1 Contrasting effects of rising CO₂ on primary production and
2 ecological stoichiometry at different nutrient levels
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1 **Abstract**

2 Although rising CO₂ concentrations are thought to promote the growth and alter the
3 carbon:nutrient stoichiometry of primary producers, several studies have reported conflicting
4 results. To reconcile these contrasting results, we tested the following hypotheses: rising CO₂
5 levels (1) will increase phytoplankton biomass more at high nutrient loads than at low
6 nutrient loads, but (2) will increase their carbon:nutrient stoichiometry more at low than at
7 high nutrient loads. We formulated a mathematical model to predict dynamic changes in
8 phytoplankton population density, elemental stoichiometry and inorganic carbon chemistry in
9 response to rising CO₂. The model was tested in chemostat experiments with the freshwater
10 cyanobacterium *Microcystis aeruginosa*. The model predictions and experimental results
11 confirmed the hypotheses. Our findings provide a novel theoretical framework to understand
12 and predict effects of rising CO₂ concentrations on primary producers and their nutritional
13 quality as food for herbivores under different nutrient conditions.

14

1 **Introduction**

2 Primary producers assimilate CO₂ by the RubisCO enzyme, which generally features very
3 low affinities for CO₂ (Badger *et al.* 1998; Giordano *et al.* 2005). Moreover, in aquatic
4 ecosystems, dissolved CO₂ concentrations can be strongly depleted during phytoplankton
5 blooms (Talling 1976; Maberly 1996; Balmer & Downing 2011). Several studies have
6 therefore predicted that the current rise in atmospheric CO₂ concentrations will increase
7 photosynthetic carbon fixation and phytoplankton growth rates (Riebesell *et al.* 1993; 2007),
8 and hence the occurrence and magnitude of phytoplankton blooms (Schippers *et al.* 2004).
9 However, this prediction may not always hold. For instance, in many waters, phytoplankton
10 growth rates and bloom development are constrained by nutrient availability (Dzialowski *et*
11 *al.* 2005; Xu *et al.* 2010; Lewandowska *et al.* 2014). If nutrients are strongly limiting, rising
12 CO₂ concentrations are unlikely to have a major impact on phytoplankton growth.

13 An increase in CO₂ availability may also affect the elemental stoichiometry of
14 primary producers (Bellerby *et al.* 2008; Van de Waal *et al.* 2010; Finkel *et al.* 2010). In
15 particular, several studies have shown that rising CO₂ concentrations increase the
16 carbon:nutrient ratio of phytoplankton (Riebesell *et al.* 2007; Verschoor *et al.* 2013), which
17 may reduce their nutritional quality for zooplankton (Urabe *et al.* 2003; Schoo *et al.* 2013).
18 However, some experimental studies do not support this expectation, as they did not find an
19 increase in carbon:nutrient stoichiometry in response to rising CO₂ concentrations
20 (Montechiaro & Giordano 2010; Gutow *et al.* 2014). Elevated CO₂ concentrations only seem
21 to change phytoplankton stoichiometry under specific conditions, for instance, at low nutrient
22 availability (Gervais & Riebesell 2001; Leonardos & Geider 2005; Li *et al.* 2012).

23 These contrasting predictions and results from previous studies indicate that the
24 response of primary producers and their elemental stoichiometry to rising CO₂ may depend
25 on the nutrient status of ecosystems. However, a coherent theoretical framework to reconcile

1 the different observations is still lacking. The nutrient-load hypothesis of Brauer *et al.* (2012)
2 may offer a useful starting point to develop such a framework. The nutrient-load hypothesis
3 describes resource limitation patterns as a function of the input of two potentially limiting
4 nutrients. Here, we consider one limiting nutrient while the role of the other nutrient is
5 replaced by inorganic carbon (Fig. 1a). This modified nutrient-load hypothesis predicts that,
6 at low pCO₂ levels but high nutrient loads, carbon will be the limiting resource for
7 phytoplankton growth. At high pCO₂ levels but low nutrient loads, the nutrient becomes
8 limiting. Systems with both high pCO₂ levels and high nutrient loads can develop dense
9 phytoplankton populations, which increase the turbidity of the water column and thereby
10 generate light-limited conditions (Fig. 1a).

