1.3)	1939.9 (±19.4)	117.9 (±8.2)	4.2 (±0.3)
Intermediate $CO_{2(aq)}$ ($n = 12$)	2474.4 (±2.6)	2477.3 (±1.1)	2003.4 (±18.5)	152.4 (±10.7)	5.7 (±0.4)
High $CO_{2(aq)}$ ($n = 12$)	2479.0 (±3.0)	2492.0 (±1.5)	2313.2 (±9.9)	641.8 (±37.2)	24.0 (±1.4)

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Table 2. Photosynthetic efficiency (F_V/F_M, arbitrary unit a.u. $n = 12$ for each treatment, except $n = 10$ for intermediate $CO_{2(aq)}$ HL) and the cellular chlorophyll-*a* contents (pg cell^{-1}, $n = 6$) of the *P. globosa* cultures in the different experimental treatments. Standard deviations are given between brackets.

	F_V/F_M (a.u.)		chlorophyll-*a* (pg cell^{-1})	
	High light	Low light	High light	Low light
Low $CO_{2(aq)}$	0.56 (±0.03)	0.71 (±0.02)	0.16 (±0.01)	0.22 (±0.02)
Intermediate $CO_{2(aq)}$	0.65 (±0.02)	0.70 (±0.03)	0.15 (±0.01)	0.23 (±0.03)
High $CO_{2(aq)}$	0.66 (±0.02)	0.70 (±0.02)	0.16 (±0.02)	0.24 (±0.03)

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Table 3. The ratio values of particulate organic carbon to chlorophyll-*a* ratio (POC:chl-*a*) in the different light and carbon treatments. Standard deviations are given between brackets, $n = 6$ for all treatments.

	High light	Low light
Low $CO_{2(aq)}$	69.1 (±4.4)	53.6 (±8.8)
Intermediate $CO_{2(aq)}$	82.4 (±11.4)	54.0 (±7.5)
High $CO_{2(aq)}$	76.2 (± 24.8)	56.1 (± 8.4)

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Table 4. Rates of nutrient uptake (fmol cell^{-1}d^{-1}) in the culture vessels and the nitrate to phosphate uptake ratios (N:P). Standard deviations are given between brackets, $n = 6$ for each treatment, except for high light, low and intermediate $CO_{2(aq)}$ where $n = 5$).

	NO_3^- (fmol cell^{-1} d^{-1})		PO_4^{3-} (fmol cell^{-1} d^{-1})		N:P	
	High light	Low light	High light	Low light	High light	Low light
Low $CO_{2(aq)}$	118.4 (±22.6)	96.2 (±17.1)	6.9 (±1.5)	5.7 (±1.0)	17.4 (±2.9)	17.1 (±3.5)
Intermediate $CO_{2(aq)}$	133.5 (±29.8)	115.1 (±18.6)	9.2 (±3.5)	7.9 (±7.5)	15.7 (±4.6)	21.6 (±10.6)
High $CO_{2(aq)}$	128.4 (±18.5)	142.3 (±22.7)	8.8 (±2.0)	9.0 (±3.2)	15.1 (±3.2)	17.0 (±5.1)

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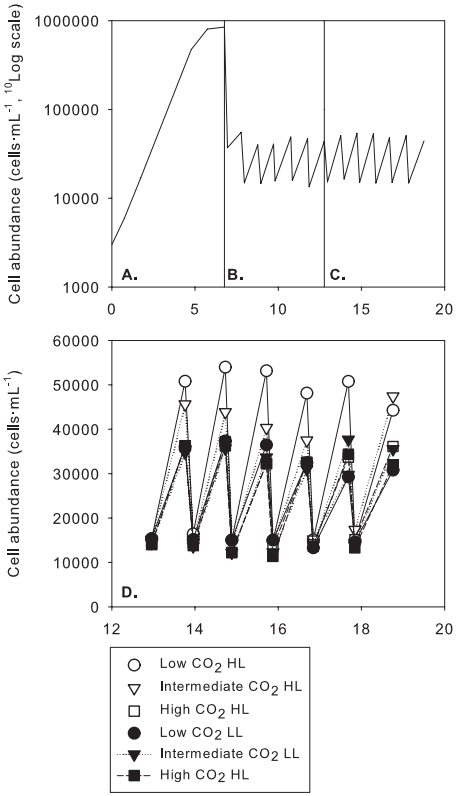

Fig. 1. The typical cell abundance during the different phases of the $CO_{2(aq)}$ and light manipulation experiments with *P. globosa*, shown for the low $CO_{2(aq)}$ high light culture in **(A)** to **(C)**. **(A)** batch phase, **(B)** pre-experimental phase and **(C)** experimental phase, as described in the materials and methods section. **(D)** shows the cell abundance during the experimental phase of all treatments. Low and intermediate $CO_{2(aq)}$ HL show a faster increase in cell abundance than the other treatments, which are similar to each other. Cell count data can be found in the Supplement, Table S1. HL denotes high light cultures and LL denotes low light cultures.

