Ocean acidification decreases the light-use efficiency in an Antarctic diatom under dynamic but not constant light

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Summary

• There is increasing evidence that different light intensities strongly modulate the effects of ocean acidification (OA) on marine phytoplankton. The aim of the present study was to investigate interactive effects of OA and dynamic light, mimicking natural mixing regimes.

• The Antarctic diatom Chaetoceros debilis was grown under two pCO₂ (390 and 1000 μatm) and light conditions (constant and dynamic), the latter yielding the same integrated irradiance over the day. To characterize interactive effects between treatments, growth, elemental composition, primary production and photophysiology were investigated.

• Dynamic light reduced growth and strongly altered the effects of OA on primary production, being unaffected by elevated pCO₂ under constant light, yet significantly reduced under dynamic light. Interactive effects between OA and light were also observed for Chl production and particulate organic carbon quotas.

• Response patterns can be explained by changes in the cellular energetic balance. While the energy transfer efficiency from photochemistry to biomass production (ΦE,C) was not affected by OA under constant light, it was drastically reduced under dynamic light. Contrasting responses under different light conditions need to be considered when making predictions regarding a more stratified and acidified future ocean.

Introduction

The Southern Ocean (SO) plays a pivotal role in the global carbon cycle (Marinov et al., 2006), strongly influencing atmospheric CO₂ concentrations on glacial–interglacial timescales (Moore et al., 2000; Sigman et al., 2010). Today, the SO takes up 15–40% of the anthropogenic CO₂ (Khatiwala et al., 2009), a large proportion of which is mediated by phytoplankton, in particular diatoms (Nelson et al., 1995; Takahashi et al., 2002). The potential for carbon sequestration via the biological pump (Volk & Hoffert, 1985) is, however, restricted through iron and light limitation (Martin, 1990; Moore et al., 2007; Feng et al., 2010). Regarding the latter, deep vertical mixing induced by strong winds leads to pronounced changes in the light regime as well as low integrated irradiances that phytoplankton cells encounter in the upper mixed layer (MacIntyre et al., 2000).

Diatoms tend to dominate under well-mixed, nutrient-rich environments where light is the main factor controlling growth rates (Sarthou et al., 2005). Even though diatom species were found to differ in their photophysiological characteristics, this group can generally be characterized by high photochemical efficiencies, low susceptibilities towards photooinhibition, and high plasticity in photoacclimation (Wagner et al., 2006; Lavaud et al., 2007; Kropuenske et al., 2009; Su et al., 2012; Li & Campbell, 2013). Overall, diatoms seem to be less compromised by fluctuating irradiances than other phytoplankton groups (van Leeuwe et al., 2005; Wagner et al., 2006; Lavaud et al., 2007; Jin et al., 2013). These physiological features can, to a large degree, explain the dominance of diatoms in natural phytoplankton assemblages exposed to deep-mixing regimes like the SO (Sarthou et al., 2005). Studies investigating the effects of dynamic light on diatoms often showed that while C : N ratios stayed constant, photosynthetic efficiencies increased and growth rates decreased compared with constant light regimes (e.g. van Leeuwe et al., 2005; Wagner et al., 2006; Kropuenske et al., 2009; Mills et al., 2010; Shatwell et al., 2012). This indicates increased costs imposed by continuous photoacclimation and/or time spent under nonoptimal configuration of the core physiological apparatus. Despite these general trends, large differences in the magnitude of responses were observed between studies. These could be caused by differences in environmental conditions (e.g. temperatures, nutrient concentrations, seawater carbonate chemistry), which may modulate phytoplankton cells’ ability to cope with fluctuating light fields (Jin et al., 2013).

Owing to the high solubility of CO₂ under low water temperatures (Sarmiento et al., 2004), the effects of increased CO₂ concentrations and decreased pH on SO phytoplankton have gained increasing attention in recent years (Tortell et al., 2008; Feng et al., 2010; Boelen et al., 2011; Hoogstraten et al., 2012a,b; Hoppe et al., 2013; Trimborn et al., 2013). The observed sensitivity of phytoplankton to these changes, commonly referred to as...
Ocean Acidification (OA), can be partially attributed to beneficial effects of an increased supply of CO₂. The carbon-fixing enzyme RubisCO has a poor affinity for CO₂, with half-saturation constants (K₅₀) being higher than the current concentrations of aquatic CO₂ (Badger et al., 1998). To overcome substrate limitation arising from this, phytoplankton employ so-called carbon concentrating mechanisms (CCMs), which increase the CO₂ concentration at the reactive site of RubisCO (Reinfelder, 2011). CCMs of diatoms include active CO₂ and HCO₃⁻ uptake, C₄-like pathways in some species, as well as the expression of carbonic anhydrase, which accelerates the inter-conversion of CO₂ and HCO₃⁻ (Morel et al., 1994; Reinfelder et al., 2000; Burkhardt et al., 2001). Even though CCMs of diatoms were found to be highly efficient in preventing carbon limitation under most conditions (e.g. Badger et al., 1998; Hopkinson et al., 2011; Trimborn et al., 2013), they are also commonly down-regulated under higher external CO₂ availability, lowering the overall metabolic costs of carbon acquisition under OA (Burkhardt et al., 2001; Rost et al., 2003; Trimborn et al., 2008).

Regarding the CO₂ sensitivity in primary production of diatom-dominated phytoplankton assemblages as well as isolated strains of the SO vary greatly between studies, indicating little to high potential for ‘CO₂ fertilization’ (e.g. Tortell et al., 2008; Feng et al., 2010; Boelen et al., 2011; Hoppe et al., 2013; Trimborn et al., 2014). Such differences in OA responses can be explained by intra- and interspecific variability (Langer et al., 2009; Trimborn et al., 2013), but also by deviating experimental conditions. Besides the impact of temperature (Tatters et al., 2013) and nutrient availability (Hoppe et al., 2013), the effect of light intensities on OA responses has been shown to be particularly important (Kranz et al., 2010; Ihnken et al., 2011; Gao et al., 2012a). In the coccolithophore Emiliania huxleyi, for example, the CO₂ sensitivity of carbon fixation and calcification was greatly enhanced under low vs high light (Rokitta & Rost, 2012). Several studies on diatoms have shown, furthermore, an increased susceptibility towards photoinhibition under elevated pCO₂ concentrations (Wu et al., 2010; McCarthy et al., 2012; Li & Campbell, 2013). Even though all of these studies increased our knowledge on the interactive effects between OA and light intensities, the transferability to processes in the ocean, where light intensities are highly dynamic, is questionable.