11 In systems with low nutrient loads, rising pCO₂ levels are predicted to shift
12 phytoplankton growth from carbon-limited to nutrient-limited conditions (indicated by the
13 black arrow in Fig. 1a). Such a shift towards nutrient limitation will not allow a large increase
14 in phytoplankton biomass (Fig. 1b), but will likely enhance the carbon:nutrient ratio of the
15 cells (Fig. 1c). In systems with high nutrient loads, rising pCO₂ levels will shift
16 phytoplankton growth from carbon-limited to light-limited conditions (indicated by the white
17 arrow in Fig. 1a). Hence, at high nutrient loads, rising pCO₂ levels may allow a much larger
18 increase in phytoplankton biomass (Fig. 1b), but are unlikely to lead to major changes in the
19 carbon:nutrient stoichiometry (Fig. 1c). Based on these graphical arguments, we put forward
20 two pairs of hypotheses. Our first pair of hypotheses is that the development of
21 phytoplankton biomass will increase substantially in response to rising pCO₂ levels in
22 eutrophic waters (Hypothesis 1a), but to a much lesser extent in oligotrophic waters
23 (Hypothesis 1b). Our second pair of hypotheses is that the carbon:nutrient stoichiometry of
24 phytoplankton will increase substantially in response to rising pCO₂ levels in oligotrophic
25 waters (Hypothesis 2a), but to a much lesser extent in eutrophic waters (Hypothesis 2b).

1 In this paper, we combine theory and experiments to test these hypotheses. To
2 establish a firm theoretical basis, we first build a stoichiometrically explicit model that allows
3 for carbon-, nutrient- and light-limited growth. For this purpose, we extend earlier models of
4 multiple resource limitation (e.g., Sterner & Elser 2002; Klausmeier *et al.* 2004; Passarge *et*
5 *al.* 2006; Brauer *et al.* 2012) by the explicit incorporation of inorganic carbon chemistry
6 (Stumm & Morgan 1996). We will test the model predictions in laboratory chemostats with
7 the freshwater cyanobacterium *Microcystis aeruginosa*, a widespread nuisance species that
8 develops dense and often toxic blooms in many lakes worldwide (Verspagen *et al.* 2006;
9 Jöhnk *et al.* 2008; Xu *et al.* 2010; Michalak *et al.* 2013). To address our hypotheses, the
10 theory and experiments will investigate the impact of rising CO₂ levels on phytoplankton
11 growth and stoichiometry at both low and high nitrogen loads.

13 **The model**

15 *Population dynamics and elemental stoichiometry*

16 We develop a model that considers a well-mixed water column, illuminated from above, with
17 a homogeneously distributed phytoplankton population. Phytoplankton fix CO₂ during
18 photosynthesis and many species, including *Microcystis*, can also utilize bicarbonate as a
19 carbon source (Giordano *et al.* 2005; Sandrini *et al.* 2014). The water column is provided
20 with a limited influx of nitrogen, which is consumed by the phytoplankton population. The
21 growing phytoplankton population absorbs light, thereby reducing light availability. Hence,
22 the model may generate carbon-limited, nitrogen-limited or light-limited conditions,
23 depending on the environmental conditions. Here we introduce the key assumptions of the
24 model, while a complete description is given in Appendix S1 of the Supporting Information.

Our model assumes a variable carbon and nitrogen content of the phytoplankton, as in the classic Droop model and related approaches in ecological stoichiometry (Droop 1973; Sterner & Elser 2002; Klausmeier *et al.* 2004). Let X denote the population density of the phytoplankton, Q_C its carbon content and Q_N its nitrogen content. Dynamic changes in phytoplankton population density and its carbon and nitrogen contents can be summarized by three differential equations:

$$\frac{dX}{dt} = \mu(Q_C, Q_N)X - DX \quad (1)$$

Commented [JMHV1]: Replaced "m" by "D".

$$\frac{dQ_C}{dt} = u_{CO_2} + u_{HCO_3} - r - \mu(Q_C, Q_N)Q_C \quad (2)$$

$$\frac{dQ_N}{dt} = u_N - \mu(Q_C, Q_N)Q_N \quad (3)$$

where $\mu(Q_C, Q_N)$ is the specific growth rate of the phytoplankton as function of its carbon and nitrogen content, D is the dilution rate, u_{CO_2} , u_{HCO_3} and u_N are the uptake rates of CO_2 , bicarbonate and nitrogen, respectively, and r is the respiration rate.

The specific growth rate increases with the carbon and nitrogen content of the phytoplankton, and depends on which of these two elements is most limiting for growth:

$$\mu(Q_C, Q_N) = \mu_{MAX} \min \left[\frac{Q_C - Q_{C,MIN}}{Q_{C,MAX} - Q_{C,MIN}}; \frac{Q_N - Q_{N,MIN}}{Q_{N,MAX} - Q_{N,MIN}} \right] \quad (4)$$

where μ_{MAX} is the maximum specific growth rate, $\min[\dots]$ is the minimum function, $Q_{C,MIN}$ and $Q_{N,MIN}$ are the minimum cellular carbon and nitrogen content required for a cell to function, and $Q_{C,MAX}$ and $Q_{N,MAX}$ are the maximum carbon and nitrogen content of the cells.

We assume that uptake rates of CO_2 , bicarbonate and nitrogen are increasing but saturating functions of the ambient concentrations of these resources described by Michaelis-Menten kinetics, and are suppressed when phytoplankton cells become satiated (Morel 1987). The energy required for carbon and nitrogen uptake comes from the light reactions of

photosynthesis, which depend on light availability. We assume that the respiration rate increases with the cellular carbon content, approaching maximum values when cells become satiated with carbon (Visser *et al.* 1997). The mathematical equations describing these relationships are presented in Appendix S1.

