Phytoplankton productivity in lakes controls the rate of synthesis of organic matter that drives energy flow through the food webs and regulates the transparency and oxygen conditions in the water. Limitation of phytoplankton productivity and biomass by nutrients and light availability is an established paradigm for lake ecosystems, whereas invasion of atmospheric CO₂ has been assumed to cover the high demands of dissolved inorganic carbon (DIC) during intense organic productivity. We challenge this paradigm, and show up to a 5-fold stimulation of phytoplankton productivity and biomass in outdoor mesocosms enriched with DIC, compared to mesocosms with lower DIC concentrations. High DIC supported phytoplankton productivity by direct algal uptake of bicarbonate, through the release of CO₂ coupled to calcification and by inducing high pH that greatly enhances atmospheric CO₂ invasion. Comparisons of 204 natural Danish lakes supported mesocosm experiments showing higher phytoplankton biomass and pH levels in hard water than soft water lakes for the same nutrient and light availabilities. The most productive lakes are nutrient-rich, hard water lakes that attain surface pHs of 10–11 and chemically enhance atmospheric CO₂ uptake 10–15-fold. Our results will help understand natural variations of lake productivity along gradients in nutrients, DIC and pH.

1. Introduction

A consensus has existed among freshwater ecologist ever since the ‘carbon-phosphorus controversy’ in the early 1970s that phytoplankton primary productivity and biomass development in lakes is determined primarily by nutrient availability and secondarily by light, whereas dissolved inorganic carbon (DIC = CO₂ + HCO₃⁻ + CO₃²⁻) plays a minor role as a limiting factor [1–5]. In this paper, we challenge the long-held paradigm in limnology that nutrients (nitrogen (N), phosphorus (P)) and/or light always limit production in lakes by asserting the importance of DIC limitation of productivity and subsequent biomass development of phytoplankton in eutrophic shallow systems.

It has been assumed that uptake of atmospheric CO₂ in the long term can match the consumption of DIC in photosynthesis and prevent limitation of phytoplankton biomass [2,6]. However, short-term DIC limitation of phytoplankton photosynthesis has been demonstrated in highly productive lakes experiencing profound CO₂ depletion and even in unproductive lakes with CO₂ concentrations close to air saturation [7–9]. To our knowledge, experiments have not been conducted across gradients in water DIC to demonstrate that CO₂ supply from the water and the atmosphere is in fact sufficiently fast to saturate primary productivity and ensure maximum biomass build-up set by nutrient availability. We regard biomass as a dynamic property regulated by productivity gains and losses, both determined by highly variable multiple factors including DIC supply [10]. The increase of alkalinity (acid neutralizing capacity (ANC)) from soft water to hard water lakes is closely positively related to DIC. It is a relationship close to 1:1 in lakes with a pH between 7.6 and 9, even though organic acids can affect alkalinity as well [11]. This is because HCO₃⁻ is the main anion (i.e. greater than 95% of DIC), the main buffer and the main carbon species in most freshwaters at air saturation [8,9]. For example, soft waters of low carbonate alkalinity (e.g.
0.1 meq. l⁻¹) at air saturation with CO₂ (389 µmol l⁻¹ at 15°C) have a pH of 7.17 and contain 118 µmol l⁻¹ DIC and 100 µmol l⁻¹ HCO₃⁻, while hard waters of high carbonate alkalinity (e.g. 2.0 meq. l⁻¹) and pH 8.46 contain 1995 µmol l⁻¹ DIC and 1956 µmol l⁻¹ HCO₃⁻ [12,13]. High pH is conducive to chemically enhanced uptake of atmospheric CO₂ by direct reaction with OH⁻ and conversion to HCO₃⁻ in the surface waters keeping the air–water CO₂ gradient maximally steep and the influx high [14,15]. This chemical enhancement (CE) increases atmospheric CO₂ uptake about 1.5-fold at pH 8 but 4.1-fold at pH 10 at 15°C relative to CO₂ uptake at pH less than 7. By contrast, uncalculated hydration of CO₂ to carbonic acid is a slow process. CE can, therefore, greatly enhance the invasion of atmospheric CO₂ (FluxCO₂) above the rates determined by the difference in partial pressure of CO₂ from air to water (pCO₂air–pCO₂wat) and the gas transfer velocity (K) set by replenishment of surface water by turbulence (FluxCO₂ = CE×K×(pCO₂air–pCO₂wat)) [14].