Regarding the potential interaction of OA and light regimes, there are only limited data in existence. Boelen et al. (2011) did not observe significant effects of pCO₂ concentrations up to 750 μatm under either constant or dynamic light for the Antarctic diatom Chaetoceros brevis. In the coccolithophore Gephyrocapsa oceanica, however, the combination of a pCO₂ of 1000 μatm and short-term (2 h) exposure to dynamic light led to lowered carbon fixation compared with ambient pCO₂ and constant light (Jin et al., 2013). In view of these conflicting results, a mechanistic understanding of the complex interactions between OA and dynamic light is required. As changes in light harvesting need to be balanced by the sum of all downstream processes, it is particularly important to focus on the interplay between the involved processes and their respective timescales. For example, comparing the short-term evolution of O₂ and production of energy carriers and reductive equivalents (ATP and NADPH) with the biomass build-up or growth on longer timescales clearly shows that both ‘ends of photosynthesis’ do not always match (Behrenfeld et al., 2008). Changes in environmental conditions, such as light regime or carbonate chemistry, will inevitably impact the balance of cellular processes, affecting the energy transfer efficiency of photosynthetic light harvesting to carbon fixation (Wagner et al., 2006; Rokitta & Rost, 2012).

In view of such considerations and earlier findings on the isolated effects of OA and dynamic light, the goal of the present study was to investigate how the energy transfer efficiency from photochemistry to biomass build-up and growth is affected by the interaction between OA and dynamic light. To do so, we acclimated the bloom-forming SO diatom species C. debilis to two pCO₂ concentrations (390 and 1000 μatm) as well as two light regimes (constant and dynamic light), the latter yielding the same integrated irradiance over the day (90 μmol photons m⁻² s⁻¹). This matrix approach was applied in order to test the hypothesis that dynamic light diminishes the beneficial effect of elevated pCO₂ often observed under constant light, and to understand the physiological mechanisms underlying the general acclimation responses.

Materials and Methods

Culture conditions

Monoclonal cultures of the diatom Chaetoceros debilis Cleve 1894 (isolated in 2004 by P. Assmy during RV Polarstern cruise ANT-XXI/3, European iron fertilization experiment (EIFEX), In-Patch, 49°36’S, 02°05’E; re-isolated by C. Hoppe in 2011) were grown in 11 glass bottles in semicontinuous dilute-batch cultures (2000–65 000 cells ml⁻¹; diluted every 4–5 d) at 3 ± 0.4°C in a 16 : 8 h, light : dark cycle. Media consisted of 0.2 μm sterile-filtered Antarctic seawater with a salinity of 34 enriched with macronutrients, yielding 180 μmol l⁻¹ nitrate, 12 μmol l⁻¹ phosphate and 108 μmol l⁻¹ silicate. Trace metals and vitamins were added according to F/2 medium (Guillard & Ryther, 1962).

For the constant light treatments (Fig. 1), an irradiance of 90 ± 10 μmol photons m⁻² s⁻¹ was applied. Also for the dynamic light treatments (Fig. 1), an average daily irradiance of 90 ± 10 μmol photons m⁻² s⁻¹ was applied. The dynamic light field was calculated assuming a spring situation with a mixed layer depth of 80 m, a mixing speed of 0.014 m s⁻¹ (Denman & Gargett, 1983), five mixing cycles d⁻¹ and an attenuation coefficient of 0.04 m⁻¹, leading to a maximum irradiance of 490 μmol photons m⁻² s⁻¹. The dynamic light modulation (Fig. 1) was controlled via the Control2000 programme of a Rumed incubator (1301; Rubarth Apparate, Laatzen, Germany). In both light treatments, irradiance was provided by identical daylight lamps (Philips Master TL-D 18W; emission peaks at wavelengths of 440, 560 and 635 nm), thus exposing the phytoplankton to the same spectral composition in all treatments. Light intensities were adjusted by neutral density screens and measured using an LI-1400 data logger (LI-Cor, Lincoln, NE, USA) equipped with a 4π sensor (Walz, Effeltrich, Germany).
Different pCO₂ conditions were achieved by continuous and gentle aeration of the incubation bottles with air of different CO₂ partial pressures (390 and 1000 μatm) as well as regular dilution of cultures with pre-aerated seawater medium to stable carbonate chemistry over the course of the experiments (Table 1) and to significant differences between pCO₂ (ANOVA, P < 0.001 for dissolved inorganic carbon (DIC), pH and pCO₂), but not between light treatments (ANOVA, P > 0.05). In the ambient treatments, pCO₂ concentrations were 382 ± 18 μatm for constant light and 400 ± 17 μatm for dynamic light. In the OA treatments, pCO₂ concentrations were 987 ± 28 μatm for constant light and 1026 ± 31 μatm for dynamic light. Over the duration of the experiment (> 5 wk), the drifts in DIC and TA compared with abiotic controls were < 2% and < 4%, respectively.

Carbonate chemistry

Samples for total alkalinity (TA; n = 14) were 0.7 μm filtered (glass fibre filters, GF/F; Whatman, Maidstone, UK) and stored in borosilicate bottles at 3°C. TA was estimated from duplicate potentiometric titration (Brewer et al., 1986) using a TitroLine alpha plus (Schott Instruments, Mainz, Germany) and corrected for systematic errors based on measurements of certified reference materials (CRMs provided by Prof. A. Dickson, Scripps, USA; batch no. 111; reproducibility ± 5 μmol kg⁻¹). DIC (n = 14) samples were filtered through 0.2 μm cellulose-acetate filters (Sartorius stedim) and stored in gas-tight borosilicate bottles at 3°C. DIC was measured colorimetrically in triplicates with a QuAAtro autoanalyser (Seal Analytical, Norderstedt, Germany; Stoll et al., 2001). The analyser was calibrated with NaHCO₃ solutions (with a salinity of 35, achieved by addition of NaCl) to achieve concentrations ranging from 1800 to 2300 μmol DIC kg⁻¹. CRMs were used for corrections of errors in instrument performance such as baseline drifts (reproducibility ± 8 μmol kg⁻¹).