Dissolved inorganic carbon

On the timescales used in our model (ranging from minutes to days) the relative availability of CO₂ and bicarbonate is essentially in equilibrium with pH, while the dissolved CO₂ concentration is usually not in equilibrium with the atmosphere. Therefore, let [DIC] denote the total concentration of dissolved inorganic carbon. Changes in [DIC] can be described by:

$$\frac{d[\text{DIC}]}{dt} = D([\text{DIC}]_{\text{IN}} - [\text{DIC}]) + \frac{g_{\text{CO}_2}}{z_{\text{MAX}}} + (r - u_{\text{CO}_2} - u_{\text{HCO}_3})X \quad (5)$$

The first term on the right-hand side of Eqn (5) describes changes in the DIC concentration due to the influx ([DIC]_{IN}) and efflux of water containing DIC. The second term describes exchange of CO₂ gas with the atmosphere, where g_{CO_2} is the CO₂ flux across the air-water interface and division by the water-column depth z_{MAX} converts the CO₂ flux per unit surface area into a volumetric CO₂ change. The third term describes how the DIC concentration increases through respiration (r) and decreases through uptake of CO₂ (u_{CO_2}) and bicarbonate (u_{HCO_3}) by phytoplankton.

We assume that the CO₂ gas exchange (g_{CO_2}) between air and water is proportional to the concentration gradient across the air-water interface, which can be quantified as the difference between dissolved CO₂ in equilibrium with the gas pressure ([CO₂^{*}]) and the actual dissolved CO₂ concentration (Siegenthaler & Sarmiento 1993; Cole & Caraco 1998):

$$g_{\text{CO}_2} = v([\text{CO}_2^*] - [\text{CO}_2]) \quad (6)$$

where ν is the gas transfer velocity. The equilibrium value $[\text{CO}_2^*]$ is calculated from Henry's law, i.e., $[\text{CO}_2^*] = K_0 p\text{CO}_2$, where $p\text{CO}_2$ is the partial pressure of CO_2 in air and K_0 is the solubility constant of CO_2 gas in water.

Uptake of CO_2 and bicarbonate not only depletes the DIC pool but also increases pH, which in turn affects the relative availability of the different forms of inorganic carbon (Stumm & Morgan 1996). CO_2 , bicarbonate and carbonate concentrations were calculated from pH by numerical solution of the underlying chemical reactions, taking into account dynamic changes in total DIC and alkalinity due to carbon and nutrient assimilation by the growing phytoplankton population (see Appendix S1 for details).

Nutrients and light

Changes in the concentration of dissolved inorganic nitrogen ([DIN]) depend on the influx ([DIN]_{IN}) and efflux of nitrogen and the uptake of nitrogen by the phytoplankton population:

$$\frac{d[\text{DIN}]}{dt} = D([\text{DIN}]_{\text{IN}} - [\text{DIN}]) - u_N X \quad (7)$$

The nitrogen concentration in the influx ([DIN]_{IN}) will be called the 'nitrogen load'.

Because carbon and nitrogen assimilation depend on light availability, the model also tracks changes in the underwater light environment. Let $I(z)$ denote the light intensity at depth z . According to Lambert-Beer's law,

$$I(z) = I_{\text{IN}} \exp(-K_{\text{BG}}z - kXz) \quad (8)$$

where I_{IN} is the incident light intensity at the water surface, K_{BG} is the background turbidity, and k is the specific light attenuation coefficient of the phytoplankton. This equation takes into account that a growing phytoplankton population increases the turbidity of the water column through self-shading, and thereby reduces the light available for further photosynthesis (Huisman & Weissing 1994; Diehl 2002; Passarge *et al.* 2006). Nitrogen is a key element in photosynthetic pigments. We therefore assume that the specific light

attenuation coefficient increases with the cellular nitrogen content (Aguirre von Wobeser *et al.* 2011). The light intensity at the bottom of the water column is defined as $I_{OUT}=I(z_{MAX})$.

Materials and methods

Species and culture conditions

We tested the model predictions with two strains of the freshwater cyanobacterium *Microcystis aeruginosa*. Strain *Microcystis* CYA140 was obtained from the Norwegian Institute for Water Research (NIVA). Strain *Microcystis* HUB5-2-4 was obtained from the Humboldt University of Berlin, Germany. The *Microcystis* strains were grown as single cells, not in colonies.

