Competition between cyanobacteria and green algae at low versus elevated CO2: who will win, and why?

Ji, X.; Verspagen, J.M.H.; Stomp, M.; Huisman, J.

Published in:
Journal of Experimental Botany

DOI:
10.1093/jxb/erx027

License
CC BY

Citation for published version (APA):

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: https://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
RESEARCH PAPER

Competition between cyanobacteria and green algae at low versus elevated CO2: who will win, and why?

Xing Ji, Jolanda M.H. Verspagen, Maayke Stomp and Jef Huisman*

Department of Aquatic Microbiology, Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, PO Box 94248, 1090 GE Amsterdam, The Netherlands

* Correspondence: j.huisman@uva.nl

Received 7 November 2016; Editorial decision 11 January 2017; Accepted 12 January 2017

Editor: Howard Griffiths, University of Cambridge

Abstract

Traditionally, it has often been hypothesized that cyanobacteria are superior competitors at low CO2 and high pH in comparison with eukaryotic algae, owing to their effective CO2-concentrating mechanism (CCM). However, recent work indicates that green algae can also have a sophisticated CCM tuned to low CO2 levels. Conversely, cyanobacteria with the high-flux bicarbonate uptake system BicA appear well adapted to high inorganic carbon concentrations. To investigate these ideas we studied competition between three species of green algae and a bicA strain of the harmful cyanobacterium Microcystis aeruginosa at low (100 ppm) and high (2000 ppm) CO2. Two of the green algae were competitively superior to the cyanobacterium at low CO2, whereas the cyanobacterium increased its competitive ability with respect to the green algae at high CO2. The experiments were supported by a resource competition model linking the population dynamics of the phytoplankton species with dynamic changes in carbon speciation, pH and light. Our results show (i) that competition between phytoplankton species at different CO2 levels can be predicted from species traits in monoculture, (ii) that green algae can be strong competitors under CO2-depleted conditions, and (iii) that bloom-forming cyanobacteria with high-flux bicarbonate uptake systems will benefit from elevated CO2 concentrations.

Key words: Carbon dioxide, climate change, competition model, CO2-concentrating mechanism, cyanobacteria, green algae, harmful algal blooms, lakes, Microcystis aeruginosa.

Introduction

Cyanobacterial blooms have become a major water quality problem in many eutrophic lakes worldwide (Chorus and Bartram, 1999; Verspagen et al., 2006; Guo, 2007; Michalak et al., 2013). They produce taste and odor compounds that may interfere with the recreational function of lakes and the preparation of drinking water (Chorus and Bartram, 1999; Watson et al., 2008). Moreover, cyanobacteria can produce a variety of toxins, causing liver, digestive and neurological diseases when ingested by waterfowl, pets, cattle, and humans (Carmichael, 2001; Codd et al., 2005; Huisman et al., 2005; Merel et al., 2013). Hence, an improved understanding of the environmental conditions that favor the dominance of cyanobacteria over eukaryotic phytoplankton species is desirable.

Dense cyanobacterial blooms often deplete the dissolved CO2 concentration in surface waters, sometimes down to less than 0.1 μmol l−1 corresponding to pCO2 less than 3 parts per million (ppm) (Lazzarino et al., 2009; Balmer and Downing, 2011). CO2 depletion by dense blooms induces...
high pH values, above 9 or even 10 (Talling, 1976; Ibelings and Maberly, 1998; Verspagen et al., 2014b). At these pH values, most dissolved inorganic carbon (DIC) is in the form of bicarbonate, and with increasing pH an increasing fraction of DIC is converted to carbonate. Under these conditions, CO₂ availability can become an important limiting factor for photosynthesis. Cyanobacteria have developed a highly efficient CO₂-concentrating mechanism (CCM) to take up CO₂ and bicarbonate as inorganic carbon (Cᵋ) source, and to augment the intracellular CO₂ level around the ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) enzyme responsible for carbon fixation (Price et al., 2008; Raven et al., 2012; Burnap et al., 2015). It has therefore been hypothesized that cyanobacteria are superior competitors at low CO₂ levels, and will dominate waters in which the dissolved CO₂ concentration has been depleted (Shapiro, 1990, 1997). Conversely, eukaryotic phytoplankton might be better competitors at high CO₂ levels. This classic paradigm has received support from several competition experiments between cyanobacteria and green algae (Caraco and Miller, 1998; Low-Décarie et al., 2011, 2015; but see Verschoor et al., 2013). If this paradigm is true, the logical corollary is that rising CO₂ levels will particularly benefit eukaryotic phytoplankton species at the expense of cyanobacteria.

Yet, recent insights indicate that this classic paradigm might be too simple. Although the details of the eukaryotic CCM are not yet fully understood, green algae can also deploy a sophisticated CCM well adapted to low CO₂ levels (Moroney and Ynalvez, 2007; Wang et al., 2011; Meyer and Griffiths, 2013). Furthermore, recent studies have revealed a striking genetic and phenotypic diversity in the CCM of harmful cyanobacteria (Sandrini et al., 2014, 2015b; Visser et al., 2016). All harmful freshwater cyanobacteria investigated so far contain two CO₂ uptake systems and the ATP-dependent bicarbonate uptake system BCT1. In addition, however, some cyanobacteria deploy the high-affinity but low-flux bicarbonate uptake system SbtA, whereas other cyanobacteria deploy the low-affinity but high-flux bicarbonate uptake system BicA (Sandrini et al., 2014). Therefore, three Cᵋ uptake genotypes can be distinguished: (i) sbtA strains (high-affinity specialists), (ii) bicA strains (high-flux specialists), and (iii) bicA+sbtA strains (CCM generalists). These three genotypes were first described for the genus Microcystis (Sandrini et al., 2014, 2016), but similar genetic diversity also exists within other harmful cyanobacterial genera such as Dolichospermum (formerly known as Anabaena) and Planktothrix (Visser et al., 2016).