Seawater pH_total (n = 14) was measured potentiometrically with a two-point calibrated glass reference electrode (IOline; Schott Instruments). An internal TRIS-based reference standard (Dickson et al., 2007) was used to correct for variability in electrode performance (reproducibility ± 0.015 pH units). Following suggestions by Hoppe et al. (2012), seawater carbonate chemistry (including pCO₂) was calculated from TA and pH using CO₂SYS (Pierrot et al., 2006). The dissociation constants of carbonic acid of Mehrbach et al. (1973; refitted by Dickson & Millero, 1987) were used for the calculations. Dissociation constants for KH₂SO₄ were taken from Dickson (1990).

Growth, elemental composition and production rates

Samples for cell counts were fixed with Lugols solution (1% final concentration) and counted on a light microscope (Axio Observer.D1; Zeiss) after 24 h sedimentation time in 10 ml Utermöhl chambers (Hydro-Bios, Kiel, Germany; > 1700 cells counted per sample). Samples for determination of Chl a were filtered onto 0.6 μm glass-fibre filters (GF/F; Whatman), immediately placed into liquid nitrogen and stored at −80°C until analysis. Chl a was subsequently extracted in 8 ml 90% acetone (2–3 h at 4°C). After removal of the filter, concentrations were determined on a fluorometer (TD-700; Turner Designs, Sunnyvale, CA, USA), using an acidification step (1 M HCl) to determine phaeopigments (Knap et al., 1996). Growth rate determinations started 1–2 d after redilution from daily Chl a sampling (n = 3) over 4 d (consecutive) within the first 15 min of the dark phase and were calculated as

| Treatment | DIC (μmol kg⁻¹) | TA (μmol kg⁻¹) | pH_total | pCO₂ (μatm) |
|-----------|-----------------|----------------|-----------|-------------|
| Constant light | 390 μatm CO₂ | 2092 ± 15 | 2250 ± 27 | 8.05 ± 0.02 | 382 ± 18 |
| | 1000 μatm CO₂ | 2202 ± 29 | 2258 ± 18 | 7.66 ± 0.03 | 987 ± 28 |
| Dynamic light | 390 μatm CO₂ | 2101 ± 27 | 2263 ± 33 | 8.03 ± 0.02 | 400 ± 17 |
| | 1000 μatm CO₂ | 2203 ± 25 | 2252 ± 27 | 7.65 ± 0.02 | 1026 ± 31 |

DIC, dissolved inorganic carbon. CO₂ partial pressure (pCO₂) was calculated from total alkalinity (TA) and pH_total at 3°C and a salinity of 34 using CO₂SYS (Pierrot et al., 2006), and concentrations of 12 and 108 μmol kg⁻¹ for phosphate and silicate, respectively.
where \([\text{Chl}]_1\) and \([\text{Chl}]_2\) denote the Chl concentrations at the sampling days \(t_1\) and \(t_2\), respectively, and \(\Delta t\) is the corresponding incubation time in d.

Particulate organic carbon (POC) and nitrogen (PON) were measured after filtration onto precombusted (15 h, 500°C) glass-fibre filters (GF/F 0.6 µm nominal pore size; Whatman). Filters were stored at −20°C and dried for at least 12 h at 60°C before sample preparation. Analysis was performed using a CHNS-O elemental analyser (Euro EA 3000; HEKAtech). Contents of POC and PON were corrected for blank measurements and normalized to filtered volume and cell densities to yield cellular quotas. Biogenic silica (BSi) was determined spectrophotometrically after treatment with a molybdate solution as described in Koroleff (1983). Production rates of Chl, POC, PON and BSi were calculated by multiplying the cellular quota with the growth rate of the respective culture. In order to diminish possible short-term effects arising from changes in irradiance fields in the dynamic treatments, all samples were taken within the first 30 min of the dark phase.

### Chl-specific net primary production

Chl-specific net primary production (NPP) rates were determined in triplicate by incubation of 20 ml of culture with 20 µCi NaH\(^{14}\)CO\(_3\) spike (53.1 mCi mmol\(^{-1}\); Perkin Elmer, Waltham, MA, USA) in 20 ml glass scintillation vials for 24 h under experimental conditions. From these incubations, 0.1 ml aliquots were immediately removed, mixed with 15 ml of scintillation cocktail (Ultima Gold AB; PerkinElmer) and counted after 2 h with a liquid scintillation counter (Tri-Carb 2900TR; PerkinElmer) to determine the total amount of added NaH\(^{14}\)CO\(_3\) (DPM\(_{100\%}\)). For blank determination (DPM\(_{0\%}\)), one replicate was immediately acidified with 0.5 ml of 6 M HCl. After 24 h of incubation, \(^{14}\)C incorporation was stopped by adding 0.5 ml of 6 M HCl to each vial. The entire sample was then left to degas and dry in a custom-built chamber. When samples were completely dry (1–2 d), 5 ml milli-Q water were added to resuspend the sample. Subsequently, 15 ml of scintillation cocktail (Ultima Gold AB; PerkinElmer) were added and samples were measured after 2 h with a liquid scintillation counter (Tri-Carb 2900TR; PerkinElmer). NPP rates (µg C (µg Chl\(^{-1}\)) d\(^{-1}\)) were calculated as

\[
\text{NPP} = \frac{[\text{DIC}] \times (\text{DPM}_{\text{sample}} - \text{DPM}_{0\%} 	imes 1.05)}{(\text{DPM}_{100\%} \times t \times [\text{Chl}])}
\]

where \([\text{DIC}]\) and \([\text{Chl}]\) denote the concentrations of DIC and Chl in the sample, respectively. DPM\(_{\text{sample}}\) denotes the disintegrations min\(^{-1}\) (DPM) in the samples, DPM\(_{0\%}\) reflects the blank value, DPM\(_{100\%}\) denotes the DPM of the total amount of NaH\(^{14}\)CO\(_3\) added to the samples, and \(t\) is the duration of the incubation.

### Variable Chl fluorescence

Photophysiological characteristics, based on photosystem II (PSII) variable Chl fluorescence, were measured using a fast repetition rate fluorometer (FRRF; FastOcean PTX; Chelsea Technologies, West Molesey, UK) in combination with a FastAct Laboratory system (Chelsea Technologies). The excitation wavelength of the fluorometer’s light-emitting diodes (LEDs) was 450 nm, and the applied light intensity was 1.3 × 10\(^{22}\) photons m\(^{-2}\) s\(^{-1}\). The FRRF was used in single turnover mode, with a saturation phase comprising 100 flashlets on a 2 µs pitch and a relaxation phase comprising 40 flashlets on a 50 µs pitch. All measurements \((n=3)\) were conducted in a temperature-controlled chamber at 3 ± 0.3°C.