The experiments were carried out in laboratory-built chemostats specifically designed for phytoplankton studies (Huisman *et al.* 1999; Passarge *et al.* 2006). Each chemostat consisted of a flat culture vessel with an optical path length (“mixing depth”) of $z_{MAX} = 5$ cm and an effective working volume of 1.7 L. The chemostat vessel was continuously illuminated from one side by white fluorescent tubes (Philips PL-L 24W/840/4P, Philips Lighting, Eindhoven, The Netherlands) using a low incident light intensity of $I_{IN} = 50 \pm 1$ $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ to enable light-limited conditions. The chemostats were supplied with a nutrient-rich mineral medium (Van de Waal *et al.* 2009), in which we modified the nitrate concentration to induce nitrogen-limited conditions (see below). The chemostats were maintained at a constant temperature using a metal cooling finger connected to a Colora thermocryostat, and were aerated with sterilized (0.2 μm Millex-FG Vent Filter, Millipore, Billerica, MA, USA) N_2 gas enriched with different CO_2 concentrations using Brooks Mass Flow Controllers (Brooks Instrument, Hatfield, PA, USA). The gas mixture was dispersed from the bottom of the chemostat vessel in fine bubbles at a constant gas flow rate of 25 L h⁻¹.

¹. Experiments without phytoplankton showed that the gas transfer velocity (v) was proportional to the gas flow rate (a), i.e., $v = b a$, where the constant of proportionality b was estimated for each chemostat separately.

Experiments

First, we ran three chemostat experiments with *Microcystis* CYA140 under carbon-limited, nitrogen-limited and light-limited conditions, respectively, to investigate whether the model could capture the transient dynamics from inoculation at low phytoplankton population density to a full-grown steady state. Carbon-limited conditions were induced by a high nitrogen concentration (12.0 mmol L⁻¹ NaNO₃⁻) supplied by the mineral medium, but a low pCO₂ of 200 ppm in the gas flow. Nitrogen-limited conditions were induced by a low nitrogen concentration (0.4 mmol L⁻¹ NaNO₃⁻) in the mineral medium and an intermediate pCO₂ of 400 ppm. Light-limited conditions were induced by a high nitrogen concentration (12.0 mmol L⁻¹ NaNO₃⁻) in the mineral medium and a high pCO₂ of 1200 ppm in the gas flow (Table S1). The chemostats had a dilution rate of $D = 0.011 \text{ h}^{-1}$, were maintained at a temperature of 21 °C, and were sampled every other day.

Next, we studied the steady states of twelve chemostats of *Microcystis* HUB5-2-4. The chemostats were provided with either a low (0.4 mmol L⁻¹ NaNO₃⁻) or high (12.0 mmol L⁻¹ NaNO₃⁻) nitrogen concentration in the mineral medium, and a range of different pCO₂ concentrations (0.5, 50, 100, 400, 800, 2800 ppm) in the gas flow (Table S1). The chemostats had a dilution rate of $D = 0.00625 \text{ h}^{-1}$ and were maintained at a temperature of 24 °C. The steady states were sampled every other day and monitored for at least ten days.

Measurements

1 The incident light intensity (I_{IN}) and the light intensity transmitted through the chemostat
2 vessel (I_{OUT}) were measured with a LI-COR LI-250 quantum photometer (LI-COR
3 Biosciences, Lincoln, NE, USA) at 10 randomly chosen positions on the front and back
4 surface of the chemostat vessel, respectively.

5 DIC concentrations were determined by sampling 15 mL of culture suspension, which
6 was immediately filtered over 0.45 μm membrane filters (Whatman, Maidstone, UK). DIC
7 was subsequently analyzed by phosphoric acid addition on a Model 700 TOC Analyzer (OI
8 Corporation, College Station, TX, USA), with a detection limit of 0.15 ppm. Temperature
9 and pH were measured with a SCHOTT pH meter (SCHOTT AG, Mainz, Germany).
10 Concentrations of dissolved CO_2 , bicarbonate and carbonate were calculated from DIC and
11 pH (Stumm & Morgan 1996), based on the dissociation constants of inorganic carbon
12 corrected for temperature and salinity (Table S2). Alkalinity was determined in a 50 mL
13 sample that was titrated in 0.1 to 1 mL steps with 10 mmol L^{-1} HCl to a pH of 3.0. The
14 alkalinity was subsequently calculated using Gran plots (Stumm & Morgan 1996).

15 Residual nitrate and phosphate concentrations in the chemostats were determined in
16 triplicate by sampling 15 mL of culture suspension, which was immediately filtered over 0.45
17 μm membrane filters (Whatman, Maidstone, UK) and the filtrate was stored at -20°C . Nitrate
18 concentrations were analyzed using a Skalar SA 400 autoanalyzer (Skalar Analytical B.V.,
19 Breda, The Netherlands), and phosphate concentrations were analyzed
20 spectrophotometrically (Murphy & Riley 1962).

21 Phytoplankton population density, both as cell numbers and total biovolume, was
22 determined in triplicate using a Casy 1 TTC cell counter with a 60 μm capillary (Schärfe
23 System GmbH, Reutlingen, Germany). Cell size varied considerably during the experiments,
24 ranging from 31-66 $\mu\text{m}^3 \text{ cell}^{-1}$ for *Microcystis* CYA140 and from 21-74 $\mu\text{m}^3 \text{ cell}^{-1}$ for

1 *Microcystis* HUB5-2-4. We therefore used the total biovolume (i.e. the summed volume of all
2 cells per litre of water) as a measure of phytoplankton population density.