Laboratory selection experiments with mixtures of several Microcystis strains found that the strain composition shifted from bicA+sbtA strains at low pCO₂ to bicA strains at high pCO₂ (Sandrini et al., 2016). Similarly, in a eutrophic lake, bicA+sbtA strains were dominant when Cᵋ concentrations in the lake were depleted during a dense cyanobacterial bloom, but were replaced by bicA strains when Cᵋ concentrations increased later in the season (Sandrini et al., 2016). These results show that the genetic composition of cyanobacterial communities adapts to changes in Cᵋ availability by means of natural selection, favoring CCM generalists at low CO₂ levels while favoring high-flux specialists at high CO₂. In natural waters, however, cyanobacteria compete not only amongst each other, but also against eukaryotic phytoplankton. How the competitive abilities of different cyanobacterial Cᵋ uptake genotypes perform against eukaryotic species such as green algae has not yet been investigated.

Resource competition theory provides a theoretical framework to understand and predict how changes in resource availability may affect the species composition (Tilman, 1982; Huisman and Weissing, 1994; Grover, 1997). This body of theory uses the kinetic traits of species measured in monoculture to predict the dynamics and outcome of competition for limiting resources in species mixtures. Resource competition models have been successfully applied to predict competition for nutrients and light, both in qualitative and quantitative terms (Tilman, 1977; Sommer, 1985; Huisman et al., 1999; Litchman et al., 2004; Stomp et al., 2004; Passarge et al., 2006). Competition for inorganic carbon is conceptually more complicated, however, because the species compete for two resources (CO₂ and bicarbonate) whose concentrations depend not only on resource uptake but also on pH and alkalinity. Moreover, pH and alkalinity depend in turn on a variety of biogeochemical processes and also change in response to the carbon and nutrient uptake activity by the phytoplankton community (Talling, 1976; Wolf-Gladrow et al., 2007; Verspagen et al., 2014b).

This study investigated competition between a harmful cyanobacterium (Microcystis PCC 7806) and three species of green algae (Monoraphidium griffithii, Scenedesmus obliquus, and Chlorella vulgaris) at low and at high CO₂ concentrations. Microcystis PCC 7806 is a bicA strain (Sandrini et al., 2014), and is therefore expected to be a relatively weak competitor at low CO₂ levels but a stronger competitor at high CO₂. To investigate this hypothesis, we ran monoculture experiments of each species at both low and high CO₂ levels (100 and 2000 ppm). The results of these monoculture experiments were used to parameterize a resource competition model, which predicted the competitive interactions between the species based on dynamic changes in inorganic carbon chemistry, alkalinity and pH induced by the growing phytoplankton populations. Next, competition experiments were carried out to test the model predictions. Together, the theory and experiments may help in understanding shifts in phytoplankton community composition in response to rising CO₂ levels.

### Competition model: theory and development

We first developed a model to investigate competition for inorganic carbon and light among phytoplankton species. The model combined previous theoretical work on growth and competition under light-limited (Huisman and Weissing, 1994; Huisman et al., 1999; Passarge et al., 2006) and carbon-limited conditions (Van de Waal et al., 2011; Verspagen et al., 2014a, b).

The model considers a well-mixed vertical water column, where depth $z$ runs from 0 at the water surface to $z_{\text{max}}$ at the
bottom of the water column. The population dynamics of the phytoplankton species are governed by their light-dependent assimilation of carbon dioxide and bicarbonate. The model assumes eutrophic conditions, in which all nutrients are in ample supply and hence do not limit phytoplankton growth. Uptake of inorganic carbon and nutrients induces dynamic changes in pH and alkalinity. These changes in pH and alkalinity affect the availability of the different inorganic carbon species, which feeds back on phytoplankton growth. The growing phytoplankton populations also increase the turbidity of the water column, thereby diminishing the light available for further photosynthesis and growth.

Species dynamics

The model assumes that the specific growth rates of the species depend on their carbon assimilation. Let $X_i$ denote the population density of phytoplankton species $i$, and let $Q_i$ denote its cellular carbon content (also known as carbon quota; sensu Droop, 1973). The population dynamics of a number of $n$ competing species can then be written as:

$$\frac{dX_i}{dt} = \mu_i (Q_i) X_i - m_i X_i \quad i = 1, \ldots, n$$  \hspace{1cm} (1)

where $\mu_i(Q_i)$ is the specific growth rate of species $i$ as an increasing function of its carbon content, and $m_i$ is its loss rate. We assume that each species requires a minimum cellular carbon content in order to function, and reaches its maximum specific growth rate when cells are satiated with carbon (see Supplementary Model S1 at JXB online). In our application, the loss rates of the species will be governed by the dilution rate of the chemostat (i.e. $m=D$).

The carbon contents of the species increase through uptake of carbon dioxide ($u_{CO_2,i}$) and bicarbonate ($u_{HCO_3,i}$), and decrease through respiration ($r_i$) and dilution of the carbon content by growth:

$$\frac{dQ_i}{dt} = u_{CO_2,i} + u_{HCO_3,i} - r_i - \mu_i (Q_i) Q_i \quad i = 1, \ldots, n$$  \hspace{1cm} (2)

We assume that uptake rates of CO$_2$ ($u_{CO_2,i}$) and bicarbonate ($u_{HCO_3,i}$) are increasing functions of the ambient CO$_2$ and bicarbonate concentration according to Michaelis–Menten kinetics. Carbon uptake and assimilation require energy from the light reactions of photosynthesis, and therefore the carbon uptake rates also depend on the photosynthetic activity of the cells and hence on light availability. Furthermore, we incorporated a simple negative feedback loop in which carbon uptake rates decrease with increasing cellular carbon content, such that carbon uptake systems are active under carbon-limiting conditions (Eisenhut et al., 2007; Burnap et al., 2015; Wang et al., 2015) and down-regulated when cells become satiated with carbon (Beardall and Giordano, 2002; Sandrini et al., 2015a). Respiration rates ($r_i$) of the species increase with their cellular carbon content, approaching maximum values when cells become satiated with carbon. The mathematical equations describing these relationships are presented in Supplementary Model S1.