Carbon supply to photosynthesis of freshwater organisms is not solely linked to CO₂, but often to a much higher carbon supply rate of HCO₃⁻. Firstly, HCO₃⁻ is used in photosynthesis by an array of carbon concentrating mechanisms (CCMs) that have evolved in most groups of aquatic photoorganisms including cyanobacteria, uni- and multicellular algae and higher plants [16–18]. Secondly, when HCO₃⁻ is not used directly, it constitutes a large inorganic carbon pool that is converted either passively to CO₂ in the water or catalytically on the surface of photoorganisms to replenish CO₂ consumption [19]. Thirdly, HCO₃⁻ releases free CO₂ by coupled photosynthesis and CaCO₃ precipitation because calcification generates protons for HCO₃⁻ conversion to free CO₂ (Ca²⁺ + 2 HCO₃⁻ → CaCO₃ + CO₂) [20]. Calcification is used directly by many algae, corals and higher plants [18–20] to ensure CO₂ supply for photosynthesis. When the solubility products of CaCO₃ minerals are surpassed, calcification takes place passively in the water surrounding photosynthesizing organisms [2]. Fourthly, increasing HCO₃⁻ elevates pH in the water at air saturation, and as CO₂ and HCO₃⁻ are used in intensive photosynthesis (HCO₃⁻ → CO₂ (assimilated) + OH⁻ (released)), pH rises to higher maximum levels (e.g. 10,11) in high-alkaline hard waters than in low-alkaline soft waters (e.g. 8,9) and thereby enhances CO₂ uptake from the atmosphere [21]. We have evaluated the importance of increasing ANC (and closely related DIC) across a range from soft waters to hard waters. We quantified carbon supply to phytoplankton production from these three sources: (i) directly from DIC, (ii) CO₂ release from calcification, and (iii) CO₂ invasion from the atmosphere. The first two sources both derive from DIC in the water. We performed this test in well-replicated, outdoor mesocosms with phytoplankton, simulating the physical CO₂ exchange capacity across lake surfaces at intermediate and high turbulence kept constant by submerged pumps [22,23]. Furthermore, we used another dataset from 204 temperate lakes for statistical evaluation of the relationships between phytoplankton summer biomass and concentrations of potentially limiting factors (P and DIC). The natural lakes included a broad range from soft water to hard water lakes and from oligotrophic to eutrophic lakes.

The aim of the study was to test the importance of DIC limitation on phytoplankton productivity and subsequent biomass development in eutrophic shallow systems. We hypothesize that CO₂ supply from the water and the atmosphere is not sufficiently fast to saturate primary productivity and biomass development. We further hypothesize that organic productivity and resulting biomass development is higher in hard waters compared with soft waters. This is so when production and biomass development are supported directly by larger DIC pools in alkaline hard waters and development of high pH leads to profound CE of CO₂ invasion from the atmosphere. The present effort is the first to consolidate data from a broad range of water DIC levels in both mesocosms and natural lakes with different nutrient regimes and air–water CO₂ exchange, providing new insights into the overall complexity and functioning of lake productivity.

2. Material and methods

(a) Mesocosms

Mesocosm experiments were performed three times from April to September. All three experiments were performed in 18 cylindrical, shallow (0.9 m, 0.15 m², 0.09 m³) well-mixed Perspex mesocosms placed outdoors. All experiments had six different treatments in three replicates. The range of alkalinites (ANCs) and coupled DIC concentrations tested (0.05–5.6 mmol l⁻¹), were within the range observed in natural Danish lakes. We avoided experiments during mid-summer to prevent problems with excessive solar heating. The average temperature in the three experiments was 12.3, 19.3 and 15.9°C, which corresponded well to the temperature in Danish lakes during summer. Longer days and higher incident irradiances and temperatures in summer will tend to increase organic productivity and the extent of inorganic carbon limitation of organic productivity and upper bounds of biomass development compared with data reported here. The day length during the experiments was 13.7, 16.9 and 13.8 h. Mean daily irradiances were 40.2, 19.5 and 21.6 mol photons m⁻² d⁻¹ during the three experiments.

In the three experiments reported here, we tested the influence of variable alkalinity and corresponding DIC levels on phytoplankton productivity as accumulation of total organic carbon (TOC) and biomass development under nutrient-rich conditions (experiment 1) and under nutrient-poor versus nutrient-rich conditions (experiment 2). Both these experiments were performed at intermediate gas transfer velocity (piston velocity, K = 2.1 cm h⁻¹) typical of moderate wind exposure in lakes. The third experiment was performed at low and high ANC, three levels of nutrient richness and uniformly high gas transfer velocity (K = 7.6 cm h⁻¹) typical of high wind exposure and surface turbulence in lakes [22,23].