3 Samples for cellular carbon, nitrogen, phosphorus and sulfur content were pressurized
4 at 10 bar to collapse the gas vesicles of *Microcystis* and subsequently centrifuged for 15 min
5 at 2,000 *g*. After discarding the supernatant, the pellet was resuspended in demineralised
6 water, and centrifuged for 5 min at 15,000 *g*. The supernatant was discarded, pellets were
7 stored at -20°C and subsequently freeze-dried and weighted to determine dry weight. The
8 carbon, nitrogen and sulfur content of homogenised freeze-dried cell powder were analysed
9 using a Vario EL Elemental Analyzer (Elementar Analysensysteme GmbH, Hanau,
10 Germany). To determine the phosphorus content, cells were oxidized with potassium
11 persulfate for 1 h at 100°C (Wetzel & Likens 2000), and phosphate concentrations were
12 subsequently analyzed spectrophotometrically (Murphy & Riley 1962).

13

14 *Parameter estimation*

15 System parameters such as incident light intensity, composition of the mineral medium,
16 dilution rate, and CO₂ concentration in the gas flow were experimentally controlled (Table
17 S1). Growth parameters of the phytoplankton were estimated by fitting the time courses
18 predicted by the model to the time courses of the variables measured during the experiments.
19 These measured variables included population size (*X*), cellular carbon and nitrogen content
20 (*Q_C* and *Q_N*), light transmission (*I_{OUT}*), DIC concentrations, alkalinity and pH. In the
21 experiments with *Microcystis* HUB5-2-4, the specific light attenuation coefficient (*k*) was
22 found to increase linearly with the cellular nitrogen content *Q_N* (Table S3 and Fig. S1). The
23 model fits were based on minimization of the residual sum of squares, following the same
24 procedures as in earlier studies (Huisman *et al.* 1999; Passarge *et al.* 2006). The parameter
25 estimates are summarized in Table S3.

Results

Dynamics under carbon, nitrogen and light limitation

We first studied growth of *Microcystis* CYA140 under carbon-, nitrogen- and light-limited conditions. This showed that the changes in population density, light penetration, carbon and nitrogen availability, pH and C:N stoichiometry were specific for each limitation (Fig. 2). Population densities increased and reached steady state within 15-30 days (Fig. 2a-c). The steady-state population density was lowest in the nitrogen-limited and highest in the light-limited chemostat (Fig. 2b,c). Dense phytoplankton populations absorbed more light. Hence, light intensity transmitted through the water column (I_{OUT}) was highest in the nitrogen-limited and lowest in the light-limited chemostat (Fig. 2b,c).

Assimilation of CO₂ by the growing phytoplankton populations decreased the dissolved CO₂ concentration and increased pH (Fig. 2d-f). Under carbon-limited conditions, the dissolved CO₂ concentration was depleted by 98% to $< 0.2 \mu\text{mol L}^{-1}$ and the pH increased to 10 (Fig. 2d). In contrast, the dissolved CO₂ concentration was reduced by only 30-40% to $\sim 10 \mu\text{mol L}^{-1}$ while the pH increased by only 0.5 units under nitrogen- and light-limited conditions (Fig. 2e,f).

The growing phytoplankton populations consumed nitrate (Fig. 2g-i). Under carbon- and light-limited conditions, the residual nitrate concentration remained high (Fig. 2g,i), whereas under nitrogen-limited conditions the nitrate concentration was depleted to $\sim 5 \mu\text{mol L}^{-1}$ at steady state (Fig. 2h).

The availability of nitrogen, carbon and light strongly affected the phytoplankton C:N stoichiometry, which decreased to 5.7 ± 0.2 under carbon-limited and 4.9 ± 0.1 under light-limited conditions (Fig. 2g,i). In contrast, their molar C:N ratio increased to 10.9 ± 0.6 under nitrogen-limited conditions (Fig. 2h).

The model not only predicted the steady-state conditions, but also adequately captured the transient dynamics under these wide-ranging growth conditions (compare symbols and lines in Fig. 2).

Steady-state patterns along a pCO₂ gradient

We studied steady-state patterns in twelve chemostats with *Microcystis* HUB5-2-4, supplied with a range of pCO₂ levels in the gas flow at either a low or high nitrate concentration in the mineral medium (Fig. 3). The steady-state population density increased with rising pCO₂ levels. At low nitrogen loads, the population density leveled off at pCO₂ > 100 ppm, while at high nitrogen loads the population density increased further and became twice as high at pCO₂ > 300 ppm (Fig. 3a). Due to the increasing phytoplankton population, light penetration I_{OUT} decreased with rising pCO₂ (Fig. 3b). At low nitrogen loads, I_{OUT} remained fairly high at ~17 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ for pCO₂ > 100 ppm, while at high nitrogen loads I_{OUT} was reduced to only 3 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$.

