

Patterns of CO₂ concentration and inorganic carbon limitation of phytoplankton biomass in agriculturally eutrophic lakes

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ABSTRACT

Lake eutrophication is a pervasive problem globally, particularly serious in agricultural and densely populated areas. Whenever nutrients nitrogen and phosphorus do not limit phytoplankton growth directly, high growth rates will rapidly lead to biomass increases causing self-shading and light-limitation, and eventually CO₂ depletion. The paradigm of phytoplankton limitation by nutrients and light is so pervasively established, that the lack of nutrient limitation is ordinarily interpreted as sufficient evidence for the condition of light limitation, without considering the possibility of limitation by inorganic carbon. Here, we firstly evaluated how frequently CO₂ undersaturation occurs in a set of eutrophic lakes in the Pampa plains. Our results confirm that conditions of CO₂ undersaturation develop much more frequently (yearly 34%, summer 44%) in these agriculturally impacted lakes than in deep, temperate lakes in forested watersheds. Secondly, we used Generalized Additive Models to fit trends in CO₂ concentration considering three drivers: total incident irradiance, chlorophyll *a* concentration, and lake depth; in eight multi-year datasets from eutrophic lakes from Europe, North and South America, Asia and New Zealand. CO₂ depletion was more often observed at high irradiance levels, and shallow water. CO₂ depletion also occurred at high chlorophyll concentration. Finally, we identified occurrences of light- and carbon-limitation at the whole-lake scale. The different responses of chlorophyll *a* and

CO₂ allowed us to develop criteria for detecting conditions of CO₂ limitation. For the first time, we provided whole-lake evidence of carbon limitation of phytoplankton biomass. CO₂ increases and eutrophication represent two major and converging environmental problems that have additive and contrasting effects, promoting phytoplankton, and also leading to carbon depletion. Their interactions deserve further exploration and imaginative approaches to deal with their effects.

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1. Introduction

The disruption of the biogeochemical cycles of nitrogen (N) and phosphorus (P) has increased nutrient loadings to lakes, and ex-

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acerbated eutrophication (Anderson et al., 2014). Lake eutrophication is a pervasive problem globally (Ho et al., 2019), and it is particularly serious in agricultural and densely populated areas (Anderson et al., 2014; Balmer and Downing, 2011; Carpenter et al., 1998; Pacheco et al., 2013). Eutrophication was initially related to point source pollution, mainly phosphorus (Dillon and Rigler, 1974; Schindler, 1974; Schindler et al., 1973), but diffuse N and P pollutions are increasingly contributing to continued eutrophication (Beusen et al., 2016; Boardman et al., 2019). Lewis (2011) estimated that enrichment caused by anthropogenic mobilization of nutrients has increased gross primary production of lakes by about 74% globally.

In our contemporary, fast-changing world, identifying the nature of factors limiting phytoplankton growth in a water body is of considerable importance to our understanding of the ecology of aquatic systems and to water management practices (Beardall et al., 2001). Under conditions where nutrients (i.e., nitrate, ammonium, phosphate) do not limit phytoplankton growth directly, high growth rates will rapidly lead to biomass levels causing self-shading and light-limitation, and eventually CO₂ depletion (Flynn and Raven, 2016).

CO₂ can be a rate-limiting resource in eutrophic lakes, at times when photosynthetic demand exceeds diffusive influx from the atmosphere (Bindloss, 1974; King, 1970; Lehman et al., 1975; Schindler and Fee, 1973; Smith, 1938). However, since the carbon-phosphorus controversy in the early 1970's, a predominant perception has prevailed that phytoplankton primary productivity and, particularly biomass development, in lakes is determined mainly by P and N availability and secondarily by light, whereas dissolved inorganic carbon (DIC = [CO₂] + [HCO₃⁻] + [CO₃²⁻]) is assumed to play only a minor role as a limiting factor (Kalf, 2002; Schindler et al., 1973). Such perception partly rests on global assessments of CO₂ concentrations in lakes, indicating the prevalence of supersaturation (Cole et al., 1994; Sobek et al., 2005). Those estimates however, derive mostly from oligotrophic-mesotrophic systems. In contrast, Balmer and Downing (2011) provided evidence indicating that agriculturally impacted eutrophic and hypertrophic lakes depart from previous estimates and are predominantly undersaturated in inorganic carbon.

