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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 an ecologically dominating phytoplankton species in many areas around the world. 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: $4 \mu mol\,kg^{-1}$, intermediate: 6–$10 \mu mol\,kg^{-1}$ and high $CO_{2(aq)}$: 21–$24 \mu mol\,kg^{-1}$) and two different light intensities (low light, suboptimal: $80 \mu mol$ photons $m^{-2}\,s^{-1}$ and high light, light saturated: $240 \mu mol$ 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 (F_V/F_M) increased with increasing $CO_{2(aq)}$ from 0.56 to 0.66. The different light conditions affected photosynthetic efficiency and cellular 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 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*). While the first two species are found in Antarctic and Arctic waters respectively, *P. globosa* is found in temperate and tropical waters (Schoemann et al., 2005). Like the other two species, *P. globosa* is well known for its polymorphic life cycle, during which it alternately occurs as 4 different morphotypes; diploid colonial cells, diploid flagellates and two different types of haploid flagellates (Rousseau et al., 2007). Solitary cells of *P. globosa* can be grazed upon by micro-zooplankton and are susceptible to viral infection. The large colonies on the other hand, which can reach a size of up to 3 cm in diameter and can contain thousands of cells in a polysaccharide matrix, are grazed upon by meso-zooplankton, and are not prone to viral infection (Schoemann et al., 2005; Whipple et al., 2005 and references therein). It is not exactly clear what causes colony formation in *P. globosa*. Colonies are always preceded by solitary cells (Whipple et al., 2005). One of the triggers for colony formation in the Dutch coastal zone of the North Sea appears to be light, where colony formation starts when a daily irradiance threshold of $100\,W\,m^{-2}\,d^{-1}$, assuming a 12 h light period, is reached. This is comparable to approximately $40 \mu mol$ photons $m^{-2}\,s^{-1}$ (Peperzak et al., 1998; L. Peperzak, NIOZ, Texel, personal communication, 2012). However, this irradiance threshold is strain dependent, since colony formation of other strains has been observed in light conditions between 10 and $20 \mu mol$ photons $m^{-2}\,s^{-1}$

(Riegman and van Boekel, 1996 and references therein). Other possible triggers for colony formation are grazing, grazer-released info chemicals, genetic mechanisms, nutrient conditions, the presence of substrate and water turbulence (Whipple et al., 2005; Rousseau et al., 1994, 2007 and references therein). The presence of grazers increases the colony size of *P. globosa* (Tang, 2003). In the Dutch coastal North Sea and the Wadden Sea, blooms of *P. globosa* occur annually (April–June). 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. Besides being recognized as a harmful species, *Phaeocystis globosa* is also known for the production of dimethylsulfoniopropionate (DMSP). After release from the cell, part of the DMSP is enzymatically cleaved into dimethylsulfide (DMS) and acrylate (Stefels et al., 1995; Stefels, 2000). The DMS production by *P. globosa* in the Dutch coastal North Sea is largest during the start of the exponential growth phase of the bloom. However, this appears to be strain specific. In Chinese waters, DMSP production by *P. globosa* is higher in the stationary phase than in the exponential phase. Here, DMSP production and DMS release is strongly affected by the temperature and the salinity of the seawater (Shen et al., 2011). While the exact function of DMSP for the algal cell is still unknown, it is known that it serves as osmotic protection. Furthermore, in the atmosphere, the oxidation products of DMS can impact global climate by acting as condensation nuclei, changing the cloud albedo, and having a cooling effect on Earth's climate (Charlson et al., 1987; Stefels et al., 1995; Stefels, 2000; Clarke et al., 1998). This gives *P. globosa* an important role in the Earth's sulfur cycle (Schoemann et al., 2005).

The decline of a *Phaeocystis* bloom is usually characterized by two phenomena: the formation of micro-aggregates through the invasion of the mucus by bacteria; and the formation of ghost colonies, which are due to the emigration of flagellated cells from the colony into the external medium. These flagellated cells can induce a new bloom (Rousseau et al., 1994). Although colonies are not susceptible to viral infection, solitary cells are, and therefore, viral lysis is an important actor in carbon recycling. It is a major loss factor for *P. globosa* and other *Phaeocystis* species 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; Jacobsen et al., 2007). 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). The 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 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 future oceans. The large blooms of *P. globosa* assimilate significant amounts of inorganic carbon into cell material and mucus and may therefore act as a feedback mechanism to increasing carbon concentrations in the atmosphere. 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, NIOZ, Texel, personal communication, 2011).