Light conditions

The underwater light gradient is described by the Lambert–Beer law (Huisman et al., 1999):

$$I(z) = I_{in} \exp(-K_{bg} z - \sum_{i=1}^{n} k_i X_i z)$$  \hspace{1cm} (3)

where $I(z)$ is the light intensity at depth $z$, $I_{in}$ is the incident light intensity, $K_{bg}$ is the background turbidity of the water itself, and $k_i$ is the specific light attenuation coefficient of phytoplankton species $i$. We note that the light gradient changes dynamically, because the light intensity at a given depth decreases with increasing phytoplankton densities. We define $I_{out}$ as the light intensity reaching the bottom of the water column (i.e. $I_{out}=I(z_{\text{max}})$).

Dissolved inorganic carbon

Changes in the concentration of total dissolved inorganic carbon, [DIC], are described by (Verspagen et al., 2014b):

$$\frac{d[DIC]}{dt} = D([DIC]_{\text{in}} - [DIC]) + \frac{g_{CO_2}}{z_{\text{max}}} - \sum_{i=1}^{n} (u_{CO_2,i} + u_{HCO_3,i}) X_i + \sum_{i=1}^{n} r_i X_i$$  \hspace{1cm} (4)

The first term on the right-hand side of this equation describes changes through the influx ($[DIC]_{\text{in}}$) and efflux of water containing DIC. The second term describes CO$_2$ exchange with the atmosphere, where $g_{CO_2}$ is the CO$_2$ flux across the air–water interface and division by $z_{\text{max}}$ converts the flux per unit surface area into the corresponding change in DIC concentration. The third term describes uptake of dissolved CO$_2$ and bicarbonate by the photosynthetic activity of the phytoplankton community. Finally, the fourth term describes CO$_2$ release by respiration of the phytoplankton species.

The CO$_2$ flux across the air–water interface, $g_{CO_2}$, depends on the difference in partial pressure. More specifically, $g_{CO_2}$ depends on the difference between the expected concentration of dissolved CO$_2$ in water if in equilibrium with the partial pressure in the atmosphere and the actual dissolved CO$_2$ concentration (Siegenthaler and Sarmiento, 1993; Cole et al., 2010):

$$g_{CO_2} = n (K_0 p_{CO_2} - [CO_2])$$  \hspace{1cm} (5)

where $n$ is the gas transfer velocity (also known as piston velocity), $K_0$ is the solubility of CO$_2$ gas in water (also known as Henry’s constant), $p_{CO_2}$ is the partial pressure of CO$_2$ in the atmosphere, and $[CO_2]$ is the dissolved CO$_2$ concentration. In chemostats, gas transfer will depend on the gas flow rate ($a$). We therefore assume that $n=ba$, where $b$ is a constant of proportionality.

Dissolved CO$_2$, bicarbonate and carbonate concentrations, and pH were calculated from [DIC] and alkalinity (Stumm and Morgan, 1996). Assimilation of nitrate, phosphate and sulfate by phytoplankton involves proton consumption, thus increasing alkalinity (Wolf-Gladrow et al., 2007; Verspagen et al., 2014b). Therefore, the model treats alkalinity as a dynamic variable (see Supplementary Model S1 for details).
Materials and methods

Species
We performed monoculture and competition experiments with four freshwater phytoplankton species: the green algae *Monoraphidium griffithii* (Berk.) Kom.-Legn. (strain CCAP 202/15A), *Scenedesmus obliquus* (strain CCAP 276/3A) and *Chlorella vulgaris* Beyerinck (strain UTEX 259), and the cyanobacterium *Microcystis aeruginosa* (strain PCC 7806). *Microcystis PCC 7806* produces the hepatotoxins microcystin-LR and [Asp3]-microcystin-LR and the potential neurotoxins cyanopeptolin A, C and 970 (Tonk et al., 2009; Faltermann et al., 2014). All four species were unialgal but not axenic. Regular microscopic inspection confirmed that concentrations of heterotrophic bacteria remained low throughout the experiments (<1% of the total biovolume measured with a CASY TTC cell counter; OLS OMNI Life Science, Bremen, Germany).

Chemostat experiments
All experiments were conducted in laboratory-built chemostats, specifically designed to study the population dynamics of phytoplankton species (Huisman et al., 2002; Passarge et al., 2006; Verspagen et al., 2014b). The chemostats allowed full control of light conditions, temperature, pCO2 in the gas flow, and nutrient concentrations in the mineral medium. Each chemostat consisted of a flat culture side to create a unidirectional light gradient, using a constant incident light intensity (I0) of 50 ± 1 µmol photons m−2 s−1 provided by white fluorescent tubes (Philips PL-L 24W/840/4P, Philips Lighting, Eindhoven, The Netherlands). The temperature was maintained at 20 ± 1 °C with a stainless steel cooling finger inside each chemostat and connected to a Colora thermocryostat. To avoid nutrient limitation, the chemostats were provided with a very nutrient-rich mineral medium (Van de Waal et al., 2009; Verspagen et al., 2014a). The dilution rate was maintained at D=0.125 d−1.

CO2 supply
We applied two CO2 treatments. The chemostats were bubbled with gas containing a CO2 concentration of either 100 ppm (‘low pCO2’) or 2000 ppm (‘high pCO2’) (Table 1). The gas was prepared as a mixture of pressurized air from which the variable CO2 concentration was completely removed using a CO2 scrubber (Ecodry Europe NV, Leuven, Belgium) and stored at 4 ºC until analysis. DIC and sulfur content of homogenized freeze-dried cell powder the pellets were freeze-dried and weighted, and the carbon, nitrogen and sulfur content of homogenized freeze-dried cell powder were analysed with a Vario EL Elemental Analyzer (Elementar Analysensysteme GmbH, Hanau, Germany).

To determine the cellular carbon, nitrogen and sulfur content, pellets from the 50 ml falcon tube were transferred into 2 ml Eppendorf tubes, washed three times with a nutrient-free NaCl solution with a salinity equal to our medium, and stored at −20 °C. Subsequently, the pellets were freeze-dried and weighted, and the carbon, nitrogen and sulfur content of homogenized freeze-dried cell powder were analysed with a Vario EL Elemental Analyzer (Elementar Analysensysteme GmbH, Hanau, Germany).

Model parameterization
The model parameters consisted of system parameters and species parameters. System parameters are under experimental control and were regularly measured during the experiments. We had already specified several of the system parameters, such as the incident light intensity (I0), pCO2 level in the gas flow, and dilution rate (D) of the chemostats. A complete list of all system parameters is provided in Table 1.