The minimum \((F_0)\) and maximum Chl fluorescences \((F_{\text{m}})\) were estimated from iterative algorithms for induction (Kolber et al., 1998) and relaxation phase (Oxborough, 2012) after subtraction of a blank value (average of \(n=8\) measurements) in the middle of the dark phase (i.e. 4 h after offset of light). Maximum quantum yields of PSII (apparent PSII photochemical quantum efficiency; \(F_v/F_{\text{m}}\)) were calculated as

\[
F_v/F_{\text{m}} = (F_{\text{m}} - F_0)/F_{\text{m}}
\]

Photosystem II electron flux was calculated on a volume basis \((J_{\text{PSII}}\) (mol e\(^{-}\) m\(^{-2}\) d\(^{-1}\)) using the absorption algorithm (Oxborough et al., 2012). The \(J_{\text{PSII}}\) rates were converted to Chl-specific absolute rates \((\text{ETR} \text{ (mol e}^{-}\text{mol Chl}^{-1} s^{-1}))\) by dividing it by the Chl concentration of the sample at the time point of the measurement and the number of seconds per day. Chl-specific \(J_{\text{PSII}}\)-based photosynthesis–irradiance (PI) curves were conducted four times a day (1 and 8 h after the onset of light as well as directly after and 4 h after the onset of darkness) at 15 irradiance \((I)\) intensities between 6 and 650 µmol photons m\(^{-2}\) s\(^{-1}\), with an acclimation time of 90 s per light step. Following the suggestion by Silsbe & Kromkamp (2012), the light-use efficiency \((\alpha)\), and the light saturation index \((I_C)\) were estimated by fitting the data to the model by Webb et al. (1974):

\[
\text{ETR} = \alpha \times I_C \times [1 - e^{(-1 \times I_C)}]
\]

The maximum electron transport rates \((\text{ETR}_{\text{max}} \text{ (mol e}^{-}\text{mol Chl}^{-1} s^{-1}))\) were estimated after applying a beta phase fit as described by Oxborough (2012). Daily electron transport rates \((\text{ETR}_{24 \text{h}} \text{ (mol e}^{-}\text{mol Chl}^{-1} d^{-1}))\) were estimated by integrating the number of electrons transported over the 16 h light phase.\(\text{ETR}_{24 \text{h}}\) were calculated in 5 min steps of \(I\)-values of both light regimes (i.e. 90 µmol photons m\(^{-2}\) s\(^{-1}\) under constant and variable irradiances under dynamic light) using \(\alpha, I_C\) and \(\text{ETR}_{\text{max}}\) from the PI curve measured closest to the time point of interest. Chl concentrations for normalizations were corrected using the growth rate and the time difference between FRRF and Chl measurements. To estimate the energy transfer efficiency from photochemistry to biomass build-up, the electron requirement for carbon fixation \((\Phi_{\text{e,C}} \text{ (mol e}^{-}\text{mol C}^{-1}))\) was calculated for each.
treatment by dividing the ETR_{24h} by NPP (expressed as molar quantities). It should be noted that differences in the spectral composition of the light used for ETR (i.e. blue light) and NPP measurements (i.e. white light) could lead to a systematic overestimation of Φ_{OC}.

Nonphotochemical quenching of Chl fluorescence (NPQ) at irradiances of 490 and 650 μmol photons m^{-2} s^{-1} (i.e. the maximum irradiance applied in the dynamic light cycle as well as the maximum irradiance step of the PI curve) were calculated using the normalized Stern–Volmer coefficient (also termed NSV) as described in Oxborough (2012) and McKew et al. (2013):

\[ \left( \frac{F'_q}{F'_v} \right) = 1 = F'_q / F'_v \]  

Eqn 5

where \( F'_q \) was measured after each light step (with a duration of 90 s).

Statistics
All data are given as the means of the replicates ± 1 SD. To test for significant differences between the treatments, two-way ANOVAs with additional normality (Shapiro–Wilk) and post hoc (Holm–SIDak method) tests were performed. The significance level was set to 0.05. Statistical analyses were performed using the program SigmaPlot (SysStat Software Inc., San Jose, CA, USA).

Results

Chl-specific growth rates (Fig. 2a; Table 2) under constant light conditions were similarly high, being 0.53 ± 0.03 and 0.56 ± 0.03 d^{-1} in ambient and high pCO₂ treatments, respectively. Under dynamic light, growth rates were significantly lower than under constant light (ANOVA, \( F = 51; P < 0.001 \); Table S1). Also under these conditions, growth rates were unaffected by the applied pCO₂ treatments, being 0.44 ± 0.01 d^{-1} at ambient pCO₂ and 0.42 ± 0.03 d^{-1} at high pCO₂.

With respect to the amount of Chl per cell (Table 2), we observed significant effects of both pCO₂ (ANOVA, \( F = 28; P < 0.001 \)) and light treatments (ANOVA, \( F = 6; P = 0.047 \)). Under dynamic light, Chl quotas significantly decreased with increasing pCO₂ (post hoc, \( P < 0.001 \)), while they remained unaffected by OA under constant light, leading to a significant interactive effect of pCO₂ and light intensity on cellular Chl quotas (ANOVA, \( F = 21; P = 0.002 \)). Similarly, the production of Chl per cell (Fig. 2b; Table 2) was also significantly affected by both pCO₂ (ANOVA, \( F = 18; P = 0.003 \)) and light (ANOVA, \( F = 56; P < 0.001 \)). Both factors also had an interactive effect on production rates (ANOVA, \( F = 25; P = 0.001 \)), which led to a significant decrease in Chl production under dynamic light and increasing pCO₂ (post hoc, \( P < 0.001 \)). The ratio of Chl:C (Table 2) was not significantly affected by any treatment.

Cellular quotas of POC (Table 2) under constant light did not differ between ambient and high pCO₂, whereas they significantly decreased with increasing pCO₂ under dynamic light (post hoc test, \( P = 0.010 \); significant ANOVA interaction between pCO₂ and light, \( F = 9; P = 0.018 \)). Overall, POC production (Fig. 2c, Table 2) under constant light was not significantly affected by pCO₂, but was significantly reduced under dynamic vs constant light (ANOVA, \( F = 31; P < 0.001 \)). Under dynamic light conditions, POC production also significantly decreased with increasing pCO₂ (post hoc, \( P = 0.009 \)), resulting in a significant interaction term between pCO₂ and light conditions (ANOVA, \( F = 9; P = 0.018 \)).