Atmospheric carbon dioxide (CO$_2$) concentrations are increasing due to anthropogenic carbon dioxide (CO$_2$) emissions. According to current predictions, following the A1B scenario of the Intergovernmental Panel on Climate Change (IPCC), this rise will continue and atmospheric CO$_2$ concentrations will increase up to 800 µatm by the year 2100 AD (Meehl et al., 2007). As CO$_2$ is a greenhouse gas and can absorb infrared (IR) energy (Millero, 1996), this rise can lead to a surface temperature increase of 2.8 °C by 2100 AD according to this same scenario. Further important effects of this increase in atmospheric CO$_2$ concentration are increased stratification at higher latitude surface waters due to a decrease in the strength of the Meridional Overturning Circulation (MOC), and a decrease in cloud cover and precipitation in the low and mid-latitudes, whereas cloud cover and precipitation in the high latitudes will increase (Meehl et al., 2007). The combination of increased stratification and decreased cloud cover will cause phytoplankton to encounter longer periods of high light conditions. Furthermore, by the continuous exchange of CO$_2$ between the atmosphere and surface waters, a large part of the anthropogenic CO$_2$ is stored in the oceans, thereby increasing the CO$_2$ concentrations in the surface waters (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$_3^-$) concentrations, but a decrease in carbonate ion (CO$_3^{2-}$) concentrations (Millero, 1996). 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., 2012). Studies on the effects of different CO$_2$ concentrations on HAB species have been done, notably on dinoflagellates, such as *Karlodinium veneficum* (Fu et al., 2010), and diatoms; in example different *Pseudonitzschia* species (Sun et al., 2011; Tatters et al., 2012). Results of these studies show that toxicity of these species increases with increasing CO$_2$ concentrations, especially in the case of a combined effect of nutrient limitation and high CO$_2$. For *Phaeocystis globosa* a number of studies have been published on the effects of temperature increase, CO$_2$ increase and different light regimes on the species. 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. Similarly, a more recent study by Wang et al. (2010b) showed a decrease in colony size with increasing temperature. In a different study Wang et al. (2010a) focused on inorganic carbon effects, but did not include temperature or light, 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. Chen and Gao (2011) found that there is a combined effect of light, UV-radiation and CO$_2$ concentration on the photosynthetic yield and growth rate. Because the focus of this study was towards photosynthesis and growth, many other parameters, such as nutrient uptake rates and organic carbon production, have not been described in this latter study. Here we report on 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, which were chosen to be suboptimal (referred to as low light: LL 80 µmol photons m^{-2} s^{-1}) and saturating (referred to as high light, HL: 240 µmol photons m^{-2} s^{-1}) light intensity for cell division (Jahnke, 1989). 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) were used. These concentrations correspond 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, isolated by R. Koeman in 2000) was cultured in aged natural seawater originating from the Gulf of Biscay in 1998 (as described by Timmermans et al., 2001). This natural seawater was filtered upon collection and kept in the dark in 1 m^3 vessels. The seawater was filter sterilized with a Sartobran 105 filter (pre-filter 0.45 µm and end-filter 0.20 µm pore size). Because this seawater has very low nutrient concentrations (nitrate: 0.04 µmol l^{-1}, phosphate 0.03 µmol l^{-1} and silicate 0.84 µmol l^{-1}), 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(OH)$_4$. 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 consists of 61 polymethylmethacrylate (PMMA) culture vessels. These were water jacketed and temperature controlled with thermostat baths (Lauda Ecoline StarEdition RE104), set to 15.0 °C (±0.1), to ensure constant temperature in the culture vessels. The PMMA has a negligible gas diffusion coefficient for CO$_2$ and is transparent for the photosynthetic active radiation (PAR) spectrum. The tubing and ports in the lid of the culture vessels (sampling, addition of medium, and the air in- and outlet) were made of PFA tubing (Teflon, Polyfluor Plastics, The Netherlands) and nylon (Swagelok, USA), respectively, to ensure gas tight connections. The gas supply and outlet had filters attached in order to prevent particles present in the air to reach the culture vessels. Light conditions in the culture vessels were saturating light (high light intensity 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. Light was provided by Biolux cool daylight lamps (Philips, TL-D 965/18W). The culture vessels were filled with 5 l medium and 1 l air and were continuously 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 air mixtures (atmospheric air, with adjusted CO$_2$ concentration, HiQ-Line Linde gas Benelux, Schiedam, The Netherlands) 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). A Teflon coated stirrer bar connected to the lid of the culture vessel by a flexible joint, ensured continuous gently stirring of the culture at a speed of 50 rpm. Prior to the experiments, *P. 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, which was determined as described below, (early in the exponential growth phase: 1.5 × 10^4 cells ml^{-1}) and cultured semicontinuously for 6 days (pre-experimental phase, cell abundance ranging between 1.3 × 10^4 and 5.6 × 10^4 cells ml^{-1}, ∼8 generations, 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