Species parameters describe the traits of the species. Some species parameters were measured experimentally. The maximum specific growth rate (µmax) and minimum cellular carbon content (Qmin) were determined in batch cultures at an I0 of 50 ± 1 µmol photons

Table 1. System parameters applied in the experiments

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Value</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>D</td>
<td>Dilution rate</td>
<td>0.125</td>
<td>d⁻¹</td>
</tr>
<tr>
<td>zmax</td>
<td>Mixing depth</td>
<td>0.05</td>
<td>m</td>
</tr>
<tr>
<td>T</td>
<td>Temperature</td>
<td>20</td>
<td>°C</td>
</tr>
<tr>
<td>I0</td>
<td>Incident light intensity</td>
<td>50</td>
<td>µmol photons m⁻² s⁻¹</td>
</tr>
<tr>
<td>Kbg</td>
<td>Background turbidity</td>
<td>7–11</td>
<td>m⁻¹</td>
</tr>
<tr>
<td>DICn</td>
<td>DIC concentration at influx</td>
<td>0.5 and 2.0</td>
<td>mmol l⁻¹</td>
</tr>
<tr>
<td>ALKn</td>
<td>Alkalinity at influx</td>
<td>0.79 and 2.29</td>
<td>mEq l⁻¹</td>
</tr>
<tr>
<td>sal</td>
<td>Salinity</td>
<td>1.23 and 1.36</td>
<td>g l⁻¹</td>
</tr>
<tr>
<td>pCO2</td>
<td>CO2 concentration</td>
<td>100 and 2000</td>
<td>ppm</td>
</tr>
<tr>
<td>a</td>
<td>Gas flow rate</td>
<td>25</td>
<td>h⁻¹</td>
</tr>
<tr>
<td>v</td>
<td>Gas transfer velocity</td>
<td>0.24 and 0.68</td>
<td>m h⁻¹</td>
</tr>
</tbody>
</table>

a Background turbidity varied among the chemostats vessels.
b The first value refers to the low pCO2 experiments and the second value to the high pCO2 experiments.
m$^{-2}$ s$^{-1}$ and a temperature of 20 ± 1 °C. Maximum specific growth rate was measured in batch cultures aerated with gas containing a saturating CO$_2$ concentration of 10 000 ppm. The minimum cellular carbon content was measured as the cellular carbon content in un aerated dense batch cultures that were first grown for a day in medium to which no DIC was added, and were subsequently incubated overnight in the dark. The cellular N:C and S:C ratios (c$_{N,i}$ and c$_{S,i}$) were calculated from the cellular carbon, nitrogen and sulfur contents measured in the steady-state monocultures of the species. The specific light attenuation coefficients (k$_i$) of the species were estimated from the monoculture experiments using the Lambert–Beer law. For monocultures, Eqn (3) can be written as ln(I$_{in}$/I$_{out}$) = K$_{bg}$+k$_i$X$_i$. Hence, the specific light attenuation coefficient (k$_i$) was estimated as the slope of a linear regression of ln(I$_{in}$/I$_{out}$) versus the population density X$_i$ and the background turbidity (K$_{bg}$) was estimated as the intercept.

All other species parameters were estimated by fitting the model predictions to the observed dynamics in the monoculture experiments. More specifically, we fitted the time courses of population density, light transmission (I$_{out}$), pH and inorganic carbon concentrations predicted by the model to the time courses measured in the monoculture experiments, following the same methodology as in earlier studies (Huisman et al., 1999; Passarge et al., 2006; Verspagen et al., 2014b). To avoid overfitting, the model was fitted simultaneously to both the low pCO$_2$ and high pCO$_2$ monocultures, resulting in eight parameter estimates per species.

The species parameters obtained from the monoculture experiments were combined with the system parameters to predict the population dynamics and inorganic carbon chemistry in the competition experiments.

In the competition experiments, we calculated the rate of competitive displacement (RCD) from the slope of the linear regression of ln(X$_1$/X$_2$) versus time, where X$_1$ and X$_2$ are the population densities of the two competing species (Grover, 1991; Passarge et al., 2006).

### Results

#### Monoculture experiments at low pCO$_2$

At low pCO$_2$, the phytoplankton species increased until a steady state was reached with population densities (expressed as biovolumes) ranging from 370 mm$^3$ l$^{-1}$ for Chlorella to 720 mm$^3$ l$^{-1}$ for Scenedesmus (Fig. 1A–D; Supplementary Table S1). The growing phytoplankton populations reduced the light intensity penetrating through the chemostats (I$_{out}$) to 6.2–9.3 µmol photons m$^{-2}$ s$^{-1}$ and depleted the dissolved CO$_2$ concentration by several orders of magnitude to <0.01 µmol l$^{-1}$ (Fig. 1E–H). Bicarbonate concentrations decreased about one order of magnitude, and were offset by a similar increase of the carbonate concentrations, such that the total DIC concentration in the chemostats remained more or less constant. Alkalinity increased to 1.3–2.0 mEq l$^{-1}$ depending on the species (see Supplementary Table S1 at JXB online). The pH increased by more than two units, from initial values of ~8 to steady state values of ~10.7 (Fig. 1I–L). The relatively high values of I$_{out}$ in combination with severe CO$_2$ depletion and a

![Fig. 1. Monoculture experiments at low pCO$_2$ (100 ppm). (A–D) Population density (expressed as biovolume) and light intensity I$_{out}$ penetrating through the chemostat. (E–H) CO$_2$(aq), bicarbonate and carbonate concentrations. (I–L) Dissolved inorganic carbon (DIC) and pH. Different panels represent different species: (A, E, I) Monoraphidium; (B, F, J) Microcystis; (C, G, K) Scenedesmus; (D, H, L) Chlorella. Symbols indicate experimental data, and lines indicate model fits. Parameter values of the model are provided in Table 1 and Table 2.](https://academic.oup.com/jxb/article-abstract/68/14/3815/3001864?redirectedfrom=10.1093/jxb/erx155)
Sumption to maintain charge balance (Goldman and Brewer, 2014). The pH increased only slightly from ~8 to ~8.6 (Verspagen et al., 2007). A similar increase in DIC and alkalinity induced by dense phytoplankton populations was also observed in earlier chemostat studies (Verspagen et al., 2014b). The pH increased only slightly from ~8 to ~8.6 (Fig. 2I–L). The very low \( I_{\text{out}} \) values in combination with high dissolved CO\(_2\) and bicarbonate concentrations indicate that the phytoplankton growth rates in these experiments were light limited.