Cellular quotas of PON (Table 2) were significantly reduced under high vs ambient pCO₂ (ANOVA, \( F = 14; P = 0.006 \)), irrespective of the light conditions applied. Also the production of PON (Fig. 2d; Table 2) decreased significantly with decreasing pCO₂ (ANOVA, \( F = 11; P = 0.010 \)). PON production was significantly higher under constant than under dynamic light (ANOVA, \( F = 23; P = 0.001 \)). Under constant light, C:N ratios significantly increased with increasing pCO₂ (Fig. 2f; Table 2; post hoc, \( P = 0.017 \)). Under dynamic light, no such response was observed. Significant differences in C:N ratios between the light treatments were observed under high pCO₂ only, where dynamic light led to a decrease in C:N (post hoc, \( P = 0.033 \)).

Cultures exhibited a highly significant decline in the cellular quota of biogenic silica (BSi; Table 2) with increasing pCO₂ (ANOVA, \( F = 38; P < 0.001 \)). BSi quotas were furthermore lower under dynamic than under constant light (ANOVA, \( F = 9; P = 0.020 \)). We also observed a highly significant decrease in BSi production (Fig. 2e; Table 2) with increasing pCO₂ (ANOVA, \( F = 38; P < 0.001 \)). Furthermore, BSi production was significantly lower under dynamic than under constant light (ANOVA, \( F = 90; P < 0.001 \)).

Chl-specific NPP

Chl-specific NPP (Fig. 3; Table 3) under constant light increased slightly, yet insignificantly, with increasing pCO₂. Under dynamic light, NPP was lower than under constant light (ANOVA, \( F = 27; P < 0.001 \); Table S2). Under these conditions, NPP was also significantly decreased with increasing pCO₂ (post hoc, \( P < 0.001 \)), resulting in a significant interaction between pCO₂ and light conditions (ANOVA, \( F = 7; P = 0.028 \)).

Chl fluorescence-based photophysiology

The dark-acclimated quantum yield efficiency of PSII (\( F_{v}/F_{m} \)) was similar in all treatments, with values of 0.53 ± 0.01. Neither nonphotochemical quenching (NPQ) at 490 μmol photons m^{-2} s^{-1} nor maximal NPQ at 650 μmol photons m^{-2} s^{-1} (NPQ_{max}, Table 3) was significantly affected by the applied treatments (Supporting Information, Fig. S1).

The fitted parameters of night-time FRRF-based PI curves (Fig. 4) were strongly influenced by experimental treatments. The maximal electron transport rates through PSII (ETR_{max}; Table 3) increased with increasing pCO₂ (ANOVA, \( F = 17; P = 0.003 \)) and were also significantly higher under dynamic than under constant light (ANOVA, \( F = 71; P < 0.001 \)). Post hoc tests
revealed that the OA response was much more pronounced under dynamic light (post hoc, \( P = 0.022 \)) than under constant light (post hoc, \( P = 0.222 \)). No sign of photoinhibition of ETR was observed (Fig. 4). The maximum PSII light-use efficiency (\( \alpha \); Table 3) was significantly higher under OA than under ambient pCO\(_2\) (ANOVA, \( F = 14, \ P = 0.006 \)), a result that was mainly driven by the responses under dynamic light (post hoc, \( P < 0.001 \)) and not that pronounced under constant light (post hoc, \( P = 0.120 \)). In addition, \( \alpha \)-values in both pCO\(_2\) treatments were significantly higher under dynamic than under constant light (ANOVA, \( F = 35, \ P < 0.001 \)). The PSII light saturation point (\( I_K \); Table 3) was not significantly affected by the experimental treatments.

Similarly, cumulative electron transport rates over 24 h (ETR\(_{24\text{~h}}\); Table 3) were also higher under OA than under ambient pCO\(_2\) (ANOVA, \( F = 9, \ P = 0.029 \)) as well as under dynamic vs constant light (ANOVA, \( F = 7, \ P = 0.015 \)). The strongest responses were observed under high pCO\(_2\) (post hoc, \( P = 0.013 \)).

Table 2 Chlorophyll-specific growth rates, cellular quotas and production rates of Chl, particulate organic carbon (POC), particulate organic nitrogen (PON) and biogenic silica (BSi) of Chaetoceros debilis (\( n = 3; \text{mean} \pm 1 \text{SD} \)) under two pCO\(_2\) concentrations at constant and dynamic light regimes

| Parameter                     | Unit       | Constant light | Dynamic light | Constant light | Dynamic light |
|-------------------------------|------------|----------------|---------------|----------------|---------------|
| Chl-specific growth rate \( \mu (\text{d}^{-1}) \) |            | 0.53 ± 0.03    | 0.56 ± 0.03   | 0.44 ± 0.01    | 0.42 ± 0.03   |
| Cellular quota Chl (pg cell\(^{-1} \)) |            | 0.66 ± 0.04    | 0.62 ± 0.06   | 0.72 ± 0.04    | 0.45 ± 0.04   |
| POC (pg cell\(^{-1} \))       |            | 40.49 ± 4.29   | 43.66 ± 4.13  | 43.28 ± 4.91   | 30.91 ± 4.72  |
| PON (pg cell\(^{-1} \))       |            | 7.69 ± 0.39    | 6.76 ± 0.89   | 7.94 ± 0.77    | 5.65 ± 0.87   |
| BSI (pg cell\(^{-1} \))       |            | 55.2 ± 1.15    | 41.87 ± 2.89  | 47.43 ± 4.88   | 39.75 ± 1.13  |
| Production rates Chl (pg cell\(^{-1} \text{d}^{-1} \)) | | 0.35 ± 0.02    | 0.36 ± 0.03   | 0.31 ± 0.02    | 0.19 ± 0.02   |
| POC (pg cell\(^{-1} \text{d}^{-1} \)) | | 21.34 ± 2.26   | 24.32 ± 2.30  | 18.88 ± 2.14   | 12.87 ± 1.96  |
| PON (pg cell\(^{-1} \text{d}^{-1} \)) | | 4.05 ± 0.21    | 3.76 ± 0.50   | 3.47 ± 0.34    | 2.35 ± 0.36   |
| BSI (pg cell\(^{-1} \text{d}^{-1} \)) | | 29.09 ± 0.61   | 23.32 ± 1.61  | 20.69 ± 2.13   | 16.55 ± 0.47  |
| Elemental ratios C : N          |            | 6.17 ± 0.93    | 7.58 ± 0.65   | 6.35 ± 0.12    | 6.38 ± 0.04   |
| Chl : C                        |            | 61.67 ± 4.70   | 68.51 ± 5.01  | 60.44 ± 5.72   | 67.87 ± 6.56  |