Table 1. Average ($\pm$ standard deviation) concentrations of total alkalinity (A_T, $n = 12$) and dissolved inorganic carbon (DIC), both in μmol kg^{-1} and pCO$_2$ (μatm) and CO$_{2(aq)}$ (μmol kg^{-1}), in the culture vessels with *P. globosa*. Based on the measured A_T and DIC concentrations (data in Supplement, Tables S2 and S3), the pCO$_2$ (μatm) and CO$_{2(aq)}$ (μmol kg^{-1}) were calculated (see Sect. 2). The average CO$_{2(aq)}$ concentration (μmol kg^{-1}) in the supply vessel was based on the measured A_T and DIC concentration in the supply vessels.

	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})	Supply vessel CO$_{2(aq)}$ (μmol kg^{-1}, $n = 2$)
High Light						
Low CO$_{2(aq)}$ ($n = 11$)	2476.3 ($\pm$3.9)	2480.7 ($\pm$1.8)	1921.7 ($\pm$15.7)	109.6 ($\pm$6.8)	4.1 ($\pm$0.3)	5.0 ($\pm$0.2)
Intermediate CO$_{2(aq)}$ ($n = 10$)	2476.2 ($\pm$3.9)	2487.2 ($\pm$1.8)	2130.9 ($\pm$15.7)	259.0 ($\pm$6.8)	9.7 ($\pm$0.3)	12.2 ($\pm$0.2)
High CO$_{2(aq)}$ ($n = 11$)	2477.1 ($\pm$5.8)	2492.4 ($\pm$1.4)	2284.1 ($\pm$19.1)	549.4 ($\pm$52.1)	20.5 ($\pm$1.9)	23.1 ($\pm$0.7)
Low Light						
Low CO$_{2(aq)}$ ($n = 11$)	2475.8 ($\pm$3.9)	2477.7 ($\pm$1.3)	1939.9 ($\pm$19.4)	117.9 ($\pm$8.2)	4.2 ($\pm$0.3)	5.1 ($n = 1$)
Intermediate CO$_{2(aq)}$ ($n = 12$)	2474.4 ($\pm$2.6)	2477.3 ($\pm$1.1)	2003.4 ($\pm$18.5)	152.4 ($\pm$10.7)	5.7 ($\pm$0.4)	7.2 ($\pm$0.0)
High CO$_{2(aq)}$ ($n = 12$)	2479.0 ($\pm$3.0)	2492.0 ($\pm$1.5)	2313.2 ($\pm$9.9)	641.8 ($\pm$37.2)	24.0 ($\pm$1.4)	24.8 ($\pm$1.9)

the experimental conditions (as described by Riebesell et al., 2010). Dilutions were made according to cell abundance.

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, $\sim$9 generations (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).