### Monoculture experiments at high pCO\(_2\)

At high pCO\(_2\), the phytoplankton species reached population densities ranging from ~2000 mm\(^3\) l\(^{-1}\) for \( \text{Monoraphidium} \) to ~2500 mm\(^3\) l\(^{-1}\) for \( \text{Scenedesmus} \) (Fig. 2A–D; Supplementary Table S1). These values are 3.5–6 times higher than in the low pCO\(_2\) experiments (Fig. 1A–D), thus confirming that phytoplankton growth in the low pCO\(_2\) experiments was indeed carbon limited. The dense phytoplankton populations absorbed almost all incident light, reducing the light intensity penetrating through the chemostats (\( I_{\text{out}} \)) to <0.2 \( \mu \)mol photons m\(^{-2}\) s\(^{-1}\) (Fig. 2A–D). The dissolved CO\(_2\) concentration was only slightly reduced to ~30 \( \mu \)mol l\(^{-1}\) (Fig. 2E–H). By contrast, the bicarbonate and carbonate concentration and hence also the total DIC concentration increased during the experiments (Fig. 2I–L). The DIC increase was enabled by a rise in alkalinity, from 2.3 mEq l\(^{-1}\) at the start of the experiments to 6.2–8.4 mEq l\(^{-1}\) at steady state (see Supplementary Table S1). Alkalinity increased during the experiments, because the high uptake rates of nitrate, phosphate, and sulfate by the growing phytoplankton populations are accompanied by proton consumption to maintain charge balance (Goldman and Brewer, 1980; Wolf-Gladrow et al., 2007). A similar increase in DIC and alkalinity induced by dense phytoplankton populations was also observed in earlier chemostat studies (Verspagen et al., 2014b). The pH increased only slightly from ~8 to ~8.6

### Predictions derived from the monoculture experiments

The monoculture experiments showed dynamic changes in population abundances, light conditions, carbon speciation, alkalinity, and pH, which caused concomitant changes in the growth rates of the species. We tried to capture these dynamics by the development of a mathematical model. The model generally fitted well to the monoculture data, although the species parameters estimated from the monoculture experiments were not adequate to predict the dynamics of the competition experiments. Resource competition theory can provide some further insights. Consider several species competing for a single limiting resource. Each species has its own critical \( R^* \), defined as the resource availability at which the specific growth rate of a species equals its loss rate. During competition, resource availability diminishes as the resource is consumed by the species. One by one, species start to decline when resource availability is depleted below their \( R^* \) values. This process continues, until eventually the species with lowest \( R^* \) will be the superior competitor (Tilman, 1982; Grover, 1997).
In many applications, the $R^*$ value of each species is measured as the steady-state concentration of the limiting resource in monoculture (e.g. Tilman, 1981; Passarge et al., 2006; Wilson et al., 2007). In our application, however, CO$_2$ and bicarbonate provide two alternative inorganic carbon sources that are rapidly interconverted by the chemical reaction of CO$_2$ with water, which makes it difficult to measure the $R^*$ for CO$_2$ and $R^*$ for bicarbonate independently. Nevertheless, the steady-state concentrations of CO$_2$ and bicarbonate in carbon-limited monoculture may provide useful information on the competitive abilities for inorganic carbon of the species. That is, if we assume that a low steady-state CO$_2$ concentration in monoculture implies a high competitive ability for CO$_2$, then the species can be ranked according to their competitive ability for CO$_2$ as (Fig. 3A): Scenedesmus > Chlorella > Microcystis > Monoraphidium. Similarly, based on the steady-state bicarbonate concentrations, the species can be ranked according to their competitive ability for bicarbonate as (Fig. 3B): Scenedesmus > Chlorella > Microcystis > Monoraphidium. Accordingly, the ranking of the species is the same for both CO$_2$ and bicarbonate, indicating that Scenedesmus will be the best competitor for inorganic carbon, followed by Chlorella and then Microcystis, while Monoraphidium is the worst competitor for inorganic carbon.

At high CO$_2$ levels, CO$_2$ and bicarbonate were in ample supply, but light becomes a limiting resource (Fig. 2A–D). Analogous to $R^*$, competition theory predicts that the species with lowest critical light intensity ($I^*_{out}$) is the superior competitor for light (Huisman and Weissing, 1994; Huisman et al., 1999). The critical light intensities of the species were measured as the steady-state values of $I^*_{out}$ in the monoculture experiments at high pCO$_2$. Based on their critical light intensities, the species can be ranked according to their competitive ability for light as (Fig. 3C): Microcystis = Scenedesmus > Chlorella > Monoraphidium. Hence, from the monoculture data, Microcystis and Scenedesmus are predicted to be the best competitors for light with an approximately similar competitive ability, followed by Chlorella, while Monoraphidium is the worst competitor for light.

Comparison of the above species rankings indicates that Microcystis will become a stronger competitor when the species interactions shift from competition for inorganic carbon at low pCO$_2$ to competition for light at high pCO$_2$. The relative ranking among the three green algae remains unaltered with rising pCO$_2$.

**Competition experiments**

The competition experiments largely confirmed the predictions derived from the monoculture experiments. For instance, in the competition experiment at low pCO$_2$ between Monoraphidium and Scenedesmus, both species increased during the first 6 d
the dissolved CO$_2$ concentration was depleted to ~100 μmol l$^{-1}$ (Supplementary Fig. S2A). These concentrations are below the $R^*$ values for CO$_2$ and bicarbonate of *Monoraphidium* (Fig. 3A, B). Hence, as predicted, *Monoraphidium* started to decline after the first week, and was competitively displaced by *Scenedesmus* (Fig. 4A).