Sparkline boxes indicate ocean acidification responses of the measured parameters under the respective light regime. Significant light effects are indicated by asterisks (*), and interactive effects by dagger symbols (†).
and dynamic light (post hoc, \( P = 0.008 \)). The electron requirement for carbon fixation (\( \Phi_{e,C} \); Fig. 3; Table 3) was significantly higher under dynamic than under constant light (ANOVA, \( F = 28; P < 0.001 \)). In the dynamic light treatments, \( \Phi_{e,C} \) was also much higher under OA compared with ambient pCO\(_2\) (post hoc, \( P < 0.001 \)). Such an OA response was not observed under constant light conditions, where \( \Phi_{e,C} \) decreased slightly (post hoc, \( P = 0.047 \)). Overall, these responses led to a significant effect of pCO\(_2\) (ANOVA, \( F = 6; P < 0.035 \)) as well as – because of the opposing OA trends under the two light treatments – a significant interaction term between light treatments and pCO\(_2\) concentrations (ANOVA, \( F = 10; P < 0.012 \)).

**Discussion**

Dynamic light exerts high metabolic costs

Prevailing strong winds lead to deeply mixed surface layers and highly dynamic light regimes in the SO (Nelson & Smith, 1991). Phytoplankton species occurring in this environment can therefore be expected to cope well with dynamic light conditions. In fact, cellular POC and PON quotas as well as C : N and Chl : C ratios were much higher under OA compared with ambient pCO\(_2\) (post hoc, \( P < 0.001 \)). In the dynamic light treatments, \( \Phi_{e,C} \) was also much higher under OA compared with ambient pCO\(_2\) (post hoc, \( P < 0.001 \)). Such an OA response was not observed under constant light conditions, where \( \Phi_{e,C} \) decreased slightly (post hoc, \( P = 0.047 \)). Overall, these responses led to a significant effect of pCO\(_2\) (ANOVA, \( F = 6; P < 0.035 \)) as well as – because of the opposing OA trends under the two light treatments – a significant interaction term between light treatments and pCO\(_2\) concentrations (ANOVA, \( F = 10; P < 0.012 \)).
significantly higher under dynamic than under constant light (Table 3), while NPP and biomass build-up under dynamic light were significantly lowered (Figs 2, 3). This implies that under dynamic light, the overall energy transfer efficiency from photochemistry to net biomass production was substantially reduced (Wagner et al., 2006; Ihnken et al., 2011; Su et al., 2012; Jin et al., 2013).

The electron requirement of carbon fixation ($\Phi_{\text{e,C}}$) was indeed significantly higher under dynamic light conditions (Fig. 3), hinting at an increase in other electron-consuming processes such as mitochondrial respiration, photorespiration or alternative electron cycling (Prášil et al., 1996; Badger et al., 2000; Wagner et al., 2006; Waring et al., 2010; Thamtrakoln et al., 2013). While $\Phi_{\text{e,C}}$ should theoretically be 4–6 mol e$^-$/mol C$^-1$ (Genty et al., 1989; Saggert et al., 2009), the estimates for $\Phi_{\text{e,C}}$ in this study range between 3 and 6 mol e$^-$/mol C$^-1$ (Table 3). Values between 1.2 and 5.4 mol e$^-$/mol C$^-1$ have been previously observed in field studies and laboratory experiments (Saggert et al., 2009; Lawrenz et al., 2013). Values of $\Phi_{\text{e,C}}$ < 4 mol e$^-$/mol C$^-1$ have been attributed to systematic errors in the ETR calculations (Lawrenz et al., 2013). In addition, differences in temporal scales between measures (e.g. Kromkamp & Forster, 2003) as well as short acclimation times may lead to a systematic underestimation of ETRs. Regarding the latter, directional errors in $\Phi_{\text{e,C}}$ are, however, unlikely to have contributed to the differences between light regimes, as the treatment-specific variations in $\varepsilon$, $I_K$ and $ETR_{\text{max}}$ were similar at different time points (data not shown). Irrespective of a potential underestimation of $\Phi_{\text{e,C}}$, the observed trends indicate comparably low energy transfer efficiency under dynamic light, which could well explain the observed decrease in growth and NPP compared with constant light (Fig. 3). This interpretation is further corroborated by the observed $I_K$-independent behaviour of the PI curves (i.e. changes in $ETR_{\text{max}}$ and $\varepsilon$ while $I_K$ stays constant; Table 3), which can be attributed to changes in processes that decouple carbon fixation from photosynthetic electron transport through the consumption of ATP and reductants (Behrenfeld et al., 2004, 2008).

Diatom cells growing under dynamic light need to adjust their photosynthetic apparatus to achieve a balance between photoprotection at high light and effective light-harvesting at low light. No high-light stress was observed in ETR vs irradiance curves (Fig. 4), indicating successful photoprotection under all tested scenarios. In line with other studies on dynamic light (van de Poll et al., 2007; Kropuenske et al., 2009; Alderkamp et al., 2012; Su et al., 2012), we also did not observe an increase in NPQ capacity (Table 3). Successful photoprotection may be achieved by other processes such as increased connectivity between reaction centres (Trimborn et al., 2014) or the induction of alternative electron pathways (e.g. Mehler reaction, electron flow around PSII or PSI) that can supplement the xanthophyll cycle in diatoms (Prášil et al., 1996; Asada, 1999; Waring et al., 2010). These mechanisms could have contributed to the observed increase in $\Phi_{\text{e,C}}$ (Fig. 3) under dynamic vs constant light.