The notion that DIC may be an important limiting factor is also supported by the almost universal occurrence of a suite of costly carbon concentrating mechanisms (CCMs) among aquatic cyanobacteria and algae (Hammer et al., 2019, and references therein). In recent years, a growing number of studies have renewed the interest in DIC as a limiting factor of phytoplankton growth and/or biomass (Low-Décarie et al., 2014). Most evidence for the limiting role of DIC has been produced through short-term primary production incubations and/or experimental studies at laboratory, mesocosm, or pond scales, in which the concentration of CO₂ has been artificially manipulated (Burris et al., 1981; Hamdan et al., 2018; Hammer et al., 2019; Hernández-Hernández et al., 2018; Kragh and Sand-Jensen, 2018; Maberly and Gontero, 2017; Riebesell et al., 1993; Shi et al., 2017; Verspagen et al., 2014). On the other hand, obtaining lake-scale, non-manipulative evidence for DIC-limitation of phytoplankton biomass has proved to be much more elusive. A major impediment to this is the lack of an operative set of criteria that allows one to distinguish the symptoms of DIC limitation from those that characterize the condition of light limitation. The concentration of CO₂ in lakes is controlled by complex physical, chemical, and biological mechanisms. In a very general way, it represents the balance between biological CO₂ consumption (photosynthesis) and production (respiration, photorespiration); the fluxes from and to the atmosphere, the sediments and the watershed; and pH-controlled changes in carbonate equilibria (Perga et al., 2016). The current partial pressure of CO₂ at equilibrium with

the atmosphere (pCO₂ ~400 μatm, corresponding to a concentration ~ 13–15 μmol/l) serves as a reference to assess the net flux of CO₂ to and from the atmosphere (i.e., evasion vs. invasion) (Balmer and Downing, 2011; Van Dam et al., 2018a). CO₂ undersaturation is linked to the dominance of autotrophic activities over heterotrophic processes, whereas supersaturation is linked to the opposite (Balmer and Downing, 2011). But CO₂ concentration alone is insufficient to infer inorganic carbon limitation of phytoplankton biomass. The kinetic constant for CO₂ uptake of phytoplankton may vary over 3 orders of magnitude (K_{1/2}: 0.1–170 μmol/l CO₂) (Hein, 1997). Moreover, many algae possess CCMs that allow them to utilize bicarbonate (Raven et al., 2011) in addition to CO₂; although, CO₂-limitation of photosynthesis may occur even in the presence of high concentrations of HCO₃⁻ (Burris et al., 1981). CO₂ concentration can have stimulating or limiting effects on bulk phytoplankton or on certain algal groups. For example, CO₂ concentrations well above the equilibrium with the atmosphere have been shown to stimulate cyanobacteria blooms (Visser et al., 2016) and whole-ecosystem gross primary production (Hamdan et al., 2018). Yet, cyanobacteria abundance has been also shown to be negatively correlated to pCO₂, indicating that C limitation favors cyanobacteria blooms and their dominance relative to other taxa (Van Dam et al., 2018b).