At low pCO₂, phytoplankton photosynthesis depleted the dissolved CO₂ concentration to 0.1 $\mu\text{mol L}^{-1}$ and raised pH to 10 (Fig. 3c,d). The control that phytoplankton exerted on the dissolved CO₂ concentration and pH decreased at a threshold of pCO₂ > 100 ppm. Beyond this threshold, the dissolved CO₂ concentration increased while the pH decreased with rising pCO₂. Interestingly, the nitrogen load had only marginal effects on the response of dissolved CO₂ and pH to rising pCO₂ levels.

In the chemostats supplied with high nitrogen loads, the residual nitrate concentration remained high and the molar C:N ratio of the phytoplankton remained low irrespective of pCO₂ (Fig. 3e,f). In contrast, in chemostats supplied with low nitrogen loads, the residual nitrate concentration and C:N stoichiometry depended on pCO₂. Residual nitrate was

1 depleted to 10 $\mu\text{mol L}^{-1}$ at $\text{pCO}_2 > 100$ ppm, while the C:N ratio increased from ~6 at $\text{pCO}_2 <$
2 50 ppm to almost 14 at $\text{pCO}_2 > 100$ ppm.

3 The model described the steady-state data well (Fig. 3). It predicts that phytoplankton
4 growth switched from carbon to nitrogen limitation at a pCO_2 of 80-100 ppm in the
5 experiments supplied with low nitrogen, while it switched from carbon to light limitation at a
6 pCO_2 of 100-300 ppm in the experiments with high nitrogen loads.

7

8 *Model predictions at different pCO_2 levels and nitrogen loads*

9 We used the model to predict steady-state patterns along a wider range of pCO_2 levels and
10 nitrogen loads. Steady-state phytoplankton population density increased with rising pCO_2
11 levels and nitrogen load (Fig. 4a). Absorption of light by the increasing phytoplankton
12 population decreased light penetration (Fig. 4b). At high pCO_2 levels and nitrogen loads (i.e.,
13 $\text{pCO}_2 > 400$ ppm and $[\text{DIN}]_{\text{IN}} > 1.5 \text{ mmol L}^{-1}$), the increase in population density and
14 decrease in light penetration leveled off, and phytoplankton growth became limited by the
15 low availability of light.

16 At low pCO_2 levels but high nitrogen loads, phytoplankton growth depleted dissolved
17 CO_2 concentrations and raised the pH to high levels (Fig. 4c,d). Under these conditions,
18 phytoplankton growth was limited by the availability of inorganic carbon. At high pCO_2
19 levels but low nitrogen loads, phytoplankton growth depleted dissolved nitrate concentrations
20 (Fig. 4e), while the carbon:nutrient ratio of the phytoplankton was high ($\text{C:N} > 13$; Fig. 4f).
21 Under these conditions, phytoplankton growth was limited by nitrogen availability.

22

23 **Discussion**

24

1 One of the major achievements of our study is the incorporation of inorganic carbon
2 chemistry into a stoichiometrically explicit model. The experimental results show that the
3 model successfully predicted the population dynamics, nutrient and light availability, carbon
4 chemistry, pH and elemental stoichiometry during phytoplankton growth (Figs. 2-4).
5 Moreover, the model predictions and experimental results are in good agreement with the
6 hypotheses put forward in Fig. 1. More specifically, in the experiments at high nitrogen loads,
7 rising pCO₂ levels shifted phytoplankton growth from carbon-limited to light-limited
8 conditions. This resulted in a strong increase of phytoplankton biomass (Hypothesis 1a;
9 Fig. 1b and Fig. 3a), while the carbon:nitrogen ratio of the phytoplankton remained low
10 (Hypothesis 2b; Fig. 1c and Fig. 3f). Conversely, at low nitrogen loads, rising pCO₂ shifted
11 phytoplankton growth from carbon-limited to nitrogen-limited conditions. This increased
12 phytoplankton biomass to only a minor extent (Hypothesis 1b; Fig. 1b and Fig. 3a), but
13 strongly increased the carbon:nitrogen ratio of the phytoplankton (Hypothesis 2a; Fig. 1c and
14 Fig. 3f).