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 Professor 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$_2$, by the use of CO$_2$sys (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 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 at least 5 min to allow for dark adaptation. A PAM fluorometer (Pulse Amplitude
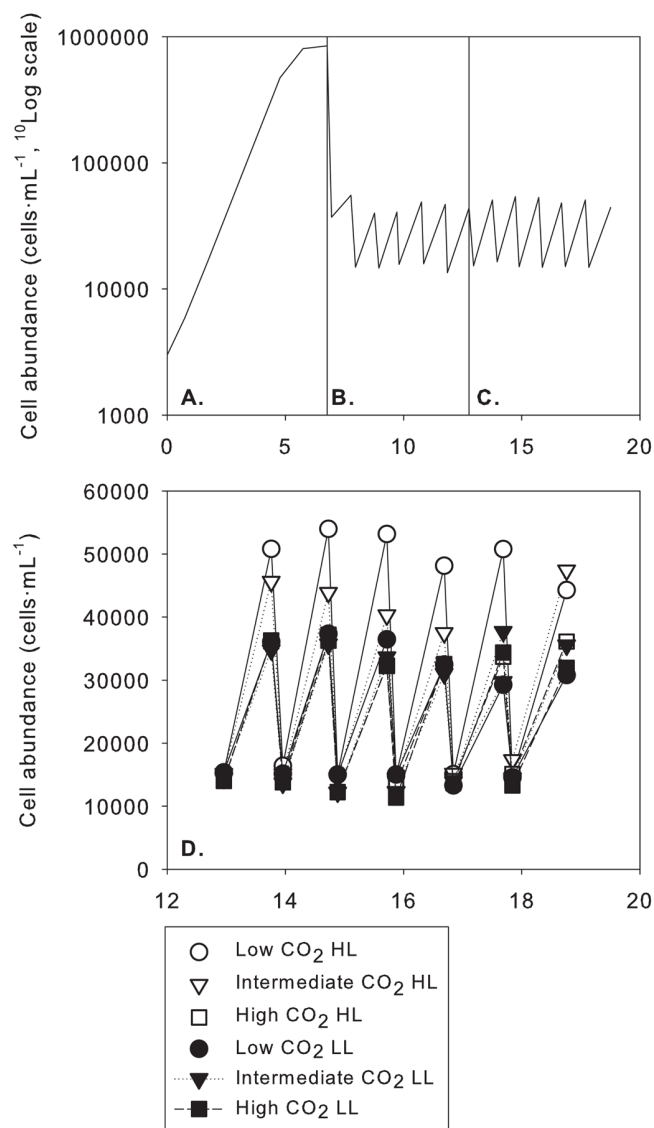
Fig. 1. The typical cell abundance during the different phases of the $CO_{2(aq)}$ and light manipulation experiments with *P. globosa,* shown for the low $CO_{2(aq)}$ high light culture in Fig. 1a to c. **(A)** Batch phase, **(B)** pre-experimental phase and **(C)** experimental phase, as described 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 supplementary files, Table S1. HL denotes high light cultures and LL denotes low light cultures.

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 $F_V = F_M - F_0$).

2.4.2 Chlorophyll *a*

Chlorophyll *a* concentrations of the cultures were determined by filtration of 150 ml sample on a 0.7 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}.

2.5 Particulate organic carbon and nitrogen

Particulate organic carbon (POC) and nitrogen (PON) were determined by the filtration of 100 ml sample on a pre-combusted 0.7 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 3 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).

3   Results

3.1   Dissolved inorganic carbon and total alkalinity

Both DIC and A_T (data available in Supplement, Tables 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 higher 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 $23.0 \, \mu\text{mol kg}^{-1}$ ($r^2 = 0.31$, $n = 16$). The average difference between $A_{T(pred)}$ and $A_{T(meas)}$ was 10.1 (± 6.7) $\mu\text{mol kg}^{-1}$. 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 $18.6 \, \mu\text{mol kg}^{-1}$ ($r^2 = 0.10$, $n = 18$). In these low light cultures, the average difference between $A_{T(pred)}$ and $A_{T(meas)}$ was 6.2 (± 5.9) $\mu\text{mol kg}^{-1}$.

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). The cells grew fast at $\mu > 0.7 \, \text{d}^{-1}$. Average specific growth rates in the HL cultures were significantly higher than in the LL cultures ($F_{(1,31)} = 5.008$, $p = 0.033$), which is largely due to the high growth rates at low $CO_{2(aq)}$ concentrations in the HL cultures (Fig. 2). In the HL cultures growth rates were highest at low $CO_{2(aq)}$ ($\mu = 1.4 \, \text{d}^{-1} \pm 0.1$) while growth rates significantly decreased with increasing $CO_{2(aq)}$ to $\mu = 1.1 \, \text{d}^{-1}$ (± 0.1) in the high $CO_{2(aq)}$ treatment ($\tau = -0.667$, $p < 0.001$, $n = 18$, Fig. 2). In the LL cultures no significant trend was observed, growth rates ranged between $\mu = 1.0 \, \text{d}^{-1}$ (± 0.1) in the low $CO_{2(aq)}$ treatment to $\mu = 1.2 \, \text{d}^{-1}$ (± 0.1) in the high $CO_{2(aq)}$ treatment (Fig. 2).