The first experiment was started on 8 April and tested the effect of different ANC levels (0.05, 0.35, 0.7, 1.4, 2.8 and 5.6 meq. l⁻¹) with high amendments of nutrients to ensure no nutrient limitation and a piston velocity of 2.1 cm h⁻¹ during 28 days. The second experiment was started on 27 May and tested three levels of ANC (0.05, 0.7 and 2.8 meq. l⁻¹) with high and low P availability and a piston velocity of 2.1 cm h⁻¹ during 31 days.

The third experiment was started on 1 September and tested two ANC levels (0.08 and 2.5 meq. l⁻¹) with high, medium and low P availability and a high piston velocity of 7.6 cm h⁻¹, during 27 days.

(b) General conditions for all experiments

The medium was prepared from de-mineralized water without organic matter and with mineral salts and trace minerals added to mimic natural freshwaters of the desired composition to ensure good growth of phytoplankton. Variable alkalinity
(ANC) and closely coupled DIC concentrations were obtained by dissolving CaCO₃ powder by purging with CO₂ in a pressurized chamber. This yielded strong solutions of Ca²⁺ and HCO₃⁻ that were added to the mesocosms in appropriate dilutions. Then CaCl₂, MgSO₄, Na₂SO₄ and K₂SO₄ were added according to the recipe of Smart & Barko [24] to obtain a composition of macronutrients resembling that of natural lake water. Trace minerals were added as 1 mg l⁻¹ of Zn and 0.2 ml l⁻¹ of cyano-trace of the Zn-metal solution [25]. Iron was added to the chambers every week as iron ammonium-citrate to a final concentration of 1.2 mg Fe l⁻¹. Inorganic nitrogen (N) was added in equal N amounts of NH₄Cl and KNO₃ and 10 times less in mass of inorganic phosphate (P) as Na₃HPO₄. Nutrients were added to reach low, medium and high concentrations (10 mg Pl⁻¹, 40 μg P l⁻¹ and 500 μg P l⁻¹) in the initial medium (table 1). Dissolved nutrient concentrations were measured weekly. In experiment 1 with uniformly high nutrient concentrations, extra amounts were added when needed to prevent concentrations from falling below 100 mg NH₄⁺ + NO₃⁻ - N l⁻¹ and 10 mg PO₄₃⁻ - P l⁻¹.

The experiments were all started by adding phytoplankton as an inoculum (0.6 μg chl. a l⁻¹) of mixed natural species.

<table>
<thead>
<tr>
<th>experiment 1</th>
<th>piston velocity (cm h⁻¹)</th>
<th>2.1</th>
<th>2.1</th>
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<tr>
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<td>1853</td>
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<tr>
<td>DICEND (µmol l⁻¹)</td>
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<td>58</td>
<td>65</td>
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<td>2339</td>
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<tr>
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<tr>
<td>CO₂END (µmol l⁻¹)</td>
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<td>2220</td>
<td>3362</td>
<td>3651</td>
<td>2832</td>
<td>2845</td>
<td></td>
</tr>
<tr>
<td>% of carbon from CO₂ invasion calculated from piston velocity</td>
<td>87</td>
<td>94</td>
<td>105</td>
<td>96</td>
<td>74</td>
<td>78</td>
<td></td>
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<tr>
<td>% of carbon from CO₂ invasion by difference from carbon precipitation</td>
<td>100</td>
<td>100</td>
<td>97</td>
<td>97</td>
<td>77</td>
<td>71</td>
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Table 1. Summarized data from three mesocosm experiments of variable piston velocity, ANC and P-supply. (TOCEND: concentration of TOC at the end of the experiment; DICEND: amount of DIC incorporated into biomass during the experiment; CO₂END: amount of CO₂ that have entered from the atmosphere at the end of the experiment. % carbon in accumulated TOC that has derived from either atmospheric CO₂ invasion or use of water DIC.)
assemblages with equal biomass contribution from four lakes, which represented extensive gradients in DIC (0.03–3 mmol l\(^{-1}\)), ANC (0–3 meq. l\(^{-1}\)) and nutrient richness (10–500 µg total phosphorus, (TP) l\(^{-1}\)). Phytoplankton was sampled in the lakes by towing a plankton net (mesh size 20 µm) vertically through the water column to obtain dense sample of high chlorophyll concentration. Water in each mesocosm was homogeneously mixed by a submerged, centrifugal pump attached to a small tile placed at the bottom of mesocosms. One type of submerged pump was used in experiment 1 and 2 (Mira, 50–400 h\(^{-1}\), 6 W), and another, larger pump to obtain stronger surface turbulence was used in experiment 3 (AquaClear 70, 200–1514 h\(^{-1}\), 21 W).