1.1. Criteria for assessing light vs. DIC limitation

An alternative approach for distinguishing conditions of light-limitation from those of DIC-limitation consists of examining trends in phytoplankton biomass and CO₂ concentration along a range of environmental drivers, and contrasting the observed trends against the predictions arising from assuming conditions of light- vs CO₂-limitation. For this purpose, incident solar irradiance (I₀) is particularly well suited: i) it is a truly independent, external variable (i.e., unaffected by in-lake processes); ii) all lakes are exposed to varying levels of I₀, and approximately the same values recur at predictable times year after year; and most important iii) the predicted responses of chlorophyll *a* (Chl*a*) and CO₂ to changes in I₀ differ (i.e., they are actually opposite), depending on the nature of limitation. The behavior of phytoplankton biomass under light-limitation has been extensively studied (Huisman et al., 2002; Laws and Bannister, 1980; Lehman et al., 1975; Scheffer, 2004) and predictions are straightforward: whenever the amount of incident light limits phytoplankton biomass, the latter should increase with increasing incident solar radiation, as has been demonstrated in laboratory experiments (Huisman, 1999; Laws and Bannister, 1980), mesocosm-scale (Llames et al., 2009), and whole lake studies (Janatani et al., 2020; Torremorell et al., 2007). CO₂ shortage on the other hand, has two major effects on algae growth rates and the potential for algae to build biomass. First, low CO₂ levels by themselves may become limiting to photosynthetic rates and phytoplankton biomass, as described above. Second, the consumption of CO₂ increases pH (basification) and could promote additional negative effects on the algae (Flynn and Raven, 2016). Therefore, increasing incident solar radiation would initially increase photosynthesis and CO₂ demand. The expected consequences are decreased CO₂ concentration and increased pH. But should CO₂ become limiting of phytoplankton, further increases of biomass with incident irradiance should not be expected, and in actuality, biomass may decrease due to impoverishment of water quality.

1.2. Objectives

The potential release of nutrient limitation of lake primary production, driven by the agriculture intensification, may have in-

creased the demand for CO₂ required for phytoplankton photosynthesis. In this paper, we first evaluate how frequently CO₂ undersaturation occurs in a set of eutrophic-hypertrophic lakes, in the Pampa plains, one of the most productive regions of the world. Secondly, we use multi-year monitoring datasets from individual eutrophic, nutrient-rich lakes in Europe, North and South America, Asia and New Zealand to analyze trends in CO₂ along three main drivers: i) incident irradiance, ii) lake depth, and iii) phytoplankton biomass (as estimated by Chla concentration). CO₂ is expected to be negatively related to incident irradiance (I_0) and Chla (i.e., increased photosynthetic demand should decrease inorganic carbon). Lake depth can be expected to have little effect on the photosynthetic demand of inorganic carbon when expressed on an area basis, but it would have a considerable positive effect on the CO₂ storage capacity of the water column. Thus, depending on daily net flow direction (to or from the atmosphere), CO₂ may decrease or increase with lake depth. Finally, our last objective was to identify situations of light-limitation and carbon-limitation at the whole-lake scale. For this purpose, we used the set of criteria outlined above to interpret the trends of Chla and CO₂ along the natural range of incident irradiance for individual eutrophic lakes.

2. Materials and methods

2.1. Study sites

For assessing the frequency of CO₂ undersaturation in agriculturally eutrophic lakes we analyzed a collection of 506 samples from 56 shallow lakes from the Pampa region of Argentina, one of the largest and most productive flatlands in the world. The lakes have experienced increasing levels of eutrophication due to the long-term agricultural practices in the area. Thirty-nine lakes are located in Buenos Aires province, the remaining 17 in La Pampa province. The collected data set includes 17 lakes that were sampled on a monthly basis for at least a year. The remaining lakes were sampled seasonally (January, April, July, and October), sporadically, or only once.

For analyzing trends in CO₂ along main drivers and identifying situations of light-limitation and carbon-limitation at the whole-lake scale we analyzed multi-year datasets from eight eutrophic lakes around the world: Acton (USA), Chascomús (Argentina), Kasumigaura (Japan), Peipsi (Estonia-Russia), Võrtsjärv (Estonia), and Waikare, Whangape and Waahi (New Zealand). These eight lakes have been continuously monitored for a long time (more than 15 years) and data is available for water transparency (Secchi disk), pH, total alkalinity, temperature, total phosphorus, total nitrogen, and chlorophyll *a* concentration. The frequency of samples displaying CO₂ undersaturation was also assessed for this set of lakes.

For detailed description of the lakes and measured variables please refer to the electronic supplementary material.