3.3   Photosynthetic efficiency and Chlorophyll *a*

The photosynthetic efficiency (F_V/F_M) varied between 0.50 and 0.75 throughout the experiment, indicating that the cells
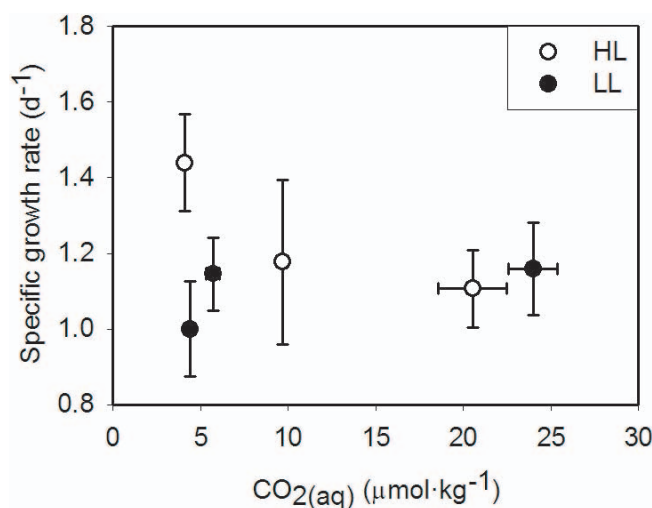
Fig. 2. Growth rates (μ, d^{-1}, $n = 6$) of *P. globosa* during the experiment versus the average $CO_{2(aq)}$ concentration between dilutions. Open circles denote the HL cultures, closed circles denote LL cultures. Error bars denote the standard deviation.

were healthy and fast growing. The F_V/F_M of the HL 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 LL cultures was significantly higher than that of the HL 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 HL conditions ($\tau = 0.475$, $p < 0.001$, $n = 34$).

Chlorophyll *a* concentrations in the *P. globosa* cells varied significantly between the two light conditions with approximately 15 pg cell^{-1} in the HL conditions as compared to higher concentrations, approximately 23 pg cell^{-1} in the LL conditions ($F_{(1,33)} = 116.246$, $p < 0.001$, $n = 36$, Table 2). In contrast, the ambient $CO_{2(aq)}$ concentrations did not affect the chlorophyll *a* concentration in *P. globosa* cells (HL: $\tau = -0.020$, $p = 0.910$, $n = 18$; LL: $\tau = 0.203$, $p = 0.240$, $n = 18$, Table 2).

3.4   Particulate organic carbon and nitrogen

The POC content of *P. globosa* ranged between 0.9 pmol C cell^{-1} in the low $CO_{2(aq)}$ HL culture and 1.1 pmol C cell^{-1} in the high $CO_{2(aq)}$ LL culture (Table 3). The cellular POC concentration was neither influenced by the different $CO_{2(aq)}$ treatments (HL: $\tau = 0.242$, $p = 0.161$, $n = 18$; LL: $\tau = 0.324$, $p = 0.070$, $n = 17$), nor by different light intensities ($F_{(1,33)} = 0.000$, $p = 1.000$, $n = 36$, Table 3).