The oxygen transfer velocity obtained by use of the two pump types was determined beforehand in five replicate mesocosms by filling them with pure, degassed water and continuously recording re-aeration of oxygen with an oxygen-temperature logger (PME miniDOT, Vista, CA, USA) until air saturation was obtained. The gas transfer velocity of CO\(_2\) is analytically more difficult to measure, but at pH 7 (where CE is negligible) it is 0.89 times that of oxygen [26]. The transfer velocity of CO\(_2\) was calculated by accounting for pH and water temperature [14] and the quoted basic physical difference of 0.89 compared with the measured oxygen transfer velocity. The basic transfer velocity at pH 7.0 and 20°C was 2.1 and 7.6 cm h\(^{-1}\), for the small and large submerged pump, respectively. Pumps were cleaned twice daily to ensure constant mixing. Development of organic biofilm on mesocosm walls was prevented by wiping them daily and suspending the material in the water.

Oxygen, water temperature and surface irradiance, in the mesocosms, was measured continuously by photosynthetic light sensors (HOBO PAR sensor (400–700 nm) S-LIA-M003, Bourne Ma, USA) and oxygen-temperature loggers (PME miniDOT, Vista, CA, USA). Every morning, pH was measured 2 h into the photoperiod by an YSI 30, 30 M/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 [27]. Every 3 days, standard analytical methods were used to measure phytoplankton chlorophyll [28], ANC [29], period [27]. Every 3 days, standard analytical methods were used to measure phytoplankton chlorophyll [28], ANC [29], period [27]. Every 3 days, standard analytical methods were used to measure phytoplankton chlorophyll [28], ANC [29], period [27].

Lakes of less than 1 m maximum depth and lakes with a high content of coloured DOC were excluded from the analysis.

(d) Statistics

All statistical tests except multiple regressions were performed in Graph Pad Prism 7 (Graph Pad Software Inc., La Jolla, CA, USA). Illustrations present mean values and confidence limits for triplicate mesocosms. All statistics are reported as confidence intervals calculated with an alpha of 0.05, unless specified otherwise. Confidence limits were calculated using the tool pack column statistics. One-way ANOVA was used in experiment 1 to analyse for the effect of ANC. Two-way ANOVA was used in experiments 2 and 3 to evaluate the effect of ANC, nutrients and ANC*nutrients. Linear relationships of TOC versus DIC (Water–Air) and CO\(_2\) invasion were regressed against each other using the analysis tool pack for linear regression using the least-squares method. Multiple regressions were performed on the data from natural lakes in R [36] using the additional package 'lattice'. The dataset on natural lakes for differences between soft water and hard water lakes with regard to Chl \(a\) versus TP relationship. Data was subdivided into nutrient-poor (below 30 mmol l\(^{-1}\) TP) and nutrient-rich (above 100 µg l\(^{-1}\) TP) and tested using unpaired Students' t-test assuming populations with the same s.d.

3. Results

(a) Acid neutralizing capacity influence at high nutrient richness

The first experiment under very eutrophic conditions varied the initial ANC (and closely similar DIC) across a 100-fold range (0.05–5.6 meq. l\(^{-1}\), figure 1). Phytoplankton growth started earlier and attained very high biomasses (600 µg chl \(a\) l\(^{-1}\)) limited by self-shading already after 20 days at high ANC (1.4–5.6 meq. l\(^{-1}\)), whereas delayed development and lower biomasses (330 µg chl \(a\) l\(^{-1}\)) formed under lower ANC (0.05–0.7 meq. l\(^{-1}\)). After 27 days, phytoplankton biomasses still remained much higher at high ANC than low ANC (one-way ANOVA, \(p < 0.0001\), electronic supplementary material, table S1 for Tukey’s multiple comparisons test). Phytoplankton biomass had reached an upper level typical of each experiment and did not change significantly towards termination of the experiments (electronic supplementary material, table S3). Organic carbon (TOC) produced by phytoplankton and present as live phytoplankton, organic detritus particles and DOC continued to accumulate throughout the 27 day long experiment in the absence of loss processes such as animal grazing, sedimentation and washout. The TOC yield was four to five times higher at the two highest ANC levels than at the two lowest (one-way ANOVA, \(p < 0.0002\) for ANC; electronic supplementary material, table S1). Final pH levels reached after about 20 days were almost two units higher at high than low ANC (one-way ANOVA, \(p < 0.0001\); electronic supplementary material, table S1). The highest final pH of 11.71 supported a 30-fold CE of atmospheric CO\(_2\) invasion in mesocosms with an initial ANC of 2.6 meq. l\(^{-1}\), while the lowest final pH of 9.35 with a 3.7-fold CE factor was in mesocosms with an initial ANC of 0.05 meq. l\(^{-1}\). ANC declined over time at high initial ANC levels (2.8 and 5.6 meq. l\(^{-1}\)) when intense photosynthesis elevated pH, converted HCO\(_3^-\) to CO\(_2^-\) and precipitated CaCO\(_3\). During the course of the experiment, carbon supply to photosynthesis initially derived