At low pCO$_2$, *Monoraphidium* was also outcompeted by *Chlorella* (Fig. 4C) and *Microcystis* (Fig. 4E). Hence, *Monoraphidium* was the weakest competitor. Furthermore, in line with expectation, *Scenedesmus* competitively displaced *Microcystis* (Fig. 4G). Contrary to expectation, *Chlorella* and *Microcystis* appeared to coexist at low pCO$_2$ (Fig. 4I). During the last 20 d of this experiment, however, *Chlorella* tended to increase slowly at the cost of *Microcystis*, although the experiment didn’t last long enough to witness the final outcome. There is indeed evidence of competitive displacement (Table 3), which indicated that *Chlorella* was indeed slowly displacing *Microcystis*. According to the competition experiments, the competitive ranking of the species at low pCO$_2$ can thus be summarized as follows: *Scenedesmus* > *Chlorella* ≈ *Microcystis* > *Monoraphidium*. This is in good agreement with the rankings based on the $R^*$ values for CO$_2$ and bicarbonate estimated in the monoculture experiments.

In their competition experiment at high pCO$_2$, both *Monoraphidium* and *Scenedesmus* increased during the first 20 d (Fig. 4B). Meanwhile, pH stabilized at ~8.5 (see Supplementary Fig. S1B at *JXB* online), the dissolved CO$_2$ concentration slightly decreased to ~20 μmol l$^{-1}$ and the bicarbonate concentration increased to >3,000 μmol l$^{-1}$ (Supplementary Fig. S2B). However, the incident light was almost completely absorbed by the dense species mixture, with <0.1 μmol photons m$^{-2}$ s$^{-1}$ penetrating through the chemostat (Supplementary Fig. S1B). This value is below the critical light intensity of *Monoraphidium* (Fig. 3C). Hence, after the first 20 d, *Monoraphidium* declined, and was gradually displaced by *Scenedesmus* (Fig. 4B).

At high pCO$_2$, *Monoraphidium* was also outcompeted by *Chlorella* (Fig. 4D) and *Microcystis* (Fig. 4F), and hence it was again the weakest competitor. Interestingly, *Scenedesmus* and *Microcystis* appeared to coexist (Fig. 4H), in agreement with the similar critical light intensities of these two species (Fig. 3C). Their coexistence at high pCO$_2$ was confirmed by the rate of competitive displacement, which did not differ significantly from zero for this species pair (Table 3). In line with expectation, *Microcystis* competitively displaced *Chlorella* (Fig. 4J). Hence, according to the competition experiments, the competitive abilities of the species at high pCO$_2$ can be ranked as follows: *Microcystis* > *Scenedesmus* > *Chlorella* > *Monoraphidium*. This matches the species ranking based on their critical light intensities in monoculture.

Also quantitatively, the population dynamics predicted by the competition model agreed well with the results of the competition experiments (compare lines versus symbols in Fig. 4, and Supplementary Figs S1 and S2), both at low pCO$_2$ and at high pCO$_2$.

**Discussion**

*Cyanobacteria versus green algae*

Both our model predictions and our experimental results contradict the classic view (Shapiro, 1990; Caraco and Miller, 1998; Low-Decarie et al., 2011, 2015) that cyanobacteria are strong competitors at low CO$_2$ levels, whereas eukaryotic phytoplankton such as green algae are better competitors at elevated CO$_2$. We found the opposite. At low CO$_2$ levels, the cyanobacterium *Microcystis* was a relatively poor competitor. It lost in competition with *Scenedesmus*, was slowly replaced by *Chlorella*, and won only against *Monoraphidium*. At high CO$_2$ levels, *Microcystis* was a stronger competitor. It won in competition with both *Monoraphidium* and *Chlorella*, and
coexisted with *Scenedesmus*. *Microcystis* was the only species that increased its competitive ranking at elevated CO$_2$; the relative ranking among the three species of green algae did not change.

**Competition at low CO$_2$**

In the competition experiments at low pCO$_2$, the growing phytoplankton populations depleted the dissolved CO$_2$ concentration within the first 1–3 weeks of the experiments and also the bicarbonate concentration declined. As a consequence, the growth rates of the phytoplankton populations slowed down, and one of the species in the experiments started to displace the other. Interestingly, both the ranking of the $R^*$ values estimated from the monocultures and the competitive replacements observed in the competition experiments show that the green algae *Scenedesmus* and *Chlorella* were stronger competitors for inorganic carbon than the cyanobacterium *Microcystis*.

These results can to a large extent be explained by the CCMs of the species. In particular, the cyanobacterium
Table 3. Rates of competitive displacement (RCD±standard error) in the competition experiments

RCD was calculated as the slope of a linear regression of ln(Xi/Xo) versus time, where Xi and Xo are the population densities of species 1 and species 2. The coefficient of determination (R²), number of data points (n) and significance (P) of the linear regression are indicated.

