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Inorganic carbon promotes photosynthesis, growth, and maximum biomass of phytoplankton in eutrophic water bodies

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Abstract

1. The traditional perception in limnology has been that phytoplankton biomass in lakes is limited by phosphorus, nitrogen, and light, but not by dissolved inorganic carbon (DIC) because CO_2 can be supplied from the atmosphere. We tested the possibility of carbon limitation of photosynthesis, growth, and biomass accumulation of phytoplankton communities across an alkalinity and DIC gradient (0.15–3.26 mM) in nutrient-rich freshwater.

2. During 47-day long experiments, we measured phytoplankton biomass, organic carbon, calcium, DIC, pH, and oxygen in indoor, constantly mixed mesocosms with either no removal or a 70% weekly removal of the biomass. Photosynthesis was measured in the morning and in the afternoon at high biomass.

3. Maximum biomass and organic carbon production increased two- to four-fold with DIC, which supported 7% of organic carbon production at low DIC and 53% at high DIC concentration, while atmospheric CO_2 uptake supplied the remainder. Weekly biomass removal increased growth rates through improved light conditions leading to enhanced total phytoplankton biomass production at high DIC. Photosynthesis was significantly higher in the morning compared to afternoon due to daily DIC depletion.

4. We conclude that phytoplankton photosynthesis, growth rate, maximum biomass, and organic carbon production can be markedly carbon limited in eutrophic lake waters. Consequently, lakes of high DIC and pH can support a faster primary production by greater DIC use and chemically enhanced atmospheric CO_2 uptake.

KEYWORDS
algal bloom, carbon limitation, DIC, eutrophication, lakes, primary production

1 | INTRODUCTION

Phytoplankton photosynthesis, growth, and maximum biomass in lakes are influenced by several physio-chemical variables; main research has focused on two key determinants—light and limiting nutrients (i.e. nitrogen, phosphorus, and occasionally silicate; Dillon & Rigler, 1974; Elser et al., 2007; Kalff, 2002; Schindler, 1974). Light

availability directly regulates phytoplankton photosynthesis and subsequent growth and is determined by incident solar irradiance, vertical light attenuation and mixing depth of the water column (Krause-Jensen & Sand-Jensen, 1998; Wofsy, 1983). Availability of nitrogen and phosphorus frequently limits formation of essential structural and catalytic cell pools and, thereby, influences rates of photosynthesis and growth as well as the maximum attainable

biomass (Bogard et al., 2017; Phillips et al., 2008; Spijkerman, 2010).

Worldwide eutrophication by nitrogen and phosphorus in the 1950–1980s confirmed their importance for the build-up of dense phytoplankton blooms causing turbid waters, oxygen depletion in bottom waters and fish kills (Kalff, 2002; Moss, 2009; Sand-Jensen et al., 2008). The key-role of nitrogen and phosphorus for setting the maximum phytoplankton biomass was supported by bottle and whole-lake experiments (Schindler et al., 1973). Early empirical models predicted the relationships between nutrient input and phytoplankton biomass with proper consideration of hydrodynamics and sediment nutrient exchange (Fee, 1979; Larsen & Mercier, 1976; Vollenweider, 1968).

The nutrient paradigm of the 1970s also led to a common agreement among freshwater ecologists that dissolved inorganic carbon (DIC: $CO_2 + HCO_3^- + CO_3^{2-}$) does not constrain the phytoplankton biomass in lakes, because depletion of CO_2 during dense phytoplankton growth induced by nutrient enrichment will stimulate CO_2 uptake from the atmosphere to cover the extra demands (Schindler, Brunskill, Emerson, Broecker, & Peng, 1972). In Schindler et al.'s (1972, 1973) whole-lake experiments in oligotrophic Canadian lakes, combined nitrogen and phosphorus enrichment substantially increased the phytoplankton biomass while organic carbon addition had no measurable impact. However, it was never tested whether supplementary addition of DIC could have increased the biomass even further. Recent laboratory and modelling work (Verspagen, van de Waal, Finke, Visser, & Huisman, 2014; Verspagen, van de Waal, Finke, Visser, Van Donk et al., 2014) as well as mesocosm studies (Bogard et al., 2017; Low-Décarie, Bell, & Fussmann, 2015) have shown that greater DIC supply stimulates short-term phytoplankton biomass development and productivity in eutrophic or eutrophied lakes of low-medium DIC content. Moreover, cyanobacterial surface blooms are stimulated by higher atmospheric CO_2 and water DIC concentrations (Ibelings & Maberly, 1998). In our study, we tested the possibility of simultaneous short-term photosynthesis and long-term growth limitation and biomass formation of phytoplankton by DIC availability in mesocosms under nutrient replete conditions in waters including the full range of typical DIC concentrations in lakes.

Massive direct use of DIC for synthesis of organic compounds in photosynthesis and the almost universal occurrence of a suite of costly carbon concentrating mechanisms (CCMs) among aquatic cyanobacteria, uni- and multicellular algae and higher plants to exploit external DIC suggest that DIC is an important limiting factor (Giordano, Beardall, & Raven, 2005; Price, Badger, Woodger, & Long, 2007; Raven, Cockell, & De La Rocha, 2008). Marked DIC limitation of photosynthesis and growth of macroscopic algae and plants has been shown by many researchers (Maberly & Madsen, 2002; Madsen, 1993; Madsen & Sand-Jensen, 1991) and can restrict ecosystem primary production whenever these macrophytes are abundant (Christensen, Sand-Jensen, & Staehr, 2013; Sand-Jensen, Binzer, & Middelboe, 2007). Some studies have confirmed short-term stimulation of phytoplankton photosynthesis by increased CO_2 concentrations in productive nutrient-rich lakes with CO_2-undersaturation

(Hein, 1997a; Spijkerman, 2010; Talling, 1985), and even in unproductive nutrient-poor lakes with CO_2-supersaturation (Jansson, Karlsson, & Jonsson, 2012). Modelling and mesocosm studies have also pointed out that low and moderately alkaline waters are susceptible to carbon limitation (Kragh & Sand-Jensen, 2018; Schippers, Lürling, & Scheffer, 2004; Verspagen, van de Waal, Finke, Visser, & Huisman, 2014; Verspagen, van de Waal, Finke, Visser, Van Donk et al., 2014). Dissolved inorganic carbon limitation of short-term phytoplankton photosynthesis does not necessarily reflect the ecologically more relevant limitation of growth rates. Short-term stimulation of photosynthesis by elevated DIC may result in a surplus of photosynthates that are either respired or released from the cell, or may reduce subsequent photosynthesis without forming additional cell products (Clark & Flynn, 2000; Goldman & Graham, 1981; Tortell, 2000).

Effects of higher DIC concentrations on primary production in lakes may not solely be linked to the direct supply of CO_2 to photosynthesis. In soft water, low-pH lakes, a high proportion of the small DIC pool (e.g. 0.01–0.1 mM) is present as free CO_2, but in hard water, high-pH lakes the by far highest proportion of the large DIC pool (>1.0 mM) is present as bicarbonate (HCO_3^-; Stumm & Morgan, 2012; Talling, 1985). Bicarbonate can be utilised in photosynthesis by an array of different active mechanisms evolved in most groups of aquatic phototrophs (Kaplan & Reinhold, 1999; McConnaughey & Whelan, 1997; Prins, Snel, Zanstra, & Helder, 1982), but even when HCO_3^- is not used directly it constitutes a large pool that is converted to CO_2 either passively or catalytically to replenish photosynthetic consumption of CO_2 (Maberly, Ball, Raven, & Sültemeyer, 2009). Bicarbonate also releases free CO_2 to aquatic phototrophs by coupled photosynthesis and $CaCO_3$ precipitation at high pH (i.e. $Ca^{2+} + 2 HCO_3^- \rightarrow CaCO_3$ (precipitation) + CO_2 (assimilation); McConnaughey et al., 1994; McConnaughey & Whelan, 1997). Photosynthetic use of CO_2 and HCO_3^-, uncoupled to direct phototrophic calcification, may drive pH up in the free water, cause $CaCO_3$ precipitation with a resulting pH decrease and HCO_3^- conversion to higher CO_2 concentrations (Bogard et al., 2017; Kranz, Wolf-Gladrow, Nehrke, Langer, & Rosta, 2010).

High HCO_3^- concentrations result in higher pH at air saturation (i.e. about pH 8.0 at 1 mM DIC and pH 7.0 at 0.1 mM DIC) and substantial photosynthetic use of CO_2 from HCO_3^- (i.e. $HCO_3^- \rightarrow CO_2 + OH^-$) generates further pH rise. High pH is conducive to chemically enhanced uptake of atmospheric CO_2 by direct reaction with OH^- and conversion to HCO_3^- in the surface waters (Portielje & Lijklema, 1995). Chemical enhancement of atmospheric CO_2 uptake is about five-fold at pH 10 and 16-fold at pH 11 relative to uptake at c. pH 7 at 20°C (Bade & Cole, 2006). For this reason, DIC limitation may be most prominent in nutrient-rich soft water lakes experiencing marked reduction of CO_2 and DIC during phytoplankton blooms, while hard water lakes may be less susceptible because of the larger DIC pool, the greater release of CO_2 from HCO_3^- by chemical equilibration and carbonate precipitation as well as a higher uptake of atmospheric CO_2 at higher pH (Bogard et al., 2017; Kragh & Sand-Jensen, 2018;

Verspagen, van de Waal, Finke, Visser, & Huisman, 2014; Verspagen, van de Waal, Finke, Visser, Van Donk et al., 2014).