In addition, the apparent insensitivity of electron transport towards high-light stress (Fig. 4) does not mean that no photodamage of reaction centres occurs. In fact, an uncoupling between PSII inactivation and the rate of electron flow has been described as a common mechanism for phytoplankton under natural light regimes (Behrenfeld et al., 1998). The uncoupling can be explained by the presence of ‘excess PSII capacity’ (i.e. more reaction centres than are actually needed), allowing for high photochemical efficiencies even if light-dependent photoactivation of PSII increases (Behrenfeld et al., 1998). This overproduction and subsequent repair of PSII, including the susceptible D1 subunit and associated proteins, imposes high metabolic costs for the phytoplankton cell (Raven, 2011). Whether or not the costs, being associated with the high-light phases of the dynamic light treatment, are compensated for by the subsequent period of low light depends on the rates of both, the changes in light intensity and D1 repair (Behrenfeld et al., 1998; Marshall et al., 2000). In the tested scenarios here, we did not observe the manifestation of photoinhibition (Fig. 4). Therefore, we postulate that a large fraction of the decline in growth and energy transfer efficiency from photochemistry to biomass production under dynamic light (Fig. 3; Table 3) results from increased metabolic costs of photoprotection and elevated D1 turnover at high light in combination with the consequences of light limitation in the low-light phases.

Ocean acidification increases energy-use efficiency under constant light

Changes in CO$_2$ supply have been shown to differentially affect SO diatoms on the species level (Boelen et al., 2011; Hoogstraten et al., 2012a,b; Trimborn et al., 2013, 2014) as well as in natural communities (Tortell et al., 2008; Feng et al., 2010; Hoppe et al., 2013). The Antarctic diatom *C. debilis* was shown to exhibit increased energy-use efficiencies (i.e. higher growth rates, but lower O$_2$ evolution) as well as decreased dark respiration under high pCO$_2$ and constant light (Trimborn et al., 2013, 2014). In the present study, C: N ratios were higher under OA and constant light, while growth rates and NPP of *C. debilis* were only slightly stimulated under these conditions (Figs 2, 3; Tables 2, 3). The differences in the CO$_2$ sensitivity of *C. debilis* most likely originate from different pCO$_2$ treatments applied in the two studies, as significant changes were mainly observed between intermediate (ambient) and low (glacial) pCO$_2$ concentrations, the latter not being investigated in the current study. Similar to our results, two other species of *Chaetoceros* also showed little or no growth response to OA, but these results apparently also depended on the applied light intensities (Boelen et al., 2011; Ihnken et al., 2011). In CO$_2$ manipulation experiments with SO phytoplankton communities, however, *Chaetoceros* was found to benefit from elevated pCO$_2$, as this genus dominated the applied OA treatments (Tortell et al., 2008; Feng et al., 2010).

Such OA responses have often been attributed to the mode of CCMs, which can differ in the ability to reach rate saturation and to respond to environmental changes as well as in the associated costs of these processes. In the case of diatoms, CCMs have been shown to be very effective avoiding carbon limitation, but also to be regulated as a function of external CO$_2$ concentration (e.g. Raven & Johnston, 1991; Trimborn et al., 2009; Hopkinsen et al., 2011). Elevated pCO$_2$ often leads to a down-regulation...
of CCM activity, thereby reducing the overall costs of carbon acquisition (Burkhardt et al., 2001; Rost et al., 2003; Hopkinson et al., 2011). Even though Trimborn et al. (2013) observed a rather constitutively expressed CCM for C. debilis, it can be speculated that the higher gross CO₂ uptake under elevated pCO₂ may have contributed to the observed stimulation in growth. In conclusion, the often documented beneficial OA effects at constant light could, to a large degree, be explained by overall lowered costs of the CCM.

Photoacclimation can also be influenced by CO₂ via the CO₂-dependent regulation of CCMs and RubisCO concentrations, as these properties can affect the amount of electrons being used during carbon fixation (Tortell, 2000; Rost et al., 2006; Reinfelder, 2011). Trimborn et al. (2014) showed strong effects of short-term exposure to low pCO₂ concentrations on various photophysiological parameters in C. debilis. In line with findings on two Thalassiosira species (McCarthy et al., 2012), ETRₘₐₓ increased with increasing pCO₂ (Table 3), indicating higher substrate saturation at RubisCO and higher activities of the Calvin cycle under OA and constant light. With respect to the balance between light and dark reaction of photosynthesis, we observed a slight decrease in Φₑ,C with increasing pCO₂ (Fig. 3; Table 3). These results could imply that the Calvin cycle acts as a better energy sink under elevated pCO₂ and constant light, as has been proposed by Trimborn et al. (2014). Such an increase in electron-use efficiency could thus explain the beneficial effects of OA (Figs 2, 3).

Dynamic light reverses the responses to ocean acidification

In line with previous findings on Chaetoceros (Boelen et al., 2011; Ihnken et al., 2011), we observed slight, yet insignificant, enhancement in growth, POC production and NPP with increasing pCO₂ under constant light (Figs 2, 3). In other studies, growth and NPP of Chaetoceros were strongly stimulated under elevated pCO₂ (Tortell et al., 2008; Feng et al., 2010; Hoppe et al., 2013; Trimborn et al., 2013). When comparing these trends with the OA responses from the dynamic light treatments, a completely different picture emerges: POC production and NPP decrease under OA by c. 30 and 50%, respectively. The putatively beneficial effects of elevated pCO₂ seem not only to be dampened, but even reversed under dynamic light as cells significantly slow down biomass production (Fig. 2). Boelen et al. (2011) did not observe any significant responses of C. brevis to either OA or dynamic light. Their results indicate a low sensitivity of this strain to increasing pCO₂ up to 750 µatm, even though POC production under OA was reduced by c. 15% in their dynamic high-light treatment. In line with our study, Jin et al. (2013) observed a decline in carbon fixation rates of the coccolithophore G. oceanica under OA and short-term exposure to dynamic light.

Surprisingly, the decline in biomass build-up in this study was observed even though electron transport through PSII was most efficient under these conditions (Table 3). In addition, there was no sign of photoinhibition after short-term exposure to irradiances up to 650 µmol photons m⁻² s⁻¹ in any of the treatments (Fig. 4). At higher irradiances, however, rETRₛ in C. debilis were found to decrease (Trimborn et al., 2014). The photophysiological results therefore suggest that the excess capacity of photosynthesis (Behrenfeld et al., 1998) was sufficient to prevent chronic photoinhibition under the applied assay irradiances (Fig. 4). As these photophysiological results do not explain the decline in POC production and NPP observed under OA and dynamic light (Figs 2, 3) and as the observed Îₖ-independent changes in the PI curves (Table 3) are indicative of changes in the demand and distribution of energy and reductive equivalents (Behrenfeld et al., 2004, 2008), the underlying reason may be associated with an imbalance between light and dark reactions of photosynthesis.