<table>
<thead>
<tr>
<th>Competition experiment</th>
<th>RCD (d⁻¹)</th>
<th>R²</th>
<th>n</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monoraphidium vs Microcystis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low pCO₂</td>
<td>-0.250 ± 0.008</td>
<td>0.99</td>
<td>14</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>High pCO₂</td>
<td>-0.043 ± 0.005</td>
<td>0.88</td>
<td>11</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Monoraphidium vs Scenedesmus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low pCO₂</td>
<td>-0.292 ± 0.021</td>
<td>0.97</td>
<td>8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>High pCO₂</td>
<td>-0.021 ± 0.002</td>
<td>0.84</td>
<td>20</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Monoraphidium vs Chlorella</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low pCO₂</td>
<td>-0.100 ± 0.008</td>
<td>0.93</td>
<td>16</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>High pCO₂</td>
<td>-0.062 ± 0.008</td>
<td>0.82</td>
<td>15</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Scenedesmus vs Microcystis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low pCO₂</td>
<td>+0.654 ± 0.071</td>
<td>0.95</td>
<td>6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>High pCO₂</td>
<td>0.000 ± 0.003</td>
<td>0.00</td>
<td>11</td>
<td>n.s.</td>
</tr>
<tr>
<td>Microcystis vs Chlorella</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low pCO₂</td>
<td>-0.039 ± 0.071</td>
<td>0.96</td>
<td>11</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>High pCO₂</td>
<td>+0.197 ± 0.025</td>
<td>0.92</td>
<td>11</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Microcystis PCC 7806 used in this study is a bicA strain (sensu Sandrini et al., 2014). It contains the bicarbonate uptake systems BicA and BCT1, but lacks the high-affinity bicarbonate uptake system SbtA. Batch experiments have shown that Microcystis PCC 7806 has a lower growth rate at low Cᵢ levels than strains that do have SbtA (Sandrini et al., 2014). BCT1 is induced when Microcystis PCC 7806 grows at low CO₂ levels (Sandrini et al., 2015a, b), but this uptake system appears to have a slightly lower affinity for bicarbonate than SbtA, at least in the cyanobacteria Synechocystis PCC 6803 and Synechococcus PCC 7002 (Price et al., 2004, 2008). Moreover, bicarbonate uptake by BCT1 is ATP dependent and therefore energetically quite expensive. The Na⁺-dependent bicarbonate uptake system BicA has a high flux rate but low affinity for bicarbonate (Price et al., 2004). Hence, the lack of SbtA offers a plausible explanation of why Microcystis PCC 7806 had a selective disadvantage under Cᵢ-limited conditions. Previous experiments have indeed shown that such bicA strains were selectively displaced by bicA+sbtA strains under Cᵢ-limited conditions (Sandrini et al., 2016). Our results show that cyanobacteria that lack the high-affinity bicarbonate uptake system SbtA are relatively poor competitors under Cᵢ-limited conditions, not only in comparison with other cyanobacteria but also in comparison with green algae such as Scenedesmus and Chlorella.

Our results do not of course imply that cyanobacteria are generally poor competitors under Cᵢ-limited conditions. Microcystis strains containing the high-affinity bicarbonate uptake system SbtA sustain higher growth rates at low Cᵢ concentrations than bicA strains such as Microcystis PCC 7806 (Sandrini et al., 2014). Moreover, recent selection experiments and lake data show that bicA+sbtA strains have a competitive advantage over bicA strains at low CO₂ levels (Sandrini et al., 2016). Whether SbtA-containing cyanobacteria or green algae such as Scenedesmus and Chlorella are better competitors at low CO₂ levels thus remains an interesting open question.

Compared with cyanobacteria, less is known about the functioning of the CCMs in green algae. Our current understanding of eukaryotic CCMs comes largely from studies with the model organism Chlamydomonas reinhardtii (Moroney and Ynalvez 2007; Spalding, 2008; Wang et al., 2011; Meyer and Griffiths, 2013). Briefly, the key components of the eukaryotic CCM are quite similar to the cyanobacterial CCM and involve active CO₂ and bicarbonate uptake, interconversion between these two Cᵢ species by carbonic anhydrases (CAs), and a microcompartment that contains RuBisCO (Wang et al., 2015). In Chlamydomonas, five Cᵢ transporters have been localized, including two confirmed bicarbonate transporters (HLA3 on the plasma membrane and LCIA on the chloroplast envelope) and three undefined Cᵢ transporters (LCII on the plasma membrane and CCAP1/2 on the chloroplast envelope). Inside the chloroplast, RuBisCO is densely packed in a microcompartment named the pyrenoid (Kuchitsu et al., 1988; Engel et al., 2015). Within the pyrenoid, bicarbonate is dehydrated to CO₂ by a CA and released to RuBisCO (Moroney and Ynalvez, 2007).

So far, there are no studies on the CCM genes of the green algal strains we used in this study, but there is some recent work on the CCM genes of a different species of Chlorella, C. pyrenoidosa, indicating that this species has a CCM similar to that of Chlamydomonas (Fan et al., 2016). For instance, when shifting C. pyrenoidosa from high CO₂ to low CO₂ conditions, CCM-related genes such as LCIA, LCIB, and HLA3 showed increased expression, similar to the response of C. reinhardtii (Fan et al., 2015). Interestingly, earlier work indicates that there is substantial variation in CCM activity within the Chlorella genus and even between different strains of the same species of Chlorella. For example, C. vulgaris strain 11 h and strain UTEX 263 seem to utilize only CO₂ as a carbon source (Miyachi et al., 1983, Tu et al., 1986), whereas C. vulgaris strain C-3, strain UTEX 259 and C. pyrenoidosa can use both CO₂ and bicarbonate (Miyachi et al., 1983). Our results indicate that the C. vulgaris strain we used can utilize bicarbonate: if this green alga could only use CO₂, it would not be able to grow in monoculture in our chemostats at a dilution rate of 0.125 d⁻¹ and dissolved CO₂ concentrations lower than 0.01 µmol l⁻¹ (Fig. 1).

Similarly, the fact that S. obliquus and M. griffithii were also able to grow at CO₂ concentrations below 0.01 µmol l⁻¹ in our chemostat experiments indicates that these green algae can also utilize bicarbonate as carbon source. Earlier studies confirm the presence of a functional CCM in a different strain of S. obliquus. WT strain D3 has increased intracellular and extracellular CA activity, as well as a higher affinity for the uptake of CO₂ and bicarbonate at low compared with high CO₂ concentrations (Palmqvist et al., 1994). Furthermore, in our experiments S. obliquus could deplete dissolved CO₂ and bicarbonate concentrations to even lower levels than Microcystis PCC 7806 (Fig. 3), and displace Microcystis in the competition experiments at low CO₂ (Fig. 4).
Similar results were obtained in CO₂-limited mesocosm experiments by Verschoor et al. (2013), where the same strain of *S. obliquus* displaced Synechocystis PCC 6803, a cyanobacterium that contains all five C₅ uptake systems (Badger et al., 2006). So far, very little is known about the CCM of *Monoraphidium griffithii*, but a related species, *M. braunii*, can photoactivate a blue-light-dependent bicarbonate transport system under CO₂-limiting conditions (Mora et al. 2002).