Phytoplankton will eventually become light limited by self-shading as the biomass grows dense (Krause-Jensen & Sand-Jensen, 1998). Dense biomass may be prevented by top-down regulated grazing control from zooplankton and benthic suspension feeders (Carpenter, Cole, Kitchell, & Pace, 1998; Carpenter, Kitchell, & Hodgson, 1985). By reducing the phytoplankton biomass, improving light availability and stimulating nutrient recirculation, grazing may enhance growth rates of phytoplankton and DIC requirements. Thus, better light and nutrient conditions through grazing could result in faster re-growth in hard water lakes compared to soft water lakes. We simulated relevant grazing losses and associated light improvements in the mesocosm experiments by weekly removal of 70% of the phytoplankton biomass, whereas we considered grazing stimulation of nutrient recirculation unimportant under the unlimited nutrient supply.

The overall objective of this study was to investigate the effects of increasing DIC concentrations on phytoplankton growth, maximum biomass, and organic carbon production on a long-term basis and photosynthesis on a daily basis. The study also aimed at evaluating the effect of biomass removal on phytoplankton growth rate and cumulative organic carbon production at varying DIC concentrations. To meet these objectives, we measured phytoplankton biomass, organic carbon, DIC, calcium, pH, and dissolved oxygen under nutrient replete conditions at low, intermediate, and high DIC levels in a mesocosm experiment over 47 days with either no removal or a 70% weekly removal of the biomass to simulate improved light conditions and re-growth generated by grazing. We also tested the influence of DIC on photosynthesis in dense phytoplankton populations in the mesocosm experiments. Those experiments were initiated in the morning and after midday in water from the mesocosms and tested at ambient, elevated and reduced DIC levels.

We hypothesise that: (1) phytoplankton growth rate and maximum biomass can be strongly carbon limited in nutrient-rich, low DIC waters; (2) ongoing biomass loss will stimulate re-growth and result in higher organic carbon production of phytoplankton with increasing DIC; and (3) photosynthesis is carbon limited on a daily basis during dense phytoplankton blooms at all DIC concentrations when very low CO_2 concentrations prevail and high CO_2 levels are confined to early in the light period.

2 | METHODS

2.1 | Experimental design and analyses

Growth responses of mixed phytoplankton assemblages at three initial DIC levels (0.15, 0.72, and 3.26 mM) and either no or a 70% weekly biomass removal were tested in triplicate in 18 cylindrical mesocosms (0.09 m^3 in volume, 0.6 m tall, 0.15 m^2 surface area) placed indoor and exposed to the same irradiance, temperature and water mixing. The three initial DIC levels corresponded to soft water lakes (0.15 mM), intermediate lakes (0.72 mM), and hard water lakes

(3.26 mM; Stumm & Morgan, 2012). The artificial lake water was made from de-mineralised water with no particulate organic carbon (POC) and very low concentration of dissolved organic carbon (DOC) according to the recipe in Smart and Barko (1985). The three levels of DIC were obtained by dissolving $CaCO_3$ in de-mineralised water by purging with CO_2 in a pressurised chamber and adding the enriched $Ca(HCO_3)_2$ solution to the mesocosms to attain the desired DIC concentrations. $Ca(HCO_3)_2$ was used because Ca^{2+} and HCO_3^- are the main macro-ions in fresh waters and calcium carbonate precipitation is a natural and very relevant ecological process. Subsequently, $CaCl_2$, $MgSO_4$, Na_2SO_4, and K_2SO_4 were added according to the scheme in Table S1 to ensure a composition of macro-ions resembling that of natural lake water. Trace minerals were added as 1 mg/L of Z8 and 0.2 ml/L of cyano-trace of the Z8-metal solution (Kotai, 1972). Iron was added to the mesocosms every week as iron ammonium citrate at a final concentration of 1.2 mg Fe/L. After adding these organic compounds and the phytoplankton inoculum (see below), mean initial DOC was 0.2 mM (±0.01, *SE*) in the mesocosms. Inorganic nutrients were added to ensure high initial concentrations of 1 mg NH_4^+-N/L and 1 mg NO_3^--N/L and 0.2 mg PO_4^{3-}-P/L. Dissolved nutrients were measured weekly and extra nutrients were added when needed to prevent concentrations from falling to limiting concentrations typically below 0.1 mg NH_4^+ + NO_3^--N/L and 0.01 mg PO_4^{3-}-P/L (Currie & Kalff, 1984; Eppley, Rogers, & McCarthy, 1969; Tilman, Kilham, & Kilham, 1982). To exemplify, mean PO_4^{3-}-P concentrations in the mesocosms were 0.10–0.44 mg/L and minimum concentrations before possible supplementary additions were 0.012–0.209 mg/L. Inorganic nutrients were measured with standard methods using an Alp-kem-RFA 300 autoanalyser (SEAL Analytical GmbH).

Incident irradiance from large light panels (Gavita Pro LEP 300, Gavita Holland BV) placed above each mesocosm was 120 μmol m^{-2} s^{-1} (PAR, 400–700 nm) in a 12-hr light:12-hr dark cycle. Water temperature varied daily from 12 to 15°C and averaged 13°C. Temperature, dissolved oxygen and surface irradiance were measured every 5 min by a logging temperature/oxygen-optode sensor (PME miniDOT) and a photon sensor (HOBO, 400–700 nm, HS-LIA-M003). Five days a week, pH was measured 2 hr after onset of the photoperiod by an YSI 30, 30M/10FT in each mesocosm. Continuous pH measurements on several days using the same equipment showed that the single measurement closely represented the mean pH for the daytime period.

Mixing of the water was provided by a small submersible centrifugal pump (Micra 6 W) attached to a small tile at a fixed angle at the bottom of the mesocosms. Pumps were cleaned twice daily to ensure constant mixing and surface turbulence. This provided homogeneity of physical, chemical, and biological conditions, including constant gas exchange rates with the atmosphere, and avoided excessive particle sedimentation. Mixing resulted in a basic O_2 exchange coefficient (i.e. piston velocity of 2.1 cm/hr) measured in the water-filled mesocosms before phytoplankton was added by depleting O_2 by bubbling with N_2 gas and followed by continuous sensor measurements the return of O_2 to air equilibrium with the submerged pumps running (see details in Kragh & Sand-Jensen, 2018).

The measured O_2 exchange coefficient was converted to the CO_2 exchange coefficient with no chemical enhancement (K) of 2.0 cm/hr according to the comparative study of Thyssen and Kelly (1985). This exchange coefficient is typical for lakes exposed to moderate wind speeds at neutral pH (Bade & Cole, 2006; Cole & Caraco, 1998). Air-water exchange rates of CO_2 are enhanced at high pH by direct chemical reaction between CO_2 and OH^- to form HCO_3^- in addition to the normal hydration of CO_2 ($CO_2 + H_2O \rightarrow H_2CO_3$; Bade & Cole, 2006).

The experiment was commenced by adding the same amount of a mixed phytoplankton assemblage to all mesocosms, which generated an initial chlorophyll a (Chl a) concentration of 4.3 µg/L. The phytoplankton assemblage was sampled in autumn with a 20-µm plankton net and a mixture from three lakes (soft water, mesotrophic Lake Grib, hard water, mesotrophic Lake Esrum, and hard water, eutrophic Lake Arresø) to make sure that a variety of species of different adaptation were present and could potentially grow at the three DIC levels. Large zooplankton and algae were removed by filtering through a 200-µm net before adding the inoculum. Chlorophyll concentrations declined during the first 1–2 days before biomass growth began. Species composition of the phytoplankton community was not studied in detailed, but small green algae (Chlorococcales) became dominant in all mesocosms. Development of an organic biofilm on mesocosm walls was prevented by wiping them daily and suspending the material in the water. Prior to sampling, particles that had settled on the bottom of mesocosms were carefully suspended in the entire water volume, which ensured a uniform sample including water, growth on walls, and settled particles. This procedure was applied to make certain that all phytoplankton and detritus that had accumulated over time was included in measurements and this permitted net growth rate of chlorophyll and organic carbon accumulation to be calculated correctly. Substantial phytoplankton biomass settled on the mesocosm bottoms towards termination of the experiments without losing their chlorophyll, which resulted in very high biomasses after resuspension and sampling.

Every 3 days, 2 hr into the light period, standard analytical methods were used to measure phytoplankton Chl a (Jespersen & Christoffersen, 1987), acid neutralising capacity (Gran, 1952), dissolved Ca^{2+} and specific conductivity (Anonymous, 1985). Dissolved inorganic carbon was measured directly on an infrared gas analyser (Vermaat & Sand-Jensen, 1987) and also calculated from measurements of acid neutralising capacity, pH, temperature, and specific conductivity according to Mackereth, Heron, and Talling (1978). Calculated and directly measured DIC concentrations were always in very close agreement (<3% deviation between mean values). Methods described by Kragh and Søndergaard (2004) were used for organic carbon measurements (TOC, POC, and DOC).