15 The conceptual framework of Fig. 1 not only allows a straightforward interpretation
16 of our experimental results, but may also help to reconcile contrasting results of several
17 earlier laboratory and field experiments. Different studies have compared different
18 hypotheses. For example, Fu *et al.* (2010) investigated our Hypotheses 1a and 1b. In line with
19 these hypotheses, Fu and colleagues found that growth rates of the harmful dinoflagellate
20 *Karlodinium veneficum* increased with increasing pCO₂ concentrations under phosphate-
21 replete but not under phosphate-limiting conditions. Li *et al.* (2012) investigated all four
22 hypotheses using the diatom *Phaeodactylum tricornutum*. They found that the specific
23 growth rate did not respond to elevated pCO₂ under nitrogen-limited conditions, while it
24 increased slightly but not significantly under nitrogen-enriched conditions. These results
25 confirm Hypothesis 1b, and are consistent with but not strong enough to confirm Hypothesis

1 1a. Furthermore, the C:N ratio of *P. tricornutum* increased from 6.7 to 8.0 when pCO₂ was
2 raised under nitrogen-limited conditions, while it remained at 5.3 – 5.5 when pCO₂ was
3 raised under nitrogen-enriched conditions. These results confirm Hypotheses 2a and 2b.

4 Montechiaro & Giordano (2010) investigated our Hypotheses 1a and 2b. They found
5 that, under nutrient replete conditions, elevated pCO₂ concentrations increased the growth
6 rates of the dinoflagellate *Protoceratium reticulatum* but had only a minor impact on
7 phytoplankton stoichiometry. In contrast, Riebesell *et al.* (2007) investigated Hypotheses 1b
8 and 2a. They spiked marine mesocosm experiments with nutrients at three different pCO₂
9 levels. After an initial increase in phytoplankton biomass, the nutrient concentrations were
10 depleted and phytoplankton biomass returned back to normal levels irrespective of the pCO₂
11 treatment. However, the stoichiometry of carbon to nitrogen drawdown increased from 6.0 at
12 low pCO₂ to 8.0 at high pCO₂, thus exceeding the Redfield carbon:nitrogen ratio of 6.6 in
13 today's ocean (Riebesell *et al.* 2007). Similar results were obtained by Verschoor *et al.*
14 (2013) in freshwater mesocosm experiments with green algae and cyanobacteria. Growth at
15 elevated pCO₂ levels led to depletion of the concentrations of dissolved inorganic nitrogen
16 and phosphorus. Elevated pCO₂ levels did not have a significant effect on phytoplankton
17 biomass, but shifted their elemental composition towards higher carbon:nutrient ratios
18 (Verschoor *et al.* 2013), thus confirming Hypotheses 1b and 2a.

19 Of course, the hypotheses presented in Figure 1 are an abstract presentation of how
20 rising pCO₂ levels may affect phytoplankton biomass and stoichiometry, emphasizing the
21 contrast between oligotrophic and eutrophic ecosystems. In reality, and also in our
22 experiments (see Fig. 4), there is a gradual transition between the different resource limitation
23 patterns. Such gradual transitions are often due to co-limitation, i.e., to interactive effects of
24 the limiting resources. For instance, in our model, light energy is required for the assimilation
25 of carbon and nitrogen, whereas nitrogen molecules are needed to develop the photosynthetic

1 machinery harvesting the available light energy. Hence, at intermediate nutrient loads both
2 the biomass and carbon:nutrient ratios of phytoplankton can increase in response to an
3 increase in pCO₂. Indeed, when the green alga *Scenedesmus acutus* was grown at P
4 concentrations corresponding to meso- to eutrophic conditions, elevated pCO₂ levels
5 stimulated algal growth and reduced algal P:C ratios (Urabe *et al.* 2003). Mesotrophic
6 systems may therefore be particularly sensitive to rising pCO₂ levels, because they can suffer
7 from the dual effects of both bloom intensification and changes in phytoplankton
8 stoichiometry.

9 Our results provide “proof of principle”, based on a combination of theory and
10 experiments. However, like all models, our model is based on a series of simplifying
11 assumptions that ignore many of the intriguing complexities of the natural world. For
12 example, we simplified cell physiology, in particular the uptake and assimilation of inorganic
13 carbon by carbon concentrating mechanisms. Cyanobacteria have five different inorganic
14 carbon uptake systems, a series of carbonic anhydrases, and specialized cellular
15 compartments known as carboxysomes where CO₂ fixation takes place (Badger *et al.* 1998,
16 Giordano *et al.* 2005, Sandrini *et al.* 2014). Incorporation of these physiological components,
17 and their regulatory mechanisms, into our model may improve understanding of the
18 physiological acclimation of phytoplankton to changing CO₂ conditions.

19 Furthermore, our study operated at the laboratory scale, with model parameters tuned to
20 the small dimensions and highly idealized conditions of chemostat experiments. This implies
21 that the critical nitrogen loads or pCO₂ levels at which we measured a transition from, say,
22 carbon-limited to nutrient-limited conditions cannot be directly applied to lakes and
23 reservoirs. For instance, light supply and CO₂ influx are expressed per unit surface area
24 whereas nutrient depletion occurs per unit volume. Hence, the critical nutrient loads and
25 pCO₂ levels will have different values for systems with dimensions that differ from our

chemostat vessels. Furthermore, we used relatively low and constant light levels, did not investigate temperature effects, and ignored the potential impacts of grazing by herbivores. Implementation of our model in more extensive ecosystem studies will require proper dimensionalization and incorporation of additional processes relevant for these ecosystems.