(c) Natural lakes

We acquired data for 204 Danish lakes on maximum water depth, ANC, TP, chlorophyll \(a\) (chl \(a\)) and Secchi transparency during summer (May–September) from the Database for Danish Environmental Parameters [34]. Data were available from 1980 to 2013. Environmental conditions in these lakes were measured according to Danish standards [35] as part of the national Danish environmental monitoring programme. The data were time-weighted and averaged from 11 to 71 samples of surface waters above the thermocline for each lake.
directly from DIC in the water and later when DIC was depleted in the water solely from atmospheric CO₂ invasion as pH and chemical enhancement rose (figures 1 and 2).

TOC accumulation during the experiment followed a hyperbolic relationship to initial ANC, reaching half the maximum TOC accumulation at 0.35–0.70 and saturation at 1.4 meq. l⁻¹ (figure 2). Inorganic carbon sources to TOC production shifted systematically in magnitude and relative importance across the ANC gradient. Direct DIC use without calcification increased almost linearly between 0.35 and 1.4 meq. l⁻¹ and declined somewhat at 2.8 and 5.6 meq. l⁻¹ as calcification increased steeply and removed part of the DIC pool. The release of CO₂ by calcification was small below 1.4 meq. l⁻¹ but increased steeply and linearly above to the maximum ANC at 5.6 meq. l⁻¹ (table 1). Atmospheric CO₂ invasion rates were small in absolute magnitude at the lowest initial ANC levels, attaining the lowest final pH levels and CE factors (figure 2). CO₂ invasion peaked at 2.8 meq. l⁻¹, which had the highest final pH and CE. Intensive calcification at the highest ANC level (5.6 meq. l⁻¹) dampened pH rise and atmospheric CO₂ invasion somewhat.

In terms of percentages of inorganic carbon supply to TOC production from different sources, CO₂ invasion from the atmosphere calculated by mass balance (subtracting the DIC loss by CaCO₃ precipitation), supplied between 84% and 98% at initial ANC levels of 0.05–2.8 meq. l⁻¹ (table 1). This proportion dropped to 40% at the highest initial ANC levels, at which the direct supply from DIC supported 60% of TOC production. The measured TOC accumulation across the ANC gradient was in close agreement with the sum of inorganic carbon supply calculated from DIC reduction (corrected for CaCO₃ precipitation) and CO₂ invasion from the atmosphere (figure 2a and electronic supplementary material, figure S1). The linear relationship between measured TOC accumulation and calculated DIC supply from the water and the atmosphere was highly significant (p < 0.0001, r² = 0.95) and followed a slope close to 1.0 (95% confidence limits (CL) : 0.88–1.01). Likewise, atmospheric CO₂ invasion estimated by mass balance and calculated directly from basic piston velocity, CE and CO₂ air–water gradient showed excellent agreement (figure 1). The linear relationship was highly significant (p < 0.0001, r² = 0.97) and had a slope close to 1.0 (95% CL : 0.89–1.06).

Thus, if CE of CO₂ invasion was not accounted for, the DIC supply from the water and atmospheric CO₂ uptake (no CE) was much less (42–98%) than the observed TOC accumulation.

(b) Acid neutralizing capacity-influence at three nutrient levels

Increasing nutrient conditions, which stimulate organic productivity and resulting phytoplankton biomass development, will increase both the likelihood and the extent of inorganic carbon limitation. Thus, stimulation of TOC production by high ANC compared to low ANC is expected to be stronger under nutrient-rich than nutrient-poor conditions. This expectation was supported by the second experiment performed at low (0.08 meq. l⁻¹) and high ANC (2.5 meq. l⁻¹) and three nutrient levels spanning a 100-fold range (figure 3a). TOC production was stimulated significantly both by increasing nutrient richness and ANC levels (two-way ANOVA p < 0.001, electronic supplementary material, table S2). At low ANC, TOC production increased 3-fold from low to high nutrient richness, while the increase was 12-fold from low to high nutrient richness at high ANC in accordance with expectations. At low and medium nutrient richness, TOC production was 1.5-fold higher at high compared to low ANC. At high nutrient richness, by contrast, high ANC increased TOC production 6-fold relative to low ANC (table 1).