2.2 | Biomass removal

About 70% of the chlorophyll biomass was removed at weekly intervals in half of the mesocosms after 19, 26, 34, and 41 days of

the experiment by using a filter system with a sufficiently high volume capacity. Weekly removal of 70% of the biomass corresponded to a relative loss rate of 0.17/day, which is within the typical range of losses in natural phytoplankton populations (0.02–0.39/day; Reynolds, Harris, & Gouldney, 1985). About 70% of the water volume in the mesocosm was siphoned into a large extra chamber, in which all particles larger than 1 µm were removed. For filtration, an inert HEPA filter composed of fiberglass was mounted on a plastic cylinder equipped with a lid with a single hole and a plastic tube as the only outlet. It was placed in a Perspex container with a tight lid at the top and an inlet tube from a large pump (Ocean Runner QR 2500, pumping capacity 3500 L/hr). The pump was placed in a bucket with 4 L of mesocosm-water and 20 g of diatom shells (HOBBY, Dohse Aquaristik GmbH & Co) were added. When the pump was started, the diatom shells settled on the surface of the HEPA filter and the re-circulated water was rinsed for particles when it passed through the diatom shell matrix, which behaves like a GF/C-filter. The pump was then submerged into the extra chamber with 70% of the mesocosm water and operated until the water was completely transparent. The rinsed water was finally returned to the original mesocosm. The removal of Chl a and POC was determined by measurements in the mesocosm before and after filtration. Additional measurements confirmed that dissolved elements (DIC, DOC, and calcium) were unaffected by the filtration.

2.3 | Growth rate and carbon balance

Relative net growth rate of the phytoplankton community (RGR) was calculated from the change in biomass (B_1 and B_2, as Chl a between consecutive samplings at day T_1 and T_2) according to:

$$RGR = \ln(B_2/B_1) : (T_2 - T_1). \tag{1}$$

The same rates were attained if RGR was calculated from changes in POC rather than chlorophyll. Thus, weight-to-weight ratios of POC to Chl a remained constant from day 13 to day 45 during the experiment. Moreover, POC to Chl a ratios were about the same at the three DIC levels in the mesocosms; that is, 22.8 ± 1.9 (95% C.L.) at initial 0.15 mM DIC, 18.9 ± 1.7 at initial 0.72 mM DIC, 19.0 ± 1.0 at initial 3.26 mM DIC (n = 47–49 at each DIC level).

The carbon balance (mM) in the mesocosms over the incubation period was calculated from the net accumulation of organic carbon (TOC-acc; TOC = POC + DOC), net decline of water DIC (DIC-decline) minus DIC loss to $CaCO_3$ precipitation ($CaCO_3$-prec), while net CO_2 input from the atmosphere (Atm-CO_2) was calculated by difference:

$$TOC\text{-}acc = DIC\text{-}decline - CaCO_3\text{-}prec + Atm\text{-}CO_2. \tag{2}$$

Thus, DIC-decline was corrected for the loss of HCO_3^- and CO_3^{2-} being incorporated into particulate $CaCO_3$ and not into organic carbon. For this purpose, we assumed that the decline in dissolved Ca^{2+} corresponded to an equal molar HCO_3^- or CO_3^{2-} decline. In mesocosms with weekly filtering (70%), the removed biomass was added

to the standing biomass when calculating the total accumulated TOC-acc. This was done by biomass determination of Chl *a* and organic carbon before and immediately after filtering. Estimated atmospheric CO_2 input from the mass balance corresponded well to CO_2 gas exchange calculated from partial pressure difference between air and water, piston velocity and chemical enhancement of CO_2 uptake at high pH (data not shown). Close agreement between mass balance estimates and direct calculations of atmospheric CO_2 uptake was shown in previous experiments in the same mesocosms (Kragh & Sand-Jensen, 2018).

2.4 | Net ecosystem production and photosynthesis–DIC relationships

Examples of net ecosystem production rate (NEP, mM O_2/hr) were calculated at the three DIC levels early and late during the experiment from oxygen concentration changes and oxygen exchange with the atmosphere over time. Oxygen concentrations were recorded every 5 min and moving averages were calculated for 12 measurements during 1 hr. Periods when oxygen sensors were out of the water for cleaning were omitted. The rate of oxygen concentration change was calculated from continuous measurements of oxygen concentrations, while the rate of oxygen exchange between the water and the atmosphere was calculated from the difference between the actual and the equilibrium concentration of oxygen in the water (O_2-act – O_2-equ) times the oxygen piston velocity (K for O_2: 2.1 cm/hr):

$$NEP = dO_2 con\ dt^{-1} + K\ (O_2 act - O_2\text{- equ}).\qquad(3)$$

To test the DIC regulation of photosynthesis on a daily basis, the photosynthetic rate as a function of DIC concentration was measured in all mesocosms after the phytoplankton biomass was well established, that is 41 days into the experiment. Water was sampled from the mesocosms just before and approximately 7 hr into the light period and three types of triplicate samples were prepared: (1) ambient water, (2) ambient water with extra 0.8 mM $KHCO_3$ added to the final solution, and (3) ambient water with DIC reduced. We used addition of $KHCO_3$ to type 2 waters because $KHCO_3$ is highly soluble, in contrast to $Ca(HCO_3)_2$, and could be added as a small aliquot from a strong solution (50 mM) without causing sample dilution and carbonate precipitation.

Type 1 and 2 waters were directly siphoned into 25-ml glass stoppered flasks. Type 3 water was transferred to 50-ml tubes, centrifuged at 300 *g* for 10 min and the supernatant removed. The algal pellet was re-suspended in DIC-poor water prepared from de-mineralised water with the same chemical composition as in the mesocosms except that no $Ca(HCO_3)_2$ had been added and the DIC content had been depleted by purging with CO_2-free air by passing atmospheric air through a CO_2 absorber (ascarite). Nonetheless, type 3 water was not completely DIC-free. Prior to the experiments, it was tested that centrifugation did not affect phytoplankton photosynthesis.

All samples were prepared in a dark chamber with infrared light as the only light source to prevent photosynthesis and DIC reduction during preparation and then incubated under stirred conditions at 15°C at an irradiance of 300 µmol m^{-2} s^{-1} for 3 hr. Incubation temperature corresponded to maximum daytime temperature in mesocosms, whereas the higher irradiance was applied to light-saturate photosynthesis. Incubation bottles were mounted on a rotating wheel under illumination in a temperature constant incubator. Dissolved oxygen was determined before and after 1, 2, and 3 hr using an Oxygen Sensor Spot situated inside the bottles and connected during hourly readings through a 2 mm polymer optical fibre to a Fibox 3 oxygen meter. Oxygen was measured by the computer program, OxyView – PST3 –V6.02 (all from PreSens Precision Sensing, Regensburg, Germany). The final Chl *a* concentration was determined in all three types of water, and net photosynthesis was calculated as the mean rate of oxygen production relative to chlorophyll over 3 hr. Dissolved inorganic carbon was measured before, and pH before and after 3 hr. The decline of DIC during incubation was calculated from the net oxygen increase applying a photosynthetic quotient of 1.1. The concentration of inorganic carbon species was calculated by multiplying the mean of initial and final DIC concentration of the bottle experiment (i.e. ambient, depleted, or enriched water) by the relative proportion of carbon species as a function of mean pH, temperature, and ionic strength (Rebsdorf, 1972). The Michaelis–Menten equation was used to determine the relationship between net photosynthesis during 3-hr incubation (NP) and mean DIC available for photosynthesis (i.e. $CO_2 + HCO_3^-$):

$$NP = NP_{max} \times DIC/(K_m + DIC),\qquad(4)$$

where NP_{max} (maximum net photosynthesis) and K_m (apparent half saturation concentration) were fitted to values from ambient, depleted and enriched waters for both the start and about 7 hr into the light period for each of the 18 mesocosms.

2.5 | Statistics

Statistics were analysed using GraphPad Prism version 7.03 (GraphPad software) and the R programming language (R Core Team, 2018). Data analysed using analysis of variance (ANOVA) and *t* tests had normally distributed residuals (D'Agostino and Pearson normality test) and homogeneous variances (Brown–Forsythe test). If assumptions were not met, data were log-transformed or ultimately tested by the non-parametric Wilcoxon matched-pairs test. Using the R package *stats*, we fitted a linear model for relative growth rate (RGR) as a function of the log of Chl *a* concentration (covariate) and initial DIC concentration (categorical variable). Residuals of the model were visually inspected for normality and variance homogeneity before analysis of variance. A significance level of 0.05 was applied in all analyses. See Table S2 for details on ANOVA tests.

3 | RESULTS

3.1 | Phytoplankton growth, DIC, and pH

Massive phytoplankton growth, DIC depletion and pH rise took place over time in all mesocosms (Figures 1–3a–c). Dissolved inorganic carbon availability had highly significant effects on both cumulative phytoplankton production (Table 1) and growth rates (Figure 4a–c), which were markedly higher in mesocosms with intermediate and high initial DIC (0.72 and 3.26 mM) compared with low initial DIC (0.15 mM) early during the experiments, in particular. Weekly biomass removal improved light conditions and increased mean growth rates (after day 19, where the biomass removal treatment was initiated) relative to mesocosms with no biomass removal (two-way ANOVA, $p < 0.001$; Figure 4a–c). Biomass removal interacted positively with increasing DIC and resulted in higher mean growth rates ($p < 0.01$).