In total, these physiological studies indicate that many green algae are well adapted to cope with C₅ limitation. Similar to cyanobacteria, many species of green algae are able to induce a CCM under CO₂-limiting conditions (Meyer and Griffiths, 2013). Furthermore, in eutrophic lakes, not only cyanobacteria but also green algae have been observed to develop dense blooms at low CO₂ concentrations and high pH (Jeppesen et al., 1990; Jensen et al., 1994; Beklioglu and Moss, 1995). The results of our competition experiments are in agreement with these physiological studies and field observations, and demonstrate that green algae can indeed be very effective competitors at low CO₂ levels.

**Competition at elevated CO₂**

The notion that *Microcystis* PCC 7806 lacks the high-affinity uptake system SbtA explains not only why it was a poor competitor at low CO₂ levels, but may also help to understand why it was more successful at high CO₂ levels. In *bicA*+*sbtA* strains of *Microcystis*, the bicarbonate uptake genes *bicA* and *sbtA* are located on the same operon and are co-transcribed (Sandrini et al., 2014). Transcription of *sbtA* will be inefficient and costly, however, when inorganic concentrations are high and hence the high-affinity but low-flux system SbtA is no longer needed. This reasoning is supported by recent selection experiments, which have shown that *bicA* strains have a competitive advantage over *bicA*+*sbtA* strains at elevated CO₂ levels (Sandrini et al., 2016).

Yet, the presence of the high-flux bicarbonate uptake system *BicA* is probably not sufficient to explain its competitive success at elevated CO₂ levels. Elevated CO₂ increased the dissolved C₅ concentrations, but also yielded 3- to 8-fold higher population densities, which in turn produced very low light availabilities (Figs 1 and 2, and Supplementary Table S1). At these high CO₂ but low light levels, carbon availability is no longer a major limiting factor and bicarbonate uptake systems tend to be down-regulated (Beardall, 1991; Sandrini et al., 2015a). Instead, the intense shading induced by the dense phytoplankton populations will favor species adapted to low light environments (Huisman et al., 1999). A range of studies have suggested that cyanobacteria are superior competitors for light (Mur et al., 1977; Reynolds et al., 1987; Huisman et al., 2004; Yang and Jin, 2008; Schwaderer et al., 2011). Comparison of the traits of the species investigated in this study shows that *Microcystis* PCC 7806 had the lowest half-saturation constant for light-limited growth (Table 2), indicating that it can sustain a relatively high growth rate at low light levels in comparison with the other species. *Scenedesmus* also had a relatively low half-saturation constant for light-limited growth, albeit higher than *Microcystis*, and had the highest maximum growth rate of all four species (Table 2). Indeed, the competitive ability of the cyanobacterium *Microcystis* increased at elevated CO₂, and together with *Scenedesmus* it became the best competitor for light (Fig. 3).

**Model predictions**

Comparison of the model predictions and experimental results shows that the impact of elevated CO₂ on phytoplankton competition can be quite accurately predicted under controlled laboratory conditions. First, the system parameters and several species parameters were measured in monoculture experiments, while the remaining species parameters were estimated from least-squares fits of the model predictions to the monoculture dynamics (Figs 1 and 2). Hence, the model is calibrated with the monoculture data. Subsequently, the parameter estimates from the monocultures were used to predict the time course and outcome of competition in the species mixtures. Accordingly, the model predictions are validated with the competition experiments (Fig. 4, and Supplementary Figs S1 and S2).

Our model and experiments are of course still a major simplification in comparison with the complexities of the real CCMs of cyanobacteria and green algae competing in natural waters. For instance, the model brushes over many of the physiological details involved in the regulation of CCMs in response to changes in carbon and light availability (e.g. Beardall and Giordano, 2002; Burnap et al., 2015). Furthermore, the experiments were limited to only a small number of phytoplankton species under controlled laboratory conditions, in isolation from a multitude of other hydrological, biogeochemical and ecological processes that are important in lakes. Species might be superior competitors for inorganic carbon or light, but if they are also preferentially grazed by zooplankton they are still unlikely to gain dominance in natural waters. Hence, further improvement of the model predictions might be obtained by further refinement of the underlying physiological and ecological processes.

Nevertheless, the results are promising. Our model and experiments seemed to capture the basic ingredients required to predict phytoplankton competition at different CO₂ levels. Similar to several previous studies (e.g. Caraco and Miller, 1998; Low-Décarie et al., 2011; Trimborn et al., 2013), we have shown that species traits measured in monoculture can be used to predict changes in phytoplankton species composition at elevated CO₂. To our knowledge, our study is the first experimental demonstration that a mathematical model can quantitatively predict dynamic changes in phytoplankton species composition, carbon speciation, pH, alkalinity and light during the competition process.

**Conclusions**

Our experimental results call for a revision of the classic paradigm that cyanobacteria are superior competitors at low CO₂ levels and high pH, whereas eukaryotic phytoplankton such as green algae are superior competitors at elevated CO₂. Such simple dichotomies do not capture the diversity of CCMs that
have been found among and within different phytoplankton taxa. First, our results demonstrate that green algae can also be very effective competitors at low CO₂ levels. Second, our results show that some cyanobacterial strains are relatively poor competitors when CO₂ is limiting but become stronger competitors at elevated CO₂. We therefore urge the CCM scientific community to further elucidate and compare the performance of different CCMs, and their selective advantages and disadvantages across a wide range of different species at the molecular, physiological and ecological level. Such a comparative approach will be essential if we are to understand and predict how the species composition of natural phytoplankton communities will respond to the anticipated rise in CO₂ levels.

Supplementary data

Supplementary data are available at JXB online.

Model S1. Detailed description of the model.

Fig. S1. Dynamic changes in light, DIC and pH during the competition experiments.

Fig. S2. Dynamic changes in carbon speciation during the competition experiments.

Table S1. Steady-state characteristics in the monoculture experiments.

Acknowledgements

We thank Hanneke van Bree, Sander de Groot, Duke van Velden, Maureen de Wit and Josan Yuw for their help in running the chemostats, Leo Hoitinga for assistance with the TOC-Varian Analyzer and Elemental Analyzer, Suzanne Naus-Wiezer of the NIOO-KNAW for assistance with flow cytometry, and the reviewers for their helpful comments. This research is supported by the Division of Earth and Life Sciences (ALW) of the Netherlands Organization for Scientific Research (NWO).

References