2.2. Carbonate system calculations and incident irradiance

Determination of $p\text{CO}_2$ and CO₂ were calculated using CO2SYS (CO2SYS_v2.1, running in Excel 2010). CO2SYS requires any two CO₂ system parameters (total alkalinity, total inorganic CO₂, and pH), water temperature and atmospheric pressure (Lewis and Wallace, 2006; Pierrot et al., 2006). The CO2SYS macro was run under the recommended Freshwater option (i.e., salinity=0). This implies that CO₂ concentrations may be slightly overestimated. Differences however become smaller at high pH, being almost negligible at pH > 8.5. Moreover, salinity changes within-lakes remained within narrow ranges, and can therefore be assumed to have little effect on the reported trends. All lakes considered in this paper occur at low elevation (see full description and summary methods in supplementary material). For each sample, an atmospheric

pressure of 10 dbar was assumed. The program was run using freshwater dissociation constants and NBS scale pH (Millero, 1979). Mean annual CO₂ atmospheric composition at Mauna Loa was obtained from <https://www.esrl.noaa.gov/gmd/ccgg/trends/data.html>. CO₂ undersaturation refers to conditions in which the partial pressure ($p\text{CO}_2$) in water was lower than the expected equilibrium value with atmospheric CO₂ for that year.

Daily incident total solar irradiance for each lake was downloaded from <https://power.larc.nasa.gov/data-access-viewer/> (Parameter: All Sky Insolation Incident on a Horizontal Surface). Daily total irradiance values were averaged for the seven-day period, previous to the sampling date for each lake, and are expressed in MJ/m². We used total solar radiation as a proxy for photosynthetically available radiation (PAR). Both variables are known to be highly correlated, with PAR representing 42–50% of total solar radiation (Baker and Frouin, 1987).

2.3. Statistical analyses

Multi-year trends of CO₂ along i) lake depth, ii) irradiance, and iii) Chla concentration were assessed using generalized additive models (GAMs) (Wood, 2017; Wood et al., 2016). GAMs were preferred over traditional time-series analysis because they better account for nonlinearity, and they do not rely on subjective model specification. Using GAMs allows the functional form of the trend to be determined from the data and the use of splines avoids bias issues at the ends of data series that affect polynomial models (Finlay et al., 2019; Simpson, 2018; Wood, 2017). Chla concentration trends along i) lake depth, and ii) irradiance as predictors, were similarly modeled using GAMs.

In all cases, fitted GAMs were estimated using maximum likelihood-based smoothness selection procedures, in particular the restricted maximum likelihood (REML). The adequacy of the initial basis dimension was checked, and if it was not sufficient, then a larger initial basis dimension was used, and the model refitted according to Wood et al. (2016). The selection of best fitted models was performed considering maximized deviance explained, adjusted R^2 , qualitative evaluation of the model fit and diagnostic plots, and the Akaike information criterion (AIC). In most cases, the scaled *t* distribution for heavy-tailed data was used as it provided the best fit and diagnostics. Uncertainty in the models were estimated under an empirical Bayesian formulation of the GAMs (Wood, 2017). Thus, the simultaneous interval for the fitted trends were estimated by generating 10,000 simulations of the trend from the posterior distribution of the fitted GAM. Each of these simulated trends is consistent with the estimated trend, but also includes the effect of the uncertainty of the smoother (spline coefficients; Wood, 2017). GAMs were estimated using the *mgcv* package (version 3.5.3, Wood 2017), uncertainty in the adjusted trends was estimated using the *gratia* package (version 0.2-8, available at <https://cran.r-project.org/package=gratia>), and graphics were plotted using the *ggplot2* package (version 1.8-24) for R (version 3.5.1, R core team, 2017).

One of our major objectives was understanding how the dependent variables (CO₂ and Chla) respond to incident solar radiation (I_0), which changes predictably along the year. From winter solstice to summer solstice (winter-spring), the gradual increase in I_0 translates into progressively higher autotrophic CO₂ demand. Conversely, from summer solstice to winter solstice (summer-fall), the relative importance of heterotrophic processes can be expected to increase, mirroring the decreasing trend in I_0 . For each lake, GAMs were first fitted to the full set of data, but considering that the patterns during winter-spring and summer-fall may differ, we also performed separate analyses for each of the above seasonal periods. In addition, three lakes (Acton, Peipsi and Võrtsjärv) often experience periods of ice-cover, which can result in the build-up of