Dissolved inorganic carbon was strongly depleted to almost zero on day 31 by the growing phytoplankton biomass in mesocosms of low and intermediate DIC (Figure 2a,b), while it had decreased to about 0.6 mM in mesocosms of high DIC (Figure 2c). Intense photosynthesis and phytoplankton growth during the first 19 days of the experiments raised mean pH (from day 19–47) to a significantly higher level at intermediate DIC (10.50 ± 0.11, mean ± 1 SE, N = 6) and high DIC (10.01 ± 0.13) compared to low DIC (8.9 ± 0.10; Two way ANOVA, $p < 0.001$; Figure 3a–c). After day 20, CO_2 concentrations
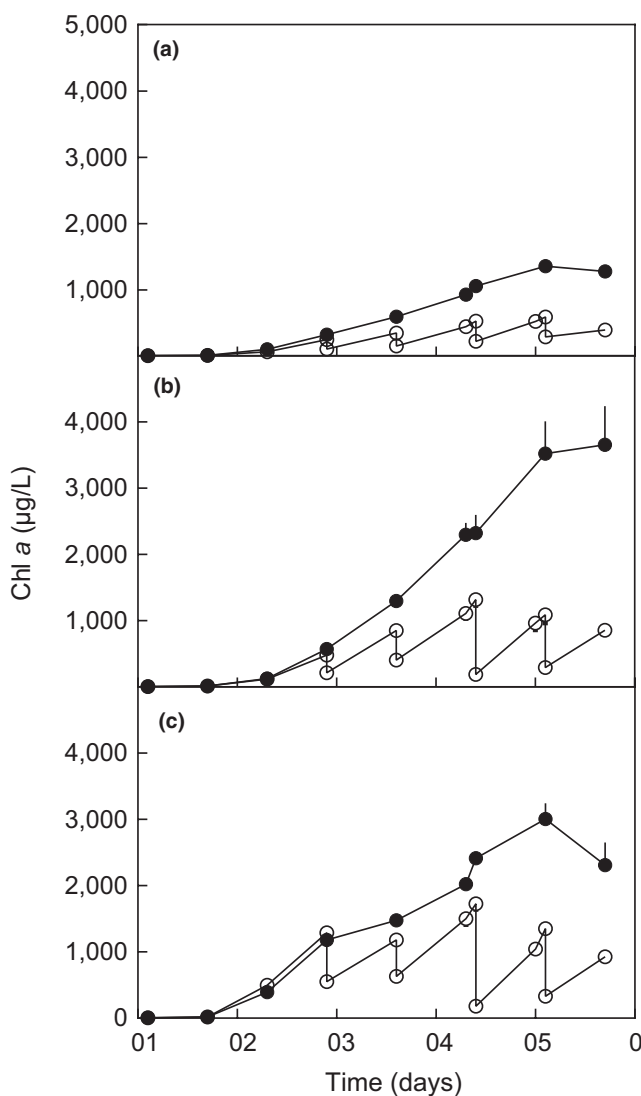

FIGURE 1 Phytoplankton biomass (chlorophyll a, Chl a) over time in mesocosms with no biomass removal (●) and weekly biomass removal (○) at three initial dissolved inorganic carbon levels of 0.15 mM (a), 0.72 mM (b), and 3.26 mM (c). Values are means (±1 SE, bars) of triplicate mesocosms. In several measurements, SE does not extend beyond the size of the symbol
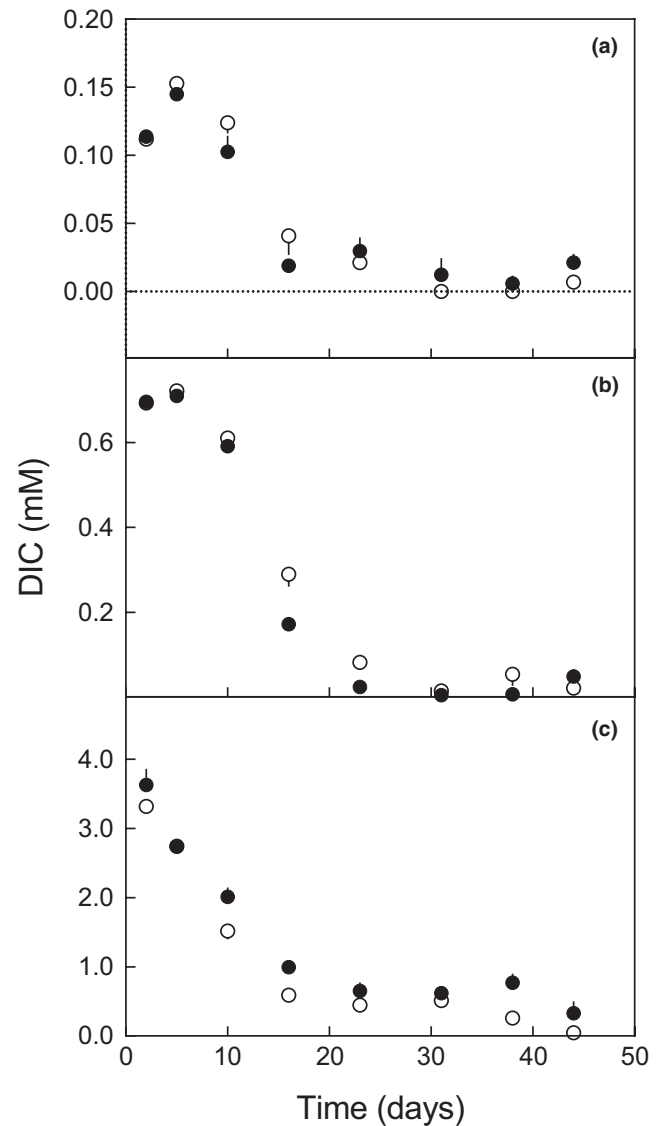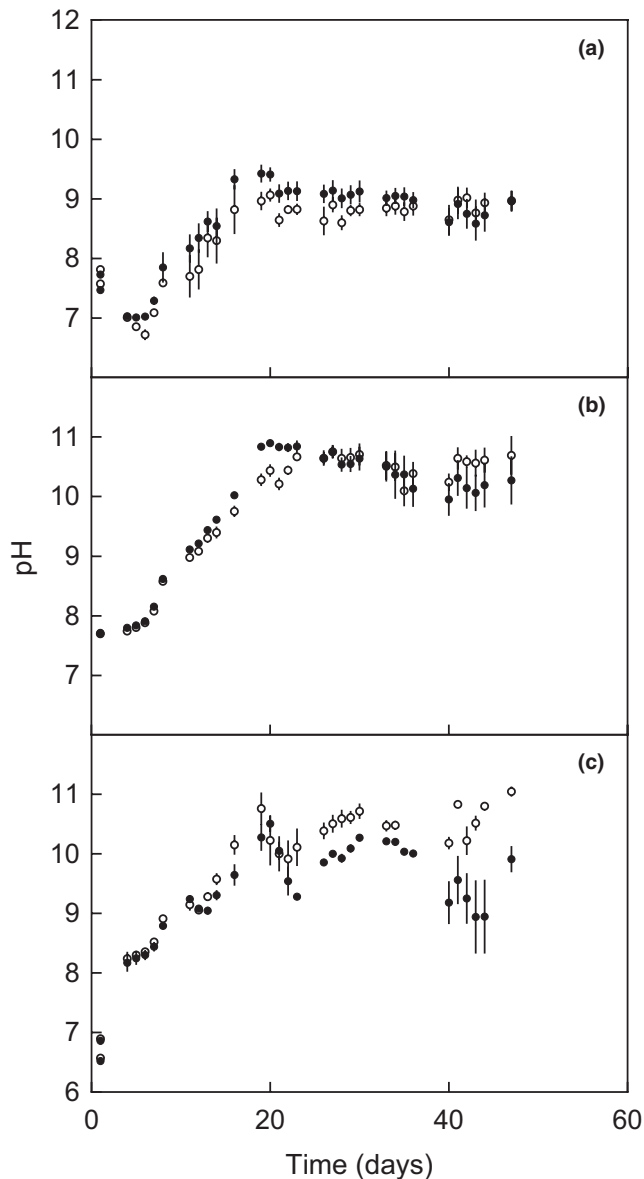
FIGURE 2 Dissolved inorganic carbon (DIC) over time in mesocosms with no biomass removal (●) and weekly biomass removal (○) at three initial DIC levels of 0.15 mM (a), 0.72 mM (b), and 3.26 mM (c). Values are means (± 1 SE, bars) of triplicate mesocosms. In several measurements, SE does not extend beyond the size of the symbol

FIGURE 3 pH over time in mesocosms with no biomass removal (●) and weekly biomass removal (○) at three initial dissolved inorganic carbon levels of 0.15 mM (a), 0.72 mM (b), and 3.26 mM (c). Values are means (±1 *SE*, bars) of triplicate mesocosms. In several measurements, *SE* does not extend beyond the size of the symbol

were extremely low in all three DIC treatments; <0.03, 0.08, and 0.77 μM, respectively compared to about 20 μM at air equilibrium.