There is increasing evidence that diatoms are more susceptible to D₁ inactivation and photoinhibition under OA than under ambient pCO₂ concentrations (Wu et al., 2010; Gao et al., 2012a; McCarthy et al., 2012). Li & Campbell (2013) observed that under OA, Thalassiosira pseudonana has enhanced growth rates under low, but not high light, a finding that is in line with studies on cyanobacteria and coccolithophores (Kranz et al., 2010; Rokitta & Rost, 2012). As photosynthesis shifts progressively from light towards carbon limitation under increasing irradiance, CCM activity also needs to be increased under these conditions (Beardall & Giordano, 2002; Rost et al., 2006). The CCM, however, is typically down-regulated under OA (Burkhardt et al., 2001; Rost et al., 2003), which could restrict the capacity to rapidly sink more electrons in the Calvin cycle or to drain excess energy by HCO₃⁻ recycling under short-term high-light stress (Tchernov et al., 1997; Rost et al., 2006). This could result in a lower capability to cope with high light and could increase photooinactivation of PSII under OA (Beardall & Giordano, 2002; Ihnken et al., 2011; Gao et al., 2012b), shifting the susceptibility to photoinhibition towards lower irradiances. The proposed mechanism implies that, under dynamic light, cells exposed to higher pCO₂ concentrations experience high-light stress for longer time periods compared with cells grown under ambient pCO₂. Under constant light, no photoacclimation to high-light phases would be needed, so that an OA-induced surplus of energy could be directly used to build more biomass (Figs 2, 3; Tortell et al., 2008; Trimborn et al., 2013). Under dynamic light, however, this extra energy may lead to higher metabolic costs for photoacclimation and D₁ repair during high-light phases, which apparently cannot be compensated by lowered operational costs of CCMs. The mechanism proposed here could collectively explain the observed higher demand for energy and reductive equivalents (i.e. Îₖ-independent increase in ETRₘₐₓ Table 3; Behrenfeld et al., 2004), as well as the decline in NPP under OA and dynamic light, ultimately leading to a strong increase in Φₑ,C (Fig. 3).

Under the conditions applied here, C. debilis seems to be able to circumvent measurable photoinhibition, even though we speculate that this comes at a high cost, especially under OA combined with dynamic light. Under higher pCO₂ concentrations as well as higher average or more dynamic irradiances, however, OA could induce measurable damage to the photosynthetic apparatus in addition to presumably high metabolic costs incurred from
D1 turnover and photosystem repair (Raven, 2011; Li & Campbell, 2013). Therefore, the modulation of OA responses probably also varies depending on the light regime applied (cf. Boelen et al., 2011). Further, the response pattern may be modulated by depth-dependent changes in the spectral composition of light (Falkowski & LaRoche, 1991), which were not investigated in the present study. In view of the generally high plasticity of photoacclimation in diatoms (Wagner et al., 2006; Lavaud et al., 2007), the interactive effects described here might be even more pronounced in other phytoplankton taxa. In any case, our data have demonstrated that a combination of OA and dynamic light could impose significantly more stress onto phytoplankton than was previously thought.

Implications for ecology and biogeochemistry

In summary, dynamic light was shown to drastically alter OA effects on an ecologically important SO diatom species, leading to a strong decline in primary production under OA and dynamic light. The observed response patterns can be explained by changes in the cellular energetic balance, as the energy transfer efficiency from photochemistry to biomass production was drastically reduced under OA and dynamic light. Given the increasing number of studies dealing with possible interactive effects of OA with high-light stress on phytoplankton (e.g. Wu et al., 2010; Gao et al., 2012a; Gao & Campbell, 2014), the importance of excess PSII capacity should be investigated in future.

Our results also have important implications for the current understanding of OA effects on marine phytoplankton. As has been shown for several environmental variables, such as temperature (e.g. Tatters et al., 2013) or nutrient concentrations (e.g. Fu et al., 2010; Hoppe et al., 2013), interactive effects need to be considered when predicting future productivity and ecosystem functioning. As a central feature of oceanic environments, dynamic light is an especially important aspect (Mitchell et al., 1991; Maclntyre et al., 2000), which has been neglected in most OA studies so far. If our results are representative, the often proposed ‘CO2 fertilization’ may be dampened or even reversed in many natural environments. In this context, it is important to consider that anthropogenic CO2 emissions do not only lead to OA, but also to a warming of the surface ocean (Sarmiento et al., 2004). A concomitant shoaling of the upper mixed layer would thus change the integrated intensity and variability of the light regimes encountered by phytoplankton cells (Rost et al., 2008; Steinacher et al., 2010), making the interactive relationship between OA and light regimes even more important to consider.

Regarding the SO, it seems likely that synergistic effects of iron limitation and dynamic light, both dominant features of this region (Boyd, 2002; de Baar et al., 2005; Alderkamp et al., 2012), jointly lower the potential benefits of OA. Under iron-enriched conditions, as in the present study, diatom taxa such as Chaetoceros and Fragilaria have been shown to dominate OA treatments under constant light, suggesting a higher potential for export production (Tortell et al., 2008; Hoppe et al., 2013). The lowered NPP under OA and dynamic light, however, questions the reliability of such predictions. The aspect of ballasting also has to be considered, as siliceous frustules make diatoms efficient vectors for carbon (Sarthou et al., 2005). In line with Milligan et al. (2004), we observed a decline in both cellular BSi quotas and production rates with increasing pCO2 (Fig. 2; Table 2), which further argues against a stimulation of the biological carbon pump. To date, the effects of dynamic light on OA responses and the underlying reasons for them, as observed in this study, were unknown. This new knowledge will change our perception of phytoplankton under climate change.

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**Supporting Information**

Additional supporting information may be found in the online version of this article.

**Fig. S1** Night-time development of nonphotochemical quenching (NPQ) with increasing irradiance under the different treatment conditions.

**Table S1** Results from two-way ANOVAs for all measured acclimation parameters

**Table S2** Results from two-way ANOVAs for Chl fluorescence-based parameters, net primary production and the electron requirement for carbon fixation

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