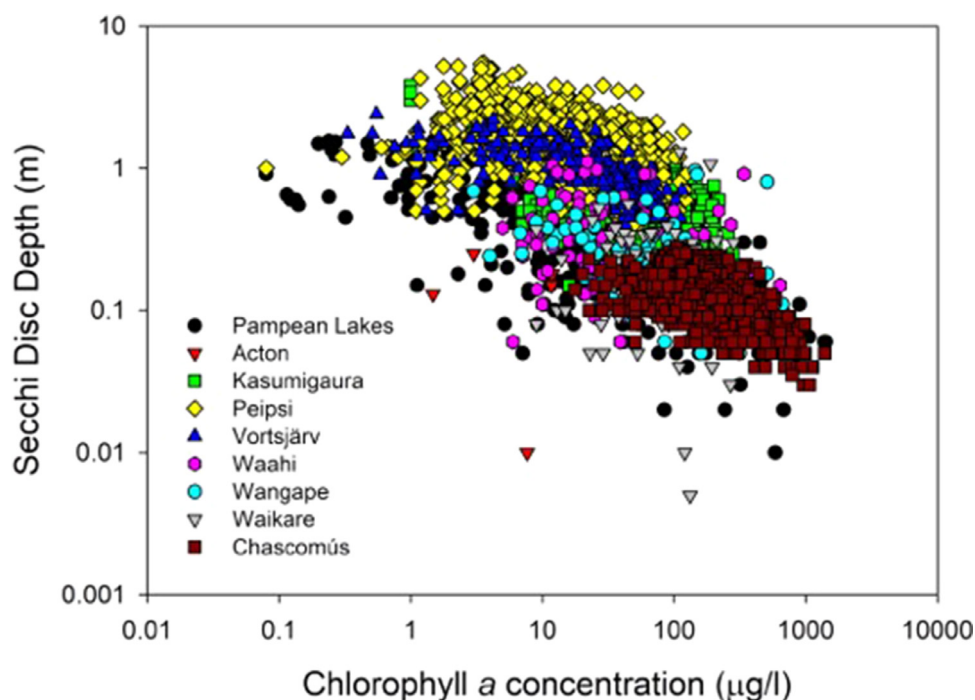


Fig. 1. Range of Secchi disc depth, as indicator of water transparency, and chlorophyll *a* concentration exhibited by the studied lakes.

CO₂ during winter and rapid degassing when the ice melts. For comparative purposes with the remaining lakes, and also because Acton and Peipsi were not sampled in winter, data during the ice cover period was excluded from the analyses. To do so, we excluded dates when water temperature was less than 2° C. This procedure captures the period before ice formation and after the ice-off date and when most CO₂ degassing has occurred. Thus, similar to previous studies (Anderson et al., 1999), the set of the samples analyzed here includes only the ice-free dates (i.e., non-restricted gas exchange). This applies only to the winter-spring period, as all lakes remained unfrozen during summer-fall.

3. Results

3.1. Frequency of CO₂ undersaturation in agriculturally eutrophic lakes

The ranges of P and N of Pampean lakes span over three orders of magnitude (Table 1). Most lakes are highly turbid (median Secchi disc depth: 0.19 m, Table 1). The proportion of samples undersaturated with CO₂ (i.e., pCO₂ in water < atmospheric pCO₂) was 34.4%. If only the samples collected in summer are considered, the proportion of samples from Pampean lakes undersaturated with CO₂ was 44%. For the multi-year dataset of eight lakes around the world, the proportion of samples displaying undersaturation with CO₂ ranged from 21% (Vörtsjärvi) to 62% (Kasumigaura) (Table 1). These lakes also span wide ranges of nutrient concentrations (Table 1), Chla concentration and water transparency (Fig. 1).

3.2. CO₂ patterns in agriculturally eutrophic lakes – analyses of multi-year monitoring datasets

Multi-year trends of CO₂ along i) lake depth, ii) incident irradiance, and iii) Chla concentration were assessed using generalized additive models (GAMs). For a full description see section S.1 in supplementary material; model selection criteria in Table S1 (suppl. mat.); and best-fit models in Fig. S1_CO₂ patterns

(suppl. mat.). For all eight lakes, the best-fit models of [CO₂] corresponded to the individual analyses by season (i.e., winter-spring and summer-fall). For Kasumigaura, Peipsi and Waahi (both seasonal periods), and Chascomús (summer-fall) the best-fit model (based on the percent of deviance explained and AIC) were the ones that included all three predictor variables (i.e. depth, total incident irradiance, and Chla). The best models for Chascomús, Waikare and Whangape (winter-spring), and Vörtsjärvi (spring) included depth and incident irradiance. Finally, the best model for Acton, Vörtsjärvi, and Waikare (summer-fall) included only incident irradiance, and that of Whangape (summer-fall) included depth and Chla. See Table 2 for a summary of significant trends.