Rates of biomass accumulation gradually declined and approached zero as the biomass increased towards a maximum level in mesocosms with no biomass removal (Figure 1a–c). This maximum biomass was maintained over at least a week emphasising that experiments were sufficiently long for biomass to reach an upper threshold, which represented the sum of phytoplankton in the water and re-suspended from the bottom. Growth continued in mesocosms with weekly biomass removal (Figure 4a–c). Relative growth rate in biomass over weekly periods declined linearly with

TABLE 1 Mean cumulated biomass production (±1 *SE*, n = 3) of the phytoplankton community during experiments at three initial dissolved inorganic carbon (DIC) levels of 0.15, 0.72, and 3.26 mM with no and weekly biomass removal. Cumulated biomass production is the maximum final biomass in mesocosms with no biomass removed and the maximum final biomass plus removed biomass in mesocosms with weekly biomass removal. Different superscript letters show significant differences between treatments (two-way ANOVA, Tukey post hoc test). Biomass accumulation was significantly influenced by DIC ($p < 0.001$), biomass removal ($p < 0.05$) and their interaction ($p < 0.05$)

	0.15 mM DIC (μg Chl a/L)	0.72 mM DIC (μg Chl a/L)	3.26 mM DIC (μg Chl a/L)
No biomass removal	1,355 ± 82[a]	3,658 ± 585[bc]	3,007 ± 239[b]
Weekly biomass removal	1,328 ± 104[a]	3,491 ± 305[bc]	4,779 ± 99[c]

Chl a, chlorophyll a

the logarithm to mean biomass (Figure 5a–c). Regression lines predicted two to three-fold higher relative growth rates in mesocosms of gradually higher initial DIC and a given biomass level. At 50 μg Chl a/L, RGR was 0.284, 0.363, and 0.610/day across the three DIC levels, while at 500 μg Chl a/L, RGR was 0.087, 0.181, and 0.233/day across DIC levels. Analysis of variance showed that the interaction between Chl a and initial DIC was significant ($p < 0.001$); that is not all three slopes were equal. By fitting the model for subsets of data, we tested if the interaction between the initial DIC concentration and Chl a was significant for low versus medium ($p = 0.376$), low versus high ($p < 0.001$), and medium versus high ($p < 0.001$) DIC concentrations. Thus, the slopes for low and medium initial DIC were both significantly different from the slope for high DIC but not different from each other. The analysis also showed that intercepts for the linear models were significantly different ($p < 0.001$) for each of the three initial DIC concentrations. A three-dimensional plot of RGR as a function of both chlorophyll and mean DIC over measuring intervals clearly revealed the growth stimulation by DIC and the growth reduction by light at increasing chlorophyll biomass and self-shading (Figure 6).

Directly measured, total production of Chl a (Table 1) over time was also significantly influenced by the initial DIC concentration (two-way ANOVA, $p < 0.001$) and weekly biomass removal ($p < 0.05$), while the significant interaction between the two factors ($p < 0.05$) indicated a stronger effect of biomass removal at higher DIC levels. This was also supported by the Tukey post hoc test. Thus, in mesocosms with weekly biomass removal, the total production of Chl a during the experiment increased three- to four-fold from low DIC (1330 μg Chl a/L) to intermediate DIC (3490 μg Chl a/L) and high DIC (4780 μg Chl a/L). Simultaneously, in mesocosms with no biomass removal, a three-fold higher maximum biomass was found at intermediate DIC (3660 μg Chl a/L) compared with that at low DIC (1360 μg Chl a/L). At high DIC, weekly biomass removal resulted in a significantly higher total biomass production (Tukey post hoc test, $p < 0.05$).
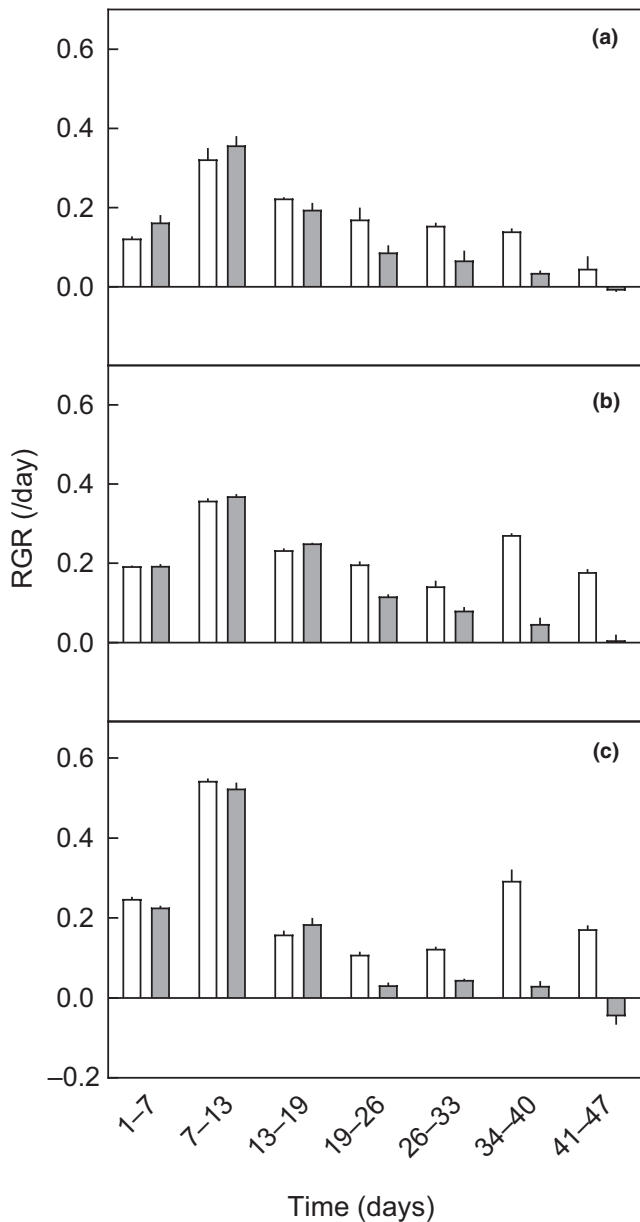
FIGURE 4 Relative growth rate (RGR, /day) of phytoplankton biomass (chlorophyll *a*) between weekly samples during the experiment in mesocosms at three initial dissolved inorganic carbon (DIC) levels of 0.15 mM (a), 0.72 mM (b) and 3.26 mM (c) and either no (grey columns) or weekly biomass removal (white columns). Columns are means and bars are ± 1 *SE* of triplicate mesocosms
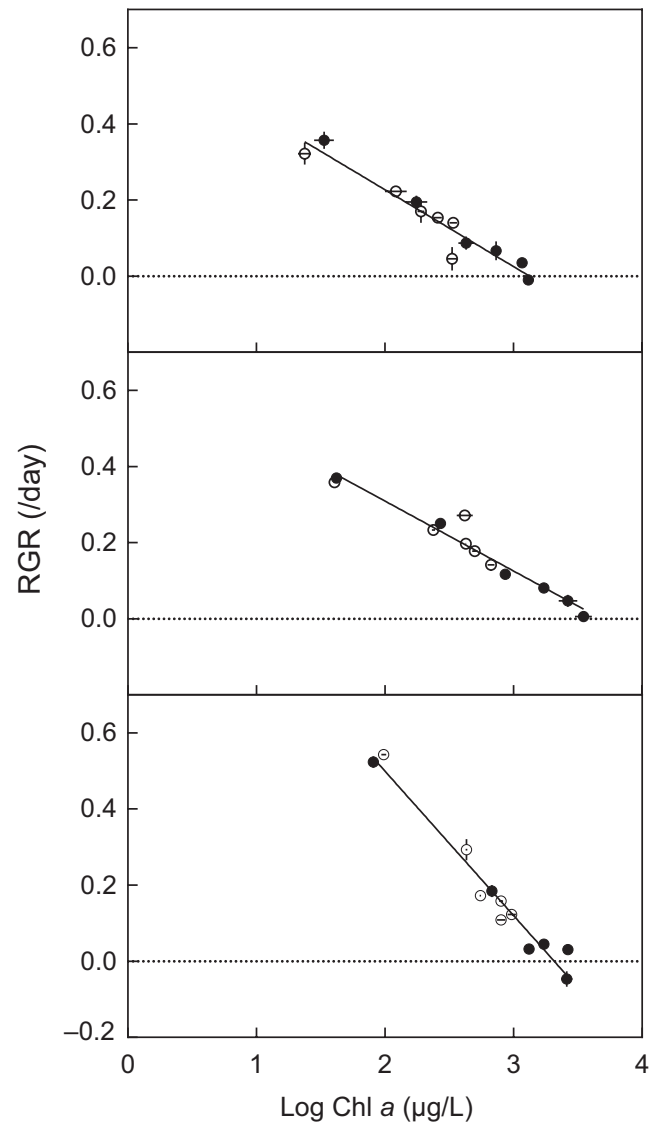
FIGURE 5 Relative growth rate (RGR, /day) of phytoplankton biomass as chlorophyll (Chl) *a* between weekly samples as a linear function of the logarithm to the biomass (Log Chl *a*) halfway through the weekly period assuming exponential growth. Results and regression equations for mesocosms with no (●) and weekly biomass removal (O) are combined for each of the three initial dissolved inorganic carbon (DIC) levels: 0.15 mM (a, RGR = −0.197 Log Chl *a* + 0.619; *p* < 0.001), 0.72 mM (b, RGR = −0.182 Log Chl *a* + 0.672; *p* < 0.001), 3.26 mM (c, RGR = −0.377 Log Chl *a* + 1.251, *p* < 0.001). Values are means (±1 *SE*, bars) of triplicate mesocosms. In several measurements, *SE* does not extend beyond the size of the symbol. Values from day 1–7 are excluded because the phytoplankton assemblage was in the stage of acclimation and establishment after inoculation before growth commenced