At low ANC, most inorganic carbon incorporated in TOC was derived from CO₂ invasion from the atmosphere, according to both mass balances and direct calculations from piston velocity, CO₂ enhancement and air–water CO₂ gradients (table 1). At low and medium nutrient richness at high ANC, photosynthesis was not sufficiently intense to elevate pH markedly. Thus, small amounts of CO₂ were in fact lost...
from water to air and all DIC incorporated in TOC derived from the water. At high nutrient richness and high ANC, phytoplankton production was profound and atmospheric CO2 supported 61 and 79% of TOC accumulation according to estimates from piston velocity and carbon mass balances, respectively.

(c) Influence of acid neutralizing capacity and surface turbulence

High organic productivity under nutrient-rich conditions requires high inorganic carbon supply rates that can be provided by high DIC concentrations, high atmospheric CO2 invasion, or both. High atmospheric CO2 invasion at moderate surface turbulence in the first and second experiments (basic piston velocity 2.1 cm h\(^{-1}\)) can only be obtained at high pH and high CE. This situation was achieved at intermediate and high ANC levels, but not at low. In the second experiment under nutrient-rich conditions, for example, pH reached a maximum of 11.2 (CE factor 15.8 at 15°C) at the high ANC level (2.5 meq. l\(^{-1}\)). At the low ANC level (0.08 meq. l\(^{-1}\)) under nutrient-rich conditions, pH only reached 9.2 and the CE factor was 2.1 or lower.

With atmospheric CO2 being the predominant carbon source to organic productivity under nutrient-rich conditions at low initial ANC, stronger water turbulence and higher piston velocity can elevate atmospheric CO2 supply. Higher piston velocity is less critical for inorganic carbon supply rates at high initial ANC, where the scope for DIC use from the water and CE of atmospheric CO2 invasion at high pH is much larger. The third experiment supported these predictions (two-way ANOVA, \(p < 0.001\) for ANC, nutrients and ANC*nutrients; electronic supplementary material, table S2; figure 3b). At low initial ANC (0.05 meq. l\(^{-1}\)) and high nutrient richness, organic productivity was 3.1-fold higher at high than low piston velocity (table 1) because the high CE factor of 10.9 at pH 10.75 ensured fast CO2 invasion even at the lower piston velocity. High piston

![Figure 2.](image2.png)  
Figure 2. Experiment 1. TOC accumulation after 27 days in hypertrophic phytoplankton mesocosms across a gradient of ANC (a) based on direct measurements (filled circles) and calculated as the sum of TOC derived from DIC decline (corrected for loss of CaCO3) and CO2 invasion from the atmosphere (open circles). TOC accumulation derived from DIC loss in % (b) and CO2 invasion from the atmosphere in % (c) are also shown separately. Values are for all three replicate mesocosms at six ANC levels.

![Figure 3.](image3.png)  
Figure 3. TOC accumulation in mesocosms after 30 days shown from experiment 2 (a) and experiment 3 (b). Experiment 2 (a) had an intermediate piston velocity of 2.1 cm h\(^{-1}\), while experiment 3 (b) was performed at another time with a higher piston velocity of 7.6 cm h\(^{-1}\). TOC accumulation measured directly are shown with filled circles and TOC determined as the sum of TOC derived from DIC decline (corrected for CaCO3 precipitation; grey-shaded area) and CO2 invasion from the atmosphere (white-shaded area) are shown with the columns. Mean values and s.d. (bars) are shown for triplicate mesocosms at each ANC level.
organic carbon measured as accumulation rate of organic and nutrients. The study revealed that net productivity of quantification of sources of inorganic carbon to organic productivity under oligo- and mesotrophic conditions. Higher organic productivity in hard waters was supported by larger DIC pools and the development of high pH leading to profound CE of CO2 invasion from the atmosphere. As a consequence, phytoplankton biomass reached higher maximum levels in nutrient-rich waters of intermediate to high levels of ANC and DIC. These results of mesocosm experiments were supported by field data from 204 meso- and eutrophic Danish lakes sampled frequently during 1990–2016 [34]. Mean phytoplankton biomasses during summer were significantly higher (ca 5-fold) in hard water lakes (greater than 0.8 meq. l\(^{-1}\)) than soft water lakes (less than 0.8 meq. l\(^{-1}\)) for the same high concentration of total P (figure 4). Soft water lakes had mean summer pH-values of 4.5–7.7 and hard water lakes reached 7.3–9.3 conducive to elevated atmospheric CO2 invasion. Multiple regression analysis confirmed that both TP and ANC contributed significantly to the increase of phytoplankton biomass among lakes.