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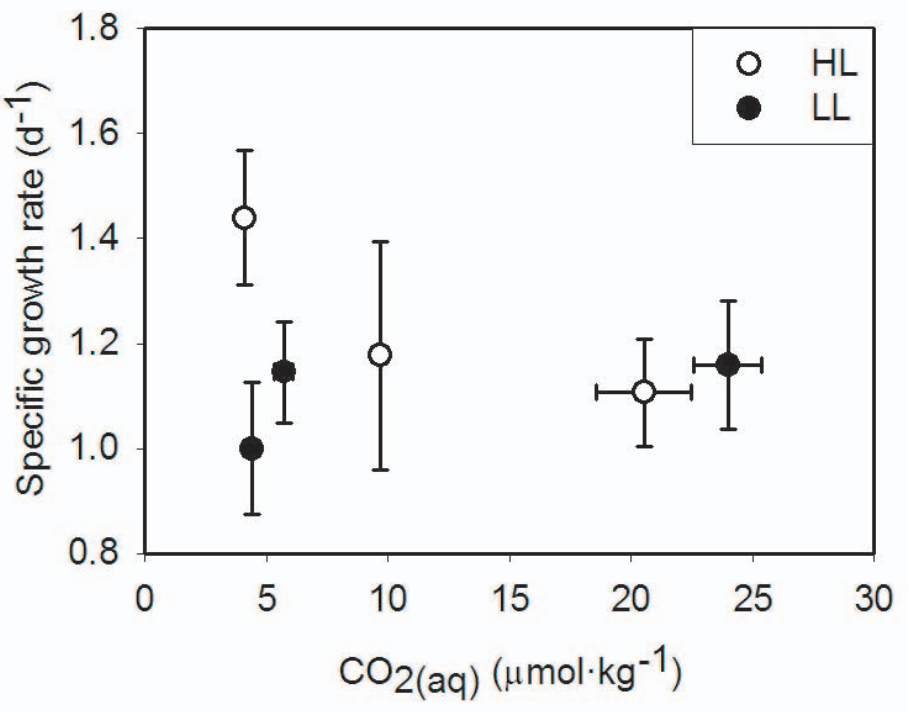

Fig. 2. Growth rates (μd^{-1}, $n = 6$) of *P. globosa* during the experiment versus the average $CO_{2(aq)}$ concentration between dilutions. Open circles denote the HL cultures, closed circles denote LL cultures. Error bars denote the standard deviation.

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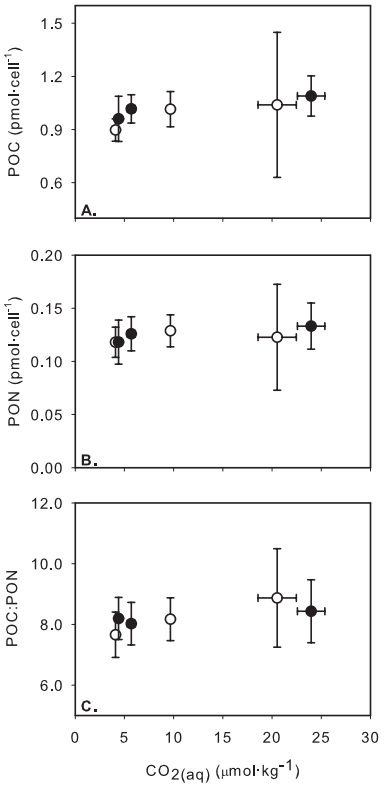

Fig. 3. Cellular particulate organic carbon (POC) and particulate organic nitrogen (PON) concentrations versus the $CO_{2(aq)}$ concentration. Open symbols denote the HL cultures, closed symbols the LL cultures. Error bars denote the standard deviation ($n = 6$). **(A)** POC (pmol cell^{-1}) concentrations in the different treatments. **(B)** PON (pmol cell^{-1}) contents in the different treatments. **(C)** POC:PON ratio in the different treatments.

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