Our results revealed different responses of CO₂ concentration to changes in lake depth, irradiance or Chla. The partial effect of lake depth on CO₂ concentration was significant for most lakes in both seasonal periods (except Waikare, during summer-fall; notice that CO₂ concentration in lake Acton was not modeled as a function of depth). In most cases CO₂ concentration increased with lake depth (Fig. 2, Table 2). But some lakes (Peipsi, both seasons; and Waikare and Kasumigaura, winter-spring) showed either decreasing, open upward parabola, or zigzagging trends (Fig. 2, Table 2).

The concentration of CO₂ for the seasonal models was significantly related to incident light, displaying, in most cases, decreasing trends. The exceptions were Whangape (summer-fall: non-significant) and Chascomús and Kasumigaura, both during summer-fall, which displayed a W-shaped trend (Fig. 2, Table 2).

The partial effect of Chla was often significant, during either both seasonal periods (winter-spring and summer-fall) or just one of them (Table 2). Whenever significant, Chla had mostly a negative effect on the concentration of CO₂ (Fig. 2), i.e., CO₂ decreased with Chla. The exceptions are Chascomús, which displayed an increasing trend in summer-fall (Fig. 2), and Peipsi (spring) and Whangape (summer-fall) that displayed zigzagging patterns (Table 2, and suppl. mat. Fig. S1_CO₂ patterns).

The patterns of pCO₂ were virtually identical to those described for the concentration of CO₂ (data not shown).

Table 1

Descriptive statistics (median, maximum and minimum) of limnological parameters estimated from the multi-year monitoring data sets for the studied lakes. The time period considered in each case is shown between brackets. For a comparison, the same descriptive statistics estimated in [Balmer and Downing \(2011\)](#) are presented. TA: total alkalinity, DIC: dissolved inorganic carbon, [CO₂]: concentration of CO₂, pCO₂: partial pressure of CO₂, N: sample size, n/a: not available.