Figure 4. Mean phytoplankton biomass (as chlorophyll \(a\)) in soft water (ANC < 0.8 meq. l\(^{-1}\)) (open circles) and hard water (ANC > 0.8 meq. l\(^{-1}\)) (closed circles) Danish lakes as a function of mean total phosphorus concentration during summer (May–October). Lines indicate type II linear regression and the light grey areas are 95% confidence bands. ANC < 0.8 meq. l\(^{-1}\), \(\log Y = 0.65*\log(X) – 0.09\), \(R^2 = 0.86\ n = 91\), ANC > 0.8 meq. l\(^{-1}\), \(\log Y = 0.96*\log(X) – 0.14\), \(R^2 = 0.86\ n = 113\).

velocity at high surface turbulence is particularly effective in supplying atmospheric CO2 to organic productivity at low ANC. Consequently, organic productivity was only 1.5-fold higher at high than low ANC at high piston velocity in experiment 3.

(d) Field phytoplankton biomass

Field phytoplankton biomass (chlorophyll \(a\)), using data from 204 Danish lakes during summer, was best explained by TP followed by ANC (figure 4). Both in soft water (less than 0.8 meq. l\(^{-1}\)) and in hard water lakes (greater than 0.8 meq. l\(^{-1}\)), phytoplankton biomass increased highly significantly with increasing TP. However, the relationship between log Chl \(a\) and log TP had a significantly steeper slope at high than low ANC. Thus, there was no significant difference in phytoplankton biomass between nutrient-poor (less than 30 \(\mu\)g TP l\(^{-1}\)) soft water and hard water lakes. Whereas in nutrient-rich (greater than 100 \(\mu\)g TP l\(^{-1}\)) more productive lakes, where inorganic carbon demands are higher, hard water lakes had significantly higher phytoplankton biomasses than soft water lakes (\(t\)-test, \(p < 0.0005\), \(n = 52\)). At 500 \(\mu\)g TP l\(^{-1}\), for example, phytoplankton biomass was fivefold higher in hard water than soft water lakes according to the regression equations.

Multiple regressions on the data yielded the significant relationship (\(p < 0.01\), \(R = 204\))

\[
\log (\text{Chl} \ a) = 0.72 \times \log(\text{TP}) – 0.10 \times \text{ANC} + 0.12 \times \log(\text{TP}) \times \text{ANC} – 0.49,
\]

showing that the stimulation of chlorophyll was strong for TP alone and ANC and TP in combination.

4. Discussion

This study adds new perspectives to the understanding and quantification of sources of inorganic carbon to organic productivity in freshwater lakes across gradients in ANC, DIC and nutrients. The study revealed that net productivity of organic carbon measured as accumulation rate of organic carbon (TOC) under high nutrient richness can be strongly limited by inorganic carbon supply rates in soft waters compared to hard waters and that limitation in part persisted under oligo- and mesotrophic conditions. Higher organic productivity in hard waters was supported by larger DIC pools and the development of high pH leading to profound CE of CO2 invasion from the atmosphere. As a consequence, phytoplankton biomass reached higher maximum levels in nutrient-rich waters of intermediate to high levels of ANC and DIC. These results of mesocosm experiments were supported by field data from 204 meso- and eutrophic Danish lakes sampled frequently during 1990–2016 [34]. Mean phytoplankton biomasses during summer were significantly higher (ca 5-fold) in hard water lakes (greater than 0.8 meq. l\(^{-1}\)) than soft water lakes (less than 0.8 meq. l\(^{-1}\)) for the same high concentration of total P (figure 4). Soft water lakes had mean summer pH-values of 4.5–7.7 and hard water lakes reached 7.3–9.3 conducive to elevated atmospheric CO2 invasion. Multiple regression analysis confirmed that both TP and ANC contributed significantly to the increase of phytoplankton biomass among lakes.

Broad-scale comparison of the carbon kinetics of phytoplankton photosynthesis has revealed that HCO3\(^-\) concentrations below 0.2 mmol l\(^{-1}\) can reduce carbon fixation directly by the limitation of photosynthetic rates. Indirectly, photosynthesis can be constrained owing to trade-offs between investment in CCMs and Rubisco activity and specificity, on the one hand, and limitations of uptake of N, P and micronutrients, on the other [9,37–39]. Reported 25–75% percentiles of half-saturation constants for CO2 fixation between 1 and 9 \(\mu\)mol l\(^{-1}\) [40] of 24 freshwater species and four natural assemblages from soft water lakes also stress that phytoplankton communities are frequently carbon limited because ambient CO2 levels during algal blooms often drop below 1, or even below 0.1 \(\mu\)mol l\(^{-1}\) [41].