Despite these caveats, extrapolation of our results to natural ecosystems indicates that rising pCO₂ concentrations will intensify phytoplankton blooms in eutrophic and hypertrophic waters. This may further enhance the productivity of these waters, but is also likely to aggravate the problems associated with phytoplankton blooms, such as deterioration of water quality, anoxia and fish kills (Hallegraeff 1993; Paerl & Huisman 2009). In particular, more intense blooms by toxic phytoplankton species, such as the freshwater cyanobacterium *Microcystis* used in this study, can make waters unsuitable for recreation, fisheries, drinking water and agricultural purposes (Verspagen *et al.* 2006; Jöhnk *et al.* 2008; Xu *et al.* 2010; Michalak *et al.* 2013). In oligotrophic waters, rising pCO₂ levels are less likely to intensify blooms, but will increase phytoplankton carbon:nutrient ratios. Such changes in elemental stoichiometry tend to reduce the nutritional quality of phytoplankton (Sterner & Elser 2002), which may lead to reduced growth rates and higher carbon excretion rates by nutrient-demanding zooplankton feeding on these phytoplankton populations (Urabe *et al.* 2003; Schoo *et al.* 2013). Increases in phytoplankton carbon:nutrient ratios can therefore alter food-web structure and may reduce the secondary production of oligotrophic waters (Van de Waal *et al.* 2010). Our results thus imply that rising atmospheric CO₂ levels will have very different consequences for eutrophic versus oligotrophic ecosystems.

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5

1 **Supporting Information**

2

3 Additional Supporting Information may be downloaded via the online version of this article
4 at Wiley Online Library (www.ecologyletters.com).

5

6 **Appendix S1** Full description of the model.

7 **Table S1** System parameters of the chemostat experiments.

8 **Table S2** Solubility and dissociation constants of dissolved inorganic carbon and phosphates
9 in water.

10 **Table S3** Parameter values estimated for the *Microcystis* strains.

11 **Figure S1** Specific light attenuation coefficient as function of the cellular nitrogen content.

12

13

1 **Figure legends**

2

3 Figure 1 Hypothesized patterns. (a) Resource limitation at different $p\text{CO}_2$ levels and nutrient
4 loads. The arrows indicate changes in resource limitation with rising $p\text{CO}_2$ levels, at low
5 nutrient loads (black arrow) and high nutrient loads (white arrow). (b) Phytoplankton
6 population density, and (c) carbon:nutrient stoichiometry of phytoplankton cells at different
7 $p\text{CO}_2$ levels. Dashed lines represent low nutrient loads, solid lines represent high nutrient
8 loads.

9

10 Figure 2 Dynamics observed under carbon-limited (left column), nitrogen-limited (middle
11 column) and light-limited conditions (right column). (a-c) Phytoplankton population density
12 (expressed as biovolume) and light intensity penetrating through the chemostat (I_{OUT}). (d-f)
13 Dissolved CO_2 concentration and pH. (g-i) Dissolved inorganic nitrogen concentration
14 ([DIN]) and phytoplankton C:N ratio. The experiments were performed with *Microcystis*
15 CYA140. Symbols represent measurements, lines represent model fits. Parameter values of
16 the model are provided in Tables S1-S3.

17

18 Figure 3 Steady-state patterns at different partial pressures of CO_2 ($p\text{CO}_2$), in experiments
19 with either low nitrogen loads (open circles and dashed lines) or high nitrogen loads (closed
20 circles and solid lines). (a) Phytoplankton population density (expressed as biovolume), (b)
21 light intensity penetrating through the chemostat (I_{OUT}), (c) dissolved CO_2 concentration, (d)
22 pH, (e) dissolved inorganic nitrogen concentration ([DIN]), and (f) phytoplankton C:N ratio.
23 The experiments were performed with *Microcystis* HUB5-2-4. Symbols represent
24 measurements, lines represent model fits. Parameter values of the model are provided in
25 Tables S1-S3.

1
2 Figure 4 Model prediction of steady-state patterns at different pCO₂ levels and nitrogen loads.
3 (a) Phytoplankton population density (expressed as biovolume), (b) light intensity penetrating
4 through the chemostat (I_{OUT}), (c) dissolved CO₂ concentration, (d) pH, (e) dissolved inorganic
5 nitrogen concentration ([DIN]), and (f) phytoplankton C:N ratio. The solid white line
6 indicates the boundary between carbon- and nitrogen-limited growth according to the
7 minimum function in Eq. 4. The dashed white line roughly indicates the transition from
8 carbon to light limitation (vertical line), and from nitrogen to light limitation (horizontal line).
9 The model predictions are based on parameter estimates obtained from our experiments with
10 *Microcystis* HUB5-2-4 (Tables S1-S3).
11