		Secchi (m)	pH	Chlorophyll a (µg/l)	Total Nitrogen (µg/l)	Total Phosphorus (µg/l)	TA(meq/ l)	DIC(µmol C/l)	[CO ₂](µmol/ l)	pCO ₂ (µatm)	** CO ₂ under- saturation (%)	N
Acton (2001-2016)	Median	0.6	8.59	57.1	3812.3	90.6	n/a	2500.2	16.7	406.2	46.3	525 / *67
	Min	0.01	7.12	1.4	437.3	41.3	n/a	1812.2	2.2	67.2		
	Max	1.27	9.3	204.1	11857.4	527.7	n/a	3578.0	440.30	10203.7		
Chascomús (2001-2018)	Median	0.12	8.82	194.7	3582.7	516.6	6491.2	n/a	25.3	579.9	27.9	1231
	Min	0.03	7.71	17.7	2.9	25.0	2688.0	n/a	3.8	81.2		
	Max	0.28	9.56	1399.1	19213.0	1775.6	16219.0	n/a	332.4	8028.7		
Vörtsjärvi (1997-2017)	Median	0.80	8.28	35.2	1200.0	41.0	3300.0	n/a	54.4	968.1	21.0	302
	Min	0.35	7.00	0.3	300.0	13.0	150.8	n/a	7.4	179.7		
	Max	2.40	8.97	116.1	3700.0	120.0	5600.0	n/a	508.9	8264.0		
Peipsi (1997-2017)	Median	1.50	8.40	19.9	n/a	42.0	2600.0	n/a	24.3	545.9	27.4	1166
	Min	0.40	7.20	0.1	n/a	11.0	1650.0	n/a	2.6	67.4		
	Max	5.50	9.20	123.8	n/a	220.0	4000.0	n/a	491.1	8030.2		
Kasumigaura (1981-2017)	Median	0.65	8.38	53.0	1177.0	99.0	n/a	1041.7	11.1	249.6	61.8	1740
	Min	0.10	6.26	60.3	13.0	1.0	n/a	200.0	0.01	0.2		
	Max	3.80	10.86	1451.0	406.0	280.0	n/a	1533.3	467.5	12588.0		
Lake Waahi (1995-2019)	Median	0.37	8.10	25.0	n/a	55.0	n/a	n/a	31.8	719.5	23.9	165
	Min	0.05	7.20	4.0	n/a	16.0	n/a	n/a	1.5	38.7		
	Max	1.33	9.30	640.0	n/a	300.0	n/a	n/a	257.6	5226.0		
Lake Waikare (1996-2019)	Median	0.16	8.40	82.0	n/a	180.0	n/a	n/a	7.7	141.8	61.2	145
	Min	0.01	6.90	9.0	n/a	26.0	n/a	n/a	0.1	2.0		
	Max	1.30	10.00	320.0	n/a	1200.0	n/a	n/a	249.4	5033.2		
Lake Whangape (2002-2019)	Median	0.22	7.90	680.0	n/a	125.0	n/a	n/a	20.4	486.2	42.6	126
	Min	0.04	6.90	3.0	n/a	26.0	n/a	n/a	0.1	2.0		
	Max	0.96	10.00	850.00	n/a	340.00	n/a	n/a	275.83	4441.15		
Pampean lakes (2002-2017) (56 lakes sampled 1 to 59 times)	Median	0.18	8.89	116.1	1071.4	309.0	8207.7	n/a	31.7	679.7	34.4	506 / *471
	Min	0.03	6.85	0.5	69.9	6.1	545.9	n/a	0.1	2.0		
	Max	1.20	10.77	1419.9	12329.6	6729.5	59010.0	n/a	2743.3	83625.7		
Balmer and Downing (2011)	Median	0.9	8.6	25	1500	73	4500	n/a	n/a	322	60	3049
	Min	<0.1	6.4	<1	0	4.6	167	n/a	n/a	0.1		
	Max	9.5	10.6	544	20600	777	10700	n/a	n/a	40392		

* N for pH, DIC, [CO₂] and pCO₂ for Acton lake and Pampean lakes

** Percentage of samples displaying CO₂ undersaturation [CO₂], pCO₂ and % CO₂ invasion for New Zealand lakes were estimated assuming constant alkalinity values of 1480 (Waahi), 660 (Waikare) and 680 (Whangape) µeq / l