The traditional view on limitation of organic productivity of phytoplankton in freshwaters should, therefore, be modified by acknowledging that inorganic carbon, in addition to the predominant limitation by nutrients and light, constrains organic productivity; particularly under high light and nutrient-rich conditions when invasion of atmospheric CO2 cannot cover high carbon demands because of low physical exchange rate with the atmosphere and low CE typical of low-pH soft waters. While productivity of lake phytoplankton and resulting maximum biomass hitherto have been related to availability of P and N in numerous publications and lake management programmes [1,2], few studies have included the obvious additional effects of light availability determined by background light attenuation and depth of mixing in the water column [4,42], and no previous studies have treated the influence of DIC availability revealed here. We confirmed that phytoplankton biomasses in natural lakes during summer were markedly higher in hard waters than soft waters experiencing the same nutrient loading and this stimulation increased along a gradient of lake trophic status.

Measurements of atmospheric CO2 invasion permitted us to evaluate the upper limits of sustained net organic productivity under nutrient-rich conditions. Daily mean CO2 uptake rates 21–27 days into the first mesocosm experiment were eight times higher (ca 165–210 mmol m\(^{-2}\) d\(^{-1}\)) in hard waters than soft waters (ca 25 mmol m\(^{-2}\) d\(^{-1}\)) at moderate gas exchange velocity, while high gas exchange velocity
doubled daily CO2 invasion rates in soft waters (ca. 50 mmol m\(^{-2}\) d\(^{-1}\)). In numerous temperate lakes during summer stratification, direct DIC supply to photosynthetic assimilation in surface waters is also constrained because input from the catchment is limited by long water retention times, while the release of DIC from degradation of organic matter in the sediment is trapped in bottom waters until autumn mixing [2]. Therefore, surface waters in hard water lakes experience the same carbon dynamics during four to six summer months of stratification as during one month in our mesocosm experiments. Lakes experience first, DIC rapidly declining and pH rising, as well as CaCO\(_3\) being precipitated and lost to deeper waters, and, finally, continued net production for several months being sustained by the invasion of atmospheric CO2 [43].

Applying the upper thresholds of CO2 invasion listed above, daily ecosystem net production could yield only about 50 mmol C m\(^{-2}\) d\(^{-1}\) in temperate soft waters and 210 mmol C m\(^{-2}\) d\(^{-1}\) in hard waters. These rates are only half the daily mean net production rates in temperate forests and croplands during summer (about 400 mmol C m\(^{-2}\) d\(^{-1}\)) [44], where the plants draw CO2 directly from the atmosphere and gas transfer velocities are not restricted by passage through an air-lake water barrier. Lake ecosystems attaining or even exceeding the mentioned upper threshold for short periods should be searched for in waters of particularly high gas transfer velocities because of strong wind and convective mixing and high CE at high temperature and high pH [22,45]. Shallow tropical soda lakes are examples of systems of particularly high phytoplankton productivities because they are well mixed, have high temperatures and pH levels regularly reach above 10–11 [21]. Temperature candidates of high productivity are shallow ponds undergoing daily stratification and warming of the upper 20–30 cm of surface waters to 25–30°C and pH rise to 9.5–10 [46]. In order to maximize the productivity and yield of phytoplankton in ponds and algal cultures for commercial use, experiences have shown that it is necessary to bypass the water barrier and increase CO2 invasion by air bubbling [47].

In conclusion, we confirm that, lake phytoplankton productivity and resulting biomass development are subject to complex regulation by inorganic carbon availability from water and atmospheric sources in addition to nutrients, light, temperature and mixing patterns. Instead of the prevailing static perception of a certain phytoplankton upper biomass size, our results support a more complex and time dynamic view on biomass development as the net result of highly dynamic gains (determined by availability of light, nutrients, inorganic carbon and temperature) as opposed to losses (encompassing senescence, grazing, sedimentation and washout). In regard to inorganic carbon supply, lake primary production is susceptible to changes in climate and land use increasing inorganic carbon inflow [48] and elevating temperature and atmospheric CO2 and, thereby, atmospheric CO2 invasion.

Data accessibility. Data are reported directly in the paper and available in the electronic supplementary material. Statistical tools and software are described in detail in the paper and can be accessed accordingly.

Authors’ contributions. Both authors contributed to the study’s conception, data acquisition, to drafting of the manuscript and approved the final submitted manuscript. Data analysis was performed by T.K.

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