The PON content of *P. globosa* ranged between 0.12 pmol N cell^{-1} in the low $CO_{2(aq)}$ HL culture and 0.13 pmol N cell^{-1} in the high $CO_{2(aq)}$ LL culture (Table 3). Similar to the POC content of the phytoplankton cells, different $CO_{2(aq)}$ treatments did also not affect the cellular PON

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 ($\pm$0.03)	0.71 ($\pm$0.02)	0.16 ($\pm$0.01)	0.22 ($\pm$0.02)
Intermediate CO$_{2(aq)}$	0.65 ($\pm$0.02)	0.70 ($\pm$0.03)	0.15 ($\pm$0.01)	0.23 ($\pm$0.03)
High CO$_{2(aq)}$	0.66 ($\pm$0.02)	0.70 ($\pm$0.02)	0.16 ($\pm$0.02)	0.24 ($\pm$0.03)

Table 3. Particulate organic carbon (POC) and nitrogen (PON) contents per cell (pmol cell^{-1}), and the ratio values of POC to PON (POC:PON) and POC to chlorophyll *a* (POC:Chl *a*) in the different light and carbon treatments. Standard deviations are given between brackets, $n = 6$ for all treatments.

	POC	PON	POC:PON	POC:Chl *a*
High Light				
Low CO$_{2(aq)}$	0.9 ($\pm$0.06)	0.12 ($\pm$0.01)	7.7 ($\pm$0.8)	69.1 ($\pm$4.4)
Intermediate CO$_{2(aq)}$	1.0 ($\pm$0.11)	0.13 ($\pm$0.02)	8.2 ($\pm$0.7)	82.4 ($\pm$11.4)
High CO$_{2(aq)}$	1.0 ($\pm$0.41)	0.12 ($\pm$0.05)	8.9 ($\pm$1.6)	76.2 ($\pm$24.8)
Low Light				
Low CO$_{2(aq)}$	1.0 ($\pm$0.13)	0.12 ($\pm$0.02)	8.2 ($\pm$0.7)	53.6 ($\pm$8.8)
Intermediate CO$_{2(aq)}$	1.0 ($\pm$0.06)	0.13 ($\pm$0.02)	8.0 ($\pm$0.7)	54.0 ($\pm$7.5)
High CO$_{2(aq)}$	1.1 ($\pm$0.11)	0.13 ($\pm$0.02)	8.4 ($\pm$1.0)	56.1 ($\pm$8.4)

content (HL: $\tau = 0.216$, $p = 0.211$, $n = 18$; LL: $\tau = 0.190$, $p = 0.272$, $n = 18$), nor did the different light conditions ($F_{(1,33)} = 0.000$, $p = 1.000$, $n = 36$, Table 3).

With little effect of both light intensities and the CO$_{2(aq)}$ concentration on the POC and PON concentration of the cells, the POC:PON ratio also remained virtually constant, ranging from 7.7 ($\pm$0.8) to 8.9 ($\pm$1.6) in the high light cultures and between 8.0 ($\pm$0.7) and 8.4 ($\pm$1.0) in the low light cultures (Table 3).

The ratio values of organic carbon (POC) to chlorophyll *a* (POC:Chl *a*) on the other hand, varied between 53.6 ($\pm$8.8) pg C pg Chl *a*$^{-1}$ in the low [CO$_{2(aq)}$] LL treatment to 82.4 ($\pm$11.4) pg C pg Chl *a*$^{-1}$ in the intermediate [CO$_{2(aq)}$] HL treatment (Table 3).

There was no significant effect of the [CO$_{2(aq)}$] on the POC:Chl *a* ratio for both the HL ($\tau = 0.229$, $p = 0.185$, $n = 18$) and the LL 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, due to lower Chl *a* concentrations per cell in the HL cultures ($F_{(1,33)} = 25.309$, $p < 0.001$, $n = 36$, Tables 2 and 3).

3.5 Nutrients

The removal of the dissolved nutrients between two consecutive dilutions was on average 0.15 ($\pm$0.07) µmol l^{-1} for phosphate and 2.34 ($\pm$0.72) µmol l^{-1} for nitrate (Supplement, Tables 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 CO$_{2(aq)}$ LL culture and the intermediate CO$_{2(aq)}$ HL culture, respectively (Table 4). Both in the HL ($\tau = 0.451$, $p = 0.025$, $n = 14$), and in the LL treatments ($\tau = 0.412$, $p = 0.017$, $n = 18$) the increasing [CO$_{2(aq)}$] stimulated phosphate uptake rates, but the variability in these 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 HL 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 LL 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