Total organic carbon accumulation increased highly significantly (*p* < 0.001, two-way ANOVA) over the course of experiments from the low DIC level (1.8–2.4 mM C) to the intermediate and high DIC levels (3.7–5.9 mM C, Figure 7). Dissolved inorganic carbon supported an increasing organic carbon production at higher initial DIC in the water (i.e. from 0.14 to about 2.6 mM C). Virtually all (93%) organic carbon accumulation (1.7–2.3 mM C) derived from atmospheric CO_2 uptake at low DIC, while higher atmospheric CO_2 uptake (3.0–4.6 mM C), chemically enhanced by maximum pH levels (Figure 3), supported about 84% of the organic carbon accumulation

at intermediate DIC (Figure 7, Table S3). At the highest initial DIC, calcium carbonate precipitated, reduced pH and decreased mean atmospheric CO_2 uptake to 47% of organic carbon accumulation. Maximum CO_2 uptake rates relative to surface area in mesocosms at intermediate DIC and no biomass removal were between 72 and 130 m^{-2} day^{-1} from day 19 to termination of the experiment. In the
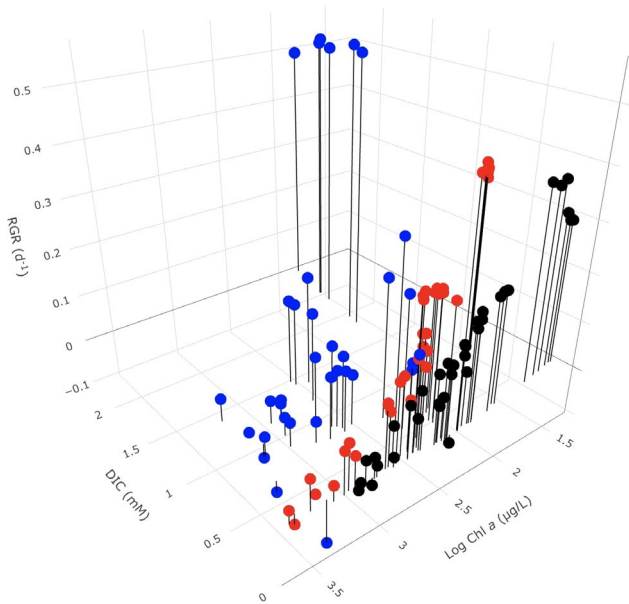
FIGURE 6 Relative growth rate (RGR, /day) as a function of mean DIC (mM) and phytoplankton biomass (Log chlorophyll [Chl] *a*) over measuring intervals for phytoplankton grown at three initial DIC levels: 0.15 mM (black symbols), 0.72 mM (red symbols) and 3.26 mM (blue symbols)
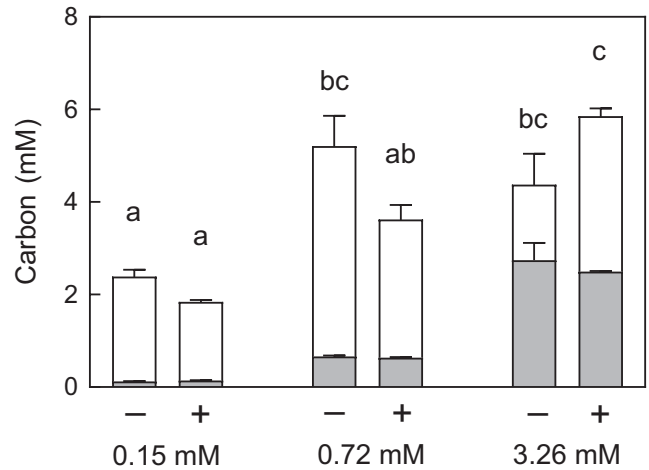

FIGURE 7 Net accumulation of organic carbon (mM C) during the course of experiments at three initial DIC levels (0.15, 0.72 and 3.26 mM) with (+) and without (−) weekly biomass removal. The contribution of declining DIC (corrected for $CaCO_3$ production) is shown in grey within the columns, while the remainder of the columns in white represents atmospheric CO_2 uptake. Values are means (±*SE*) of triplicate mesocosms. Same letters indicate no significant difference (Two-way ANOVA and Tukey post hoc test)

same period, daily CO_2 uptake rates were 46–58 mmol/m^2 at the lower initial DIC and pH.

3.2 | Net ecosystem production, phytoplankton photosynthesis, and DIC limitation

Dissolved inorganic carbon limitation of phytoplankton organic carbon production and growth rates implies that photosynthesis should also be limited. This limitation should be strongest at low initial DIC, high biomass, and late in the daily light period with particularly high pH and low CO_2. In support of these predictions, net ecosystem production (NEP) in the mesocosms peaked at the onset of light and decreased markedly during the entire light period as DIC decreased and pH and oxygen increased (Figure 8). The NEP decline during the light period was much steeper at high biomass late during experiment (day 26–29) compared with earlier during the experiment (day 15–17). Early during the experiment, ecosystem production was substantially higher at high than low initial DIC levels (Figure 8).

These patterns were confirmed by bottle experiments initiated in the morning (Figure 9a,c,e) and in the afternoon (Figure 9b,d,f). In 3-hr long bottle experiments at high biomass, net photosynthetic rates in ambient water of all six treatments were from 2.1- to 4.6-fold and significantly higher (paired *t* test, *p* < 0.001) when initiated in the morning as opposed to 7 hr later in the day at lower DIC availability. The significant relationship (linear regression, *p* < 0.001) between produced O_2 and DIC available for photosynthesis (CO_2 and HCO_3^-) was in close agreement with a typical photosynthetic O_2 to carbon molar quotient close to 1.1 (Figure 10).

Photosynthetic rates declined in all treatments when DIC was further depleted experimentally, and increased in all treatments when extra DIC was added (Figure 9a–f). Mean photosynthetic rates were seven-fold and significantly higher under elevated compared with reduced DIC concentrations (Wilcoxon matched-pairs test, *p* < 0.001). The time course showed that photosynthetic rates stopped after 1 or 2 hr into the light period in DIC depleted water, while photosynthesis continued at high rates for 3 hr in DIC-enriched water. The lower photosynthetic rates in ambient water after 7 hr light compared to rates obtained just before first light were not due to differences in photosynthetic capacity of phytoplankton communities, because maximum photosynthetic rates relative to chlorophyll did not differ significantly among the six treatments (Table 2, *p* = 0.24, one-way ANOVA). Photosynthetic rates as a function of DIC available for photosynthesis (HCO_3^-: 98.0% of DIC) followed a common hyperbolic Michaelis–Menten function (Figure S1) with half-saturation constants of 60–280 μM among treatments (Table 2, see Table S4 for additional information).

4 | DISCUSSION

Our experiments support a broader view on the regulation of phytoplankton growth than the perception of inorganic nutrients and light as the sole limiting factors. The results demonstrated all three main processes, characterising DIC limitation by physiological and ecological processes in nutrient-rich waters. The photosynthetic rate, the RGR over weekly intervals, and the net organic carbon production over 47 days were all DIC limited. Uptake of atmospheric CO_2 was very substantial and the main long-term source to organic carbon

FIGURE 8 Examples of net ecosystem production rate (NEP, mM O₂/hr) early (a, day 15–18) and late in the experiment (b, day 26–29) in mesocosms (no biomass removal) with initial dissolved inorganic carbon of 0.15 (black line), 0.72 (red line), and 3.26 mM (blue line). Light/dark periods are marked with white and grey bars, respectively
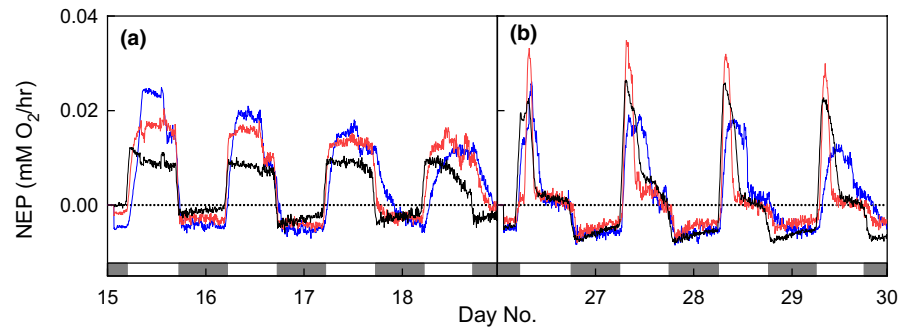
FIGURE 9 Net oxygen evolution during 3-hr phytoplankton light incubations in ambient water (●), water with depleted dissolved inorganic carbon (DIC; ○) and in DIC enriched water (+0.8 mM; □) from mesocosms with initial DIC concentrations of 0.15 (a, b), 0.72 (c, d) and 3.26 mM (e, f) DIC and no biomass removal. Incubations were initiated just before the light period (a, c, e) and about 7 hr into the light period (b, d, f) after 41 days. Values are means (±1 SE, bars, n = 2 or 3)
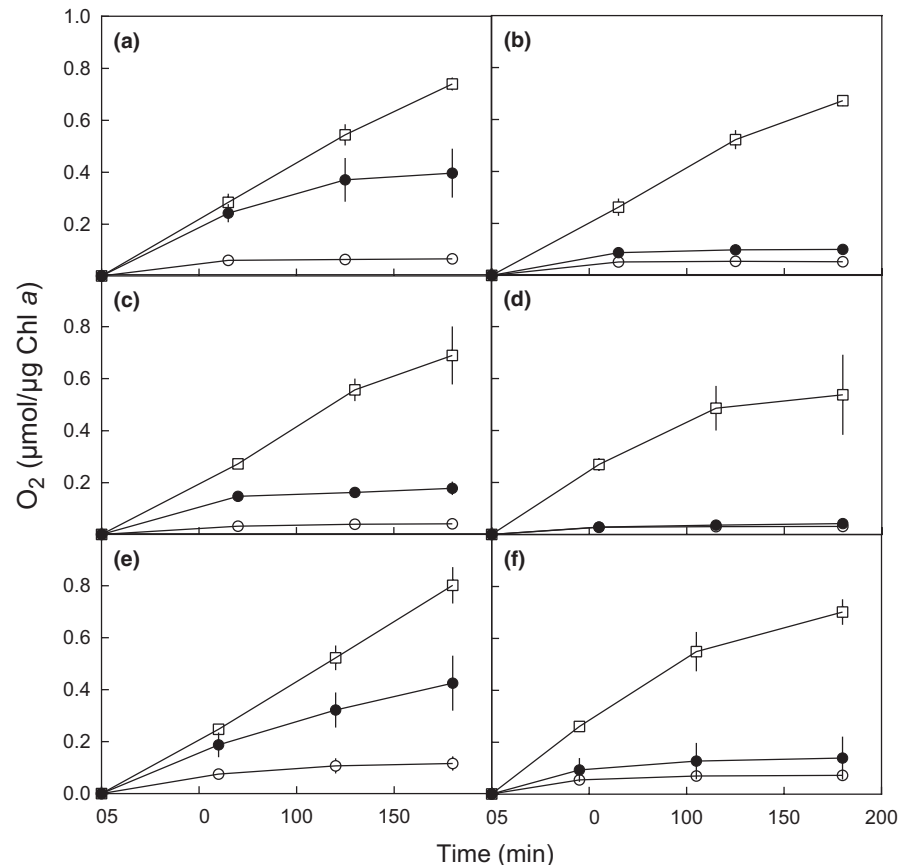
production in mesocosms of low and intermediate alkalinity and DIC. The results support recent models and experimental studies that have shown extensive growth stimulation at elevated atmospheric CO_2 and lake water bicarbonate concentrations (Bogard et al., 2017; Ibelings & Maberly, 1998; Schippers et al., 2004; Verspagen, van de Waal, Finke, Visser, & Huisman, 2014; Verspagen, van de Waal, Finke, Visser, Van Donk et al., 2014). We widen the perspectives of DIC limitation by confirming its importance to photosynthesis, growth rate and maximum biomass under nutrient-rich conditions across the full range from soft water to hard water and by evaluating the relative importance of CO_2 supply from the atmosphere and DIC in the water. Below, we discuss the mechanisms and field implications of: (1) DIC limitation of photosynthesis; (2) DIC limitation of growth rate, maximum biomass, and organic carbon production; and (3) biomass removal and light environment.