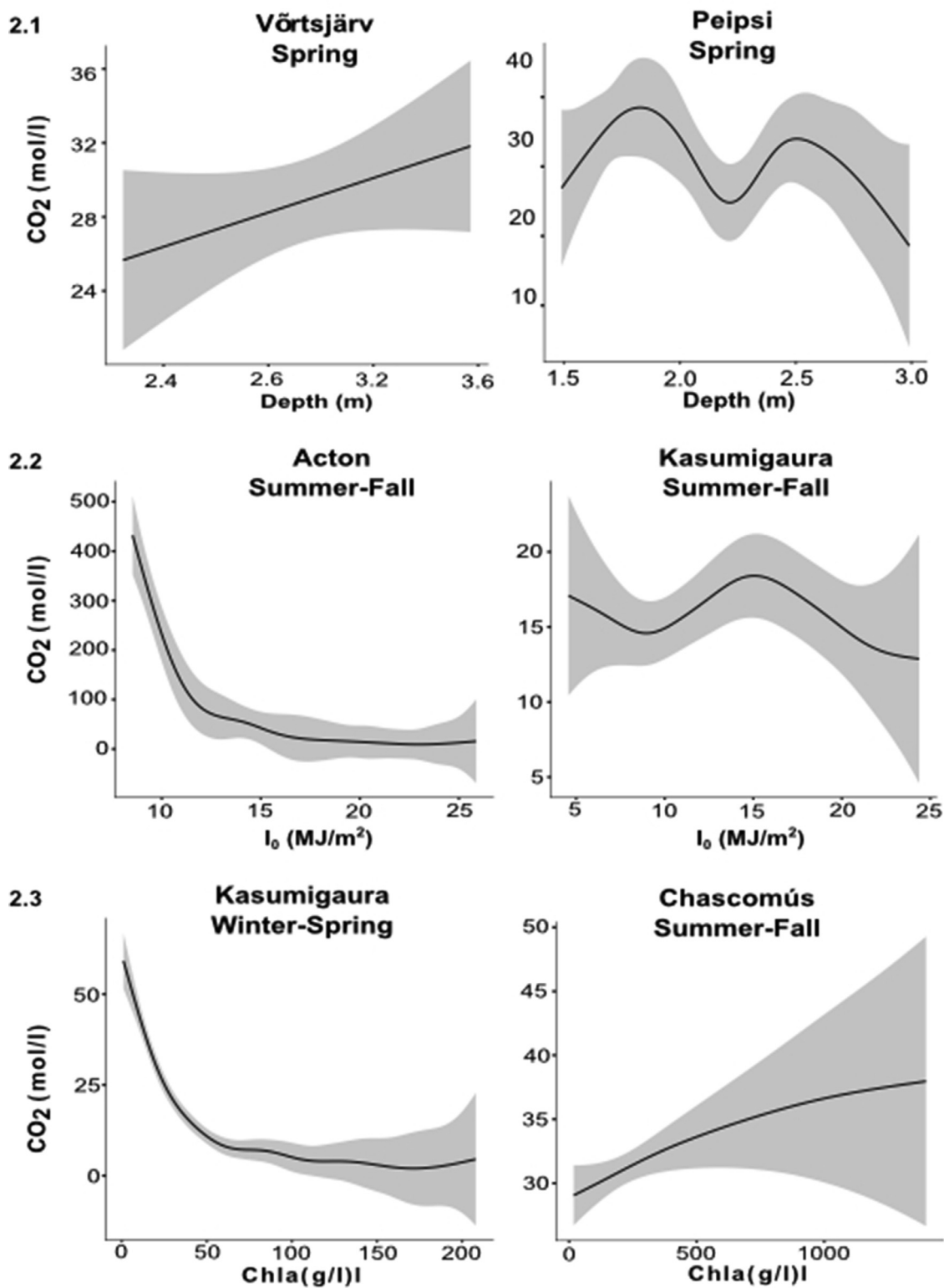


Fig. 2. Frequent trends for CO₂ concentration are shown considering the partial effect of depth (2.1), total irradiance (2.2), and chlorophyll *a* concentration (2.3). Trends were fitted using Generalized Additive Models (GAMs). Graphs show the fitted trend, and its simultaneous interval (95%) generated from the posterior distribution of the fitted GAM. Plots are partial plots of the smooth term in the model, and the y axis is the intercept plus the partial effect of the individual smooth.

3.3. Chlorophyll dynamics and C-limitation in agriculturally eutrophic lakes

Multi-year trends of Chla along i) lake depth and ii) total irradiance were also assessed using generalized additive models (GAMs)

for the whole dataset of each lake, and also to each of the seasonal periods defined previously (i.e., individual analyses by season). For a full description see section S.2 in supplementary material; model selection criteria in Table S2 (suppl. mat.); and best-fit models in Fig. S2_Chlorophyll (suppl. mat.). Each model included two predic-

Table 2

Summary of trends showing the most and least frequent trends of CO₂ concentration when the explanatory variables lake depth, incident irradiance and chlorophyll *a* concentration were considered after adjusting GAMs to the multi-year monitoring data from eight eutrophic lakes located at the northern (USA, Estonia, Japan, Russia) and the southern (New Zealand, Argentina) hemispheres. The trends correspond to the best fitting models across two seasonal periods: winter-spring and summer-fall. GAMs: Generalized Additive Models. ns: non-significant trend, not incl.: not included.

Lake	Seasonal period	Water level		Irradiance		Chlorophyll	
		Most frequent	Least frequent M-shape, U-shape, or decreasing trend ∖-shape	Most frequent	Least frequent	Most frequent	Least frequent
		Increasing trends		Decreasing trends:	W-shape	Decreasing trends