Table 4. Rates of nutrient uptake (fmol cell^{-1} d^{-1}) in the culture vessels and the nitrogen to phosphorus 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 ($\pm$22.6)	96.2 ($\pm$17.1)	6.9 ($\pm$1.5)	5.7 ($\pm$1.0)	17.4 ($\pm$2.9)	17.1 ($\pm$3.5)
Intermediate $CO_{2(aq)}$	133.5 ($\pm$29.8)	115.1 ($\pm$18.6)	9.2 ($\pm$3.5)	7.9 ($\pm$7.5)	15.7 ($\pm$4.6)	21.6 ($\pm$10.6)
High $CO_{2(aq)}$	128.4 ($\pm$18.5)	142.3 ($\pm$22.7)	8.8 ($\pm$2.0)	9.0 ($\pm$3.2)	15.1 ($\pm$3.2)	17.0 ($\pm$5.1)

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 ranged between 17.0 and 21.6 in the low light cultures and between 15.1 and 17.4 in the HL cultures (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 (HL: $\tau = -0.385$, $p = 0.055$, $n = 14$; LL: $\tau = 0.098$, $p = 0.570$, $n = 18$).

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*. 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 concentration of inorganic 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 (approx. 1 %) compared to the A_T (approx. 0.1 %). Due to the daily dilution approach, this variability still was minor and resulted in stable inorganic carbon chemistry in the cultures, with a maximum variability in the $CO_{2(aq)}$ concentrations of only 10 % within each culture. A difference in the [$CO_{2(aq)}$] was found between the intermediate $CO_{2(aq)}$ HL and LL culture, with the [$CO_{2(aq)}$] of the LL culture being lower than that of the HL culture. This was due to technical problems rather than to biological activity, since the [$CO_{2(aq)}$] in the supply vessel was also significantly lower (Table 1). However, because regular samples for the determination of the carbonate system were taken, this could be accounted for in the analysis of the results.

In their study, Kim et al. (2006) reported significant effects of particulate organic carbon concentrations on A_T measurements. Increasing [POC] led to an increased discrepancy between the measured A_T of filtered and unfiltered phyto-

plankton cultures. The slight discrepancy between the measured and the predicted A_T in our study 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). This difference in response is likely due to the [POC] in the different studies. Particulate organic carbon concentrations in our study ranged between 12.3 and 55.0 µmol l^{-1}, with an average of 38.1 µmol l^{-1}, while the [POC] in the study by Kim et al. (2006) ranged between approximately 200 and 1100 µmol l^{-1}. This shows that it is very important to maintain low cell abundance in carbon perturbation experiments in order to obtain accurate data.

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, which is comparable to the results of Wang et al. (2010a), who also did not find an increased growth rate of solitary cells of *P. globosa* in their experiments. However, their results also showed that the growth rate of colonial cells was significantly increased in the high CO_2 cultures. This demonstrates that it is necessary to study the different morphotypes of *P. globosa* in more detail, since the cells in high light cultures of our experiments grew comparatively faster as compared to the low light cultures, and here the growth rate was negatively influenced by higher [$CO_{2(aq)}$]. The results from the high light cultures corroborate the observations by Chen and Gao (2011), who also found decreasing growth rates with increasing [CO_2] in high light conditions. In contrast, under low light conditions growth rates of *P. globosa* increased with increasing CO_2 in their study. Because of the differences in the research question between the study of Chen and Gao (2011), who were interested in natural light regimes, and our study with stable light regimes, it is difficult to compare the two. Irrespective of this, our results, and the results of Wang et al. (2010a) suggest that the effects of CO_2

on growth rates of solitary *P. globosa* cells are consistent among different strains of this species.

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. These results contradict the results of Wang et al. (2010a), who showed a lower F_V/F_M at low CO_2. In their study, F_V/F_M in the batch cultures decreased during the experiment, and the difference between the two treatments only became visible after the number of colonial cells in the cultures dropped significantly. This suggests that this difference in F_V/F_M is most likely not entirely due to the different CO_2 concentrations, but more likely related to the abundance of colonial and solitary cells and perhaps nutrient and light limitation. Because the environmental conditions in batch cultures are strongly influenced by growth, we used a semi-continuous set-up, resulting in a stable F_V/F_M throughout the experiment. Similarly, cellular POC and PON concentrations were relatively stable throughout our experiments, but variability in cellular POC and PON content increased with increasing $[CO_{2(aq)}]$. Neither of the two parameters, nor the POC:PON ratios were affected by the different $CO_{2(aq)}$ treatments. This is in contrast to the findings by Wang et al. (2010a). 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, ranging between 7.7 and 8.9, were higher than the ratio's that have been previously measured in cultures or field samples of solitary *P. globosa* cells, which range between 4.0 and 6.0, but comparable to colonial stoichiometry, ranging between 6.5 and 9.7, the latter which was measured in P-deficient conditions (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:phosphorus) 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 our experimental setup. Light saturation for optimal cell division of *P. globosa* was reported to be 180 μmol photons m^{-2} s^{-1} (Jahnke, 1989;