4.1 | Dissolved inorganic carbon limitation of photosynthesis

Inorganic carbon limitation of photosynthesis was profound. It was reflected by the declining rate of oxygen evolution in the mesocosms during the course of the day as DIC was depleted and pH rose. Dissolved inorganic carbon limitation was further demonstrated in bottle experiments, which showed gradually stronger DIC limitation of photosynthesis in longer incubations and stimulation of photosynthesis when extra DIC was added. Furthermore, as a result of DIC consumption since the morning, stronger limitation of photosynthesis developed in experiments performed 7 hr into the light period. The kinetics characterising photosynthesis versus HCO_3^- concentration yielded half-saturation constants (60–280 μM) implying widespread DIC limitation as DIC concentrations dropped below 20 and
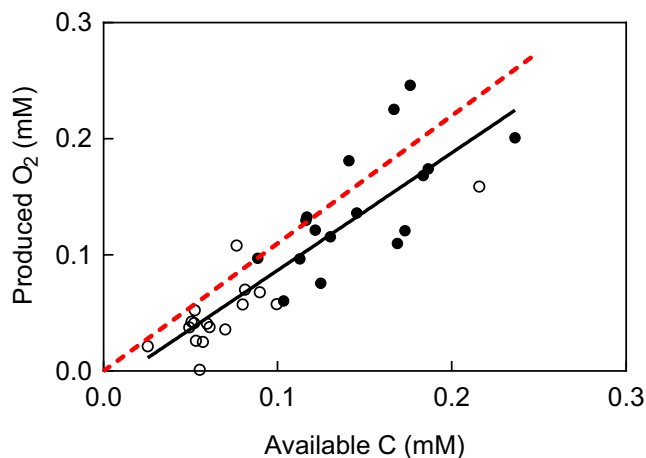
FIGURE 10 Photosynthetic oxygen production during 3-hr light incubations as function of dissolved inorganic carbon (DIC) concentration available for photosynthesis ($CO_2 + HCO_3^-$) in water collected from six types of mesocosms (i.e. three levels of initial DIC, with and without weekly biomass removal; HCO_3^- constituted >99% of photosynthetically available DIC). Incubations were made in water collected in the mesocosms just before (●) and about 7 hr (○) into the light period. The figure shows incubations with water of ambient DIC derived directly from the mesocosmos and does not include incubations with removal or additions of DIC. The common regression line (black solid line): Prod. $O_2 = 1.012 \times$ Avail. C – 0.0144 (linear regression, $p < 0.001$). The red, punctured line shows the C:O_2 ratio of 1:1.1

TABLE 2 Mean maximum net photosynthesis (NP_{max}) and apparent half saturation concentration (K_m; ±1 *SE*) of the Michaelis–Menten relationship between specific photosynthetic production (µmol O_2/ µg chl *a*/hr) and available dissolved inorganic carbon (DIC; mM, CO_2 plus HCO_3^-) in phytoplankton from the mesocosms with three levels of initial DIC concentration (i.e. 0.15, 0.72, and 3.26 mM DIC) without (–) and with (+) weekly biomass removal. Kinetics is based on 3-hr bottle experiments at ambient, DIC depleted and DIC enriched DIC levels after 0 and 7 hr into the light period. There were no significant differences among NP_{max} and K_m values of the different treatments (one-way ANOVA, $p > 0.05$). See Table S4 for detailed information on the individual Michaelis–Menten relationships

Initial DIC concentration (mM)	Weekly biomass removal	NP_{max}	K_m	*n*
0.15	–	0.28 ± 0.02	0.13 ± 0.05	3
	+	0.30 ± 0.03	0.06 ± 0.03	3
0.72	–	–[a]	–[a]	–[a]
	+	0.40 ± 0.01	0.28 ± 0.05	3
3.26	–	0.34 ± 0.01	0.23 ± 0.00	2
	+	0.41 ± 0.09	0.23 ± 0.13	3

[a]This experiment did not follow Michaelis–Menten kinetics.

strong carbon limitation may develop during algal blooms in nutrient-rich lakes when stripping ambient CO_2 levels below 1 µM in photosynthesis leads to pH rise above 9.0–9.5 (Jeppesen et al., 1998; Sorrell, Hawes, & Safi, 2013).

4.2 | Dissolved inorganic carbon limitation of growth rate, maximum biomass and production

Higher photosynthesis and organic carbon production are intimately coupled and should result in faster growth rates unless nutrient limitation prevents extra photosynthates from being allocated to new biomass and thus consumed by higher respiration or exudate release instead (Conan et al., 2007). However, nutrient limitation was not an issue in our mesocosm experiments. Indeed, during the early part of our experiments at low phytoplankton biomass, higher DIC was accompanied by two- to three-times faster growth rate. Later in the experiment at high phytoplankton biomass and light limitation, DIC stimulation of growth was only profound in mesocosms with weekly biomass removal, which improved light availability.

The maximum phytoplankton biomass was two- to three-times higher in mesocosms at intermediate DIC and maximum pH compared with mesocosms of low DIC and pH. The same profound differences were observed for the mean organic carbon production among mesocosms of low versus intermediate or high initial DIC concentrations. Intermediate initial DIC levels resulted in the highest pH due to lower buffer capacity in the water compared to high initial DIC levels and thus, the highest chemically enhanced atmospheric CO_2 uptake. This compensated for the lower initial DIC availability in the water compared to the highest initial DIC. Similar

100 µM in mesocosms of low and intermediate initial DIC, respectively. The kinetics can also account for the observed lower summer biomasses in soft water compared to hard water lakes at the same nutrient levels (Kragh & Sand-Jensen, 2018). This DIC limitation in mesocosms and photosynthesis experiments was probably not due to phytoplankton with low affinity for DIC. On the contrary, chlorococcalean green algae were dominant and most of them use HCO_3^- and have efficient CCMs (Colman, Huertas, Bhatti, & Dason, 2002). The observed half-saturation constants in our mesocosms resemble the kinetics of HCO_3^- use in freshwater phytoplankton cultures with 25–75 percentiles of 20–200 µM in 30 culture studies compiled by Hein (1997b) (Table S5).

Considering CO_2 kinetics, Hein (1997b) reported 25–75 percentiles of half-saturation constants for CO_2 (0.9–9 µM) from 24 freshwater phytoplankton species and measured summer values for natural phytoplankton assemblages in the same range (1.2–8.9 µM; Hein, 1997a). After day 20 in our mesocosm experiments, CO_2 concentrations were too low in all three DIC treatments (<0.03, 0.08, and 0.77 µM, respectively) to support appreciable photosynthesis, which must be based on HCO_3^- use with the kinetics discussed above. In natural phytoplankton assemblages from boreal lakes substantial stimulation (1.6–3.8-fold) of photosynthesis was found when CO_2 concentrations were elevated six-fold above air saturation (about 20 µM; Jansson et al., 2012, their Table 1). Overall, these results emphasise the likely importance of carbon limitation of photosynthesis and growth of natural phytoplankton communities. Particularly

DIC stimulation of organic carbon production with increasing DIC and chemical enhancement of atmospheric CO_2 uptake at high pH were documented in previous outdoor experiments in the same mesocosm setup (Kragh & Sand-Jensen, 2018).

The marked DIC limitation in mesocosms was due to high nutrient availability and accompanying high DIC requirements, which were initially supplied by water DIC and later by CO_2 uptake from the atmosphere as DIC declined and pH rose. The extensive DIC limitation was not induced by artificially low air-water exchange rates of CO_2 or high irradiances. On the contrary, the basic piston velocity of CO_2 exchange in mesocosms (2.0 cm/hr, equivalent to 0.48 m/day) was typical of small lakes (0.35–0.74 m/day and 0.3–45 ha; Cole, Bade, Bastviken, Pace, & van de Bogert, 2010) and the extensive DIC limitation developed even though daily incident photon flux on the mesocosms (i.e. 5.2 mol photon per m^2) was in the lower range of daily photon fluxes (4–50 mol photon per m^2) during summer in temperate habitats (Christensen et al., 2013).

Physio-chemical exchange rates of atmospheric CO_2 were elevated to 2.3–4.6 m/day by chemical enhancement factors of 5–10 at maximum pH of 10–10.6 (Bade & Cole, 2006). According to carbon mass balances in the mesocosms with no biomass removal at intermediate initial DIC, atmospheric CO_2 uptake rates were between 72 and 130 mmol m^{-2} day^{-1} from day 19 to termination of the experiment. In the same period, daily CO_2 uptake rates were 46–58 mmol/m^2 at the lower initial DIC and pH. High uptake rates can also be attained in small eutrophic lakes with the same surface turbulence, shallow depth and high pH (Jeppesen et al., 1998). Large eutrophic lakes with stronger wind exposure, surface turbulence and higher basic piston velocity (e.g. 0.9–1.4 m/day, Schilder et al., 2013) would tend to have lower pH (max. 9.5) as well as lower chemical enhancement factors (e.g. 2, Trolle et al., 2012). Thus, large lakes may have lower daily atmospheric CO_2 uptake rates compared to smaller lakes.

Uptake rates of atmospheric CO_2 in our mesocosms increased from low initial DIC and peaked at intermediate initial DIC. Because DIC availability in the water increased along the DIC gradient, the relative contribution of atmospheric CO_2 to organic productivity declined from absolutely dominant (93%) at low DIC to equal contribution from air (about 47%) and water at high DIC. In natural eutrophic lakes alike, restricted input of low-DIC water from the catchment and long water retention time will increase the relative contribution of atmospheric CO_2 uptake to sustain primary production. The main DIC source to primary production in our mesocosms can initially derive from the water. However, when this source is exhausted, the ongoing organic carbon accumulation is supplied by atmospheric CO_2 uptake. The same shift may take place in temperate lakes during the growth season as a result of high water and DIC input during winter and spring forming high in-lake DIC concentrations, but changing to low DIC input and intensive photosynthesis use later in summer and causing low in-lake DIC concentrations (McConnaughey et al., 1994; Müller, Meyer, & Gächter, 2016). Thus, atmospheric CO_2 can become the main source to net ecosystem production during intense phototrophic growth in nutrient-rich lakes during summer. However, the possibility of attaining high pH conducive to strong chemical enhancement of atmospheric uptake is only realised in waters of intermediate or high DIC. In soft water lakes with HCO_3^- concentrations below 0.05 mM and pH below 6.5 at air saturation (Stumm & Morgan, 2012), atmospheric CO_2 uptake is constrained by limited chemical enhancement because pH does not rise above 8–9.

Our mesocosm experiments show that net daily ecosystem production rates in eutrophic water of intermediate alkalinity and initial DIC of 0.7 mM at 10–13°C can reach upper thresholds of about 130 mmol C m^{-2} day^{-1} solely based on CO_2 invasion from the atmosphere. To attest the relevance and magnitude of this CO_2 invasion rate and net ecosystem production, we compared with estimates for a 1-m deep lake. If net ecosystem production of 130 mmol C m^{-2} day^{-1} should be supported by external water inflow containing 1.0 mM DIC, water retention time should be as short as 8 days. The majority of water bodies have much longer water retention times of several months or years (Kalff, 2002). Moreover, stream input with inorganic carbon is particularly low during summer when evapotranspiration typically exceeds rainfall (Kalff, 2002). Thus, atmospheric CO_2 input can be the most important and highly constrained external inorganic carbon source to phototrophic production in very eutrophic alkaline water bodies during summer. Maximum daily gross primary production rates of phytoplankton in 19 temperate and sub-tropical rates compiled by Kalff (2002; his Table 21-4) averaged 259 mmol C m^{-2} day^{-1}. If 50% of primary production would be respired by phytoplankton and bacteria, these rates correspond to the maximum CO_2 invasion rates in our mesocosms, which emphasise that they can support intensive lake metabolism provided surface turbulence and high pH in medium–high alkaline waters support high chemically enhanced uptake rates.

The mesocosm experiments were performed under nutrient replete conditions to evaluate carbon dynamics at highly variable alkalinities and DIC concentrations. The mesocosms only allowed external input of atmospheric CO_2 and no inorganic carbon input via streams and groundwater as in natural lakes. At the start of experiment, there was little mineralization of organic matter while, at its termination, production and decomposition of organic carbon have reached a balance. In natural lakes there is an ongoing mineralization in water and sediments involving macrozooplankton, zoobenthos, and fish not being represented in the mesocosms. Thus, compared to natural systems, the mesocosms best represent the bloom formation of phytoplankton in hypertrophic lakes during summer with low external water input and atmospheric CO_2 supporting net ecosystem production due to high pH levels (9.5–10.0) caused by intensive photosynthesis (e.g. Lake Arresø in Trolle et al., 2012). At those high summer pH levels in hypertrophic hardwater lakes, the rates of CO_2 influx and calcium carbonate formation resemble those in mesocosms. While the high organic carbon production accumulates in the mixed water column or is removed weekly by simulated grazing in the mesocosms, the net organic carbon production in natural lakes is lost to the sediment or exported via stream outlets.

4.3 | Biomass removal and light environment

Relative growth rates of phytoplankton biomass were higher in mesocosms with weekly biomass removal because co-limitation by light was reduced by biomass harvesting. Natural lakes experience an ongoing biomass loss of phytoplankton by sinking to the sediment, washout through the outlet and, in particular, grazing by zooplankton and benthic animals. In our mesocosms, weekly removal of 70% of the phytoplankton biomass corresponds to a daily relative loss rate of 0.17/day, which balanced the relative growth rate at constant biomass late during the experiment, while growth rates were higher (0.3–0.6/day) early during the experiment. Such rates of net growth and grazing losses are common in natural lake populations (e.g. 0.02–0.39/day in Reynolds et al., 1985; and 0.3–1.0/day in Forsberg, 1985). Ongoing losses may increase the importance of DIC limitation for phytoplankton photosynthesis, growth and organic carbon production due to greater DIC demands of a continuously dividing phytoplankton population in an environment with higher light availability. Without biomass removal, limitation by DIC may be most intense at an intermediate biomass level where light is suitable and the phytoplankton population sufficiently dense to deplete DIC. In contrast, intense losses could reduce the phytoplankton biomass to very low levels and elevate DIC and nutrient supply by organic decomposition and, thereby, reduce growth limitation by light, DIC and nutrients, and stimulate faster growth rates.

Mechanisms of rate limitation of phytoplankton photosynthesis and growth are complex. Low DIC concentrations can reduce carbon fixation directly by limitation of photosynthetic rates and carbon incorporation into cell products (Clark & Flynn, 2000). Indirectly, low DIC may also reduce carbon fixation by inducing proportionally greater investment in CCMs and enhance limitation of uptake of nitrogen, phosphorus, and micronutrients (Saito, Goepfert, & Ritt, 2008; Tortell, Rau, & Morel, 2000; Wirtz, 2011). In general, interactions are possible between light, DIC, nutrients, and grazing in the regulation of phytoplankton growth rate and biomass accumulation (Kranz, Levitan et al., 2010). During the 47-days long experiment, DIC became gradually more limiting as phytoplankton biomass increased and DIC was depleted. Likewise, light became more limiting as biomass and self-shading increased. Declining relative growth rates with increasing biomass (and thus lower light) and decreasing DIC reflect a coupled limitation, while an increase of either light or DIC will increase the extent of limitation by the other unmodified factor. It is possible, though not explored here, that the increase of DIC could reduce the demand on light and photosynthetic energy because less investment in active mechanisms to exploit DIC would be needed, while the increase of light would make more energy available to ensure active DIC uptake and offset DIC leakage from the cells (Giordano et al., 2005).

In conclusion, the carbon limitation of phytoplankton photosynthesis, growth rate, maximum biomass, and organic carbon production found in this study implies that inorganic carbon could be of far greater importance in eutrophic waters than previously assumed and that the extent of limitation changes across the DIC gradient from soft water to hard water lakes. Our experiments were conducted in mesocosms under ample nutrient supply and with no resupply of DIC from the sediment and external sources, which will probably increase the extent of DIC limitation. Consequently, we recommend that inorganic carbon supply is taken into account, and further tested in situ, when predicting and evaluating primary production and carbon budgets in eutrophic waters on both daily and annual scales.

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