Meyer et al., 2000), which is slightly lower that the saturating light conditions used in our experiments (240 μmol photons m^{-2} s^{-1}). The results of our experiments showed only a moderate effect of low light on the photosynthetic efficiency (F_V/F_M in LL cultures was 10–20 % higher, compared to the high light cultures), but the cells responded by increasing the chlorophyll *a* concentration by as much as 50 %. It has been shown that other *Phaeocystis* species are also affected by different light intensities and that for example *P. antarctica* is susceptible to photo-inhibition. This species needs a dark period, provided by deep mixing, for efficient photosynthesis (Mills et al., 2010; Arrigo et al., 1999). The differences in F_V/F_M in *P. globosa* due to the different light conditions in our study are comparable to findings of Mills et al. (2010) and van Leeuwe and Stefels (2007), who found a decrease of F_V/F_M in *P. antarctica* with increasing light conditions. Although in the study by Mills et al. (2010) no general effect of the irradiance level on chlorophyll concentrations was found. One of the tested strains (#1374) did show an increase in the POC:Chl *a* ratio with increasing light, which is similar to our results for *P. globosa*, although their ratios for *P. antarctica* were much higher than those of *P. globosa* in our study. Because the second strain of *P. antarctica* in the study by Mills et al. (2010) did not show an increasing POC:Chl *a* ratio, we might conclude that this is a strain specific response. The increased POC:Chl *a* ratio in our experiments was comparable to ratios measured for an Antarctic *Phaeocystis* sp. at 110 μmol photons m^{-2} s^{-1} (Stefels and Van Leeuwe, 1998). Light conditions also have shown to affect *P. pouchetii*. A study by Hegarty and Villareal (1998) has demonstrated that *P. pouchetii* reached maximum growth rates at low irradiance levels, while our results showed that *P. globosa* growth rates decreased in low light conditions, supporting the conclusion of Hegarty and Villareal (1998) that the two species differ in the relative growth potential at different light conditions.

The results of our study have shown that the effects of light on *P. globosa* are much less pronounced as compared to the other species. This could be explained by the environmental conditions in which these different species live. While *P. antarctica* and *P. pouchetii* are both cold water (polar) species, and therefore more subjected to periods of low light and darkness, *P. globosa* is a species that occurs in warmer, temperate waters, with smaller differences in light availability. Therefore, *P. globosa* is less likely to become photo-inhibited at the high light conditions in our experiments. Furthermore, our experiments have shown that in high light conditions, for example caused by increased stratification, growth rates are decreasing with increasing $[CO_{2(aq)}]$. This suggests that the primary production by *P. globosa* will decrease in the future ocean. The increased phosphate uptake rates at high $[CO_{2(aq)}]$ suggests that the cells require more nutrients at high $[CO_{2(aq)}]$. Because the Dutch coastal North Sea and Wadden Sea are phosphate controlled at present (Philippart et al., 2000; P. Ruardij, NIOZ, Texel, personal communication, 2011), the ability of *P. globosa* to

outcompete other phytoplankton species in these and other waters might decrease in the future. Because *P. globosa* is a strong DMS producer, a decrease in *P. globosa* presence would also cause a decrease in DMS production, which could lead to a decrease in the strength of the negative feedback to global warming, hence increasing temperatures further. The increasing temperature and the increasing atmospheric CO_2 concentrations will most likely amplify the effects of either one of these stress factors and can significantly change the primary production, and therefore the carbon sequestration by *P. globosa*.

Supplementary material related to this article is available online at: http://www.biogeosciences.net/9/ 1885/2012/bg-9-1885-2012-supplement.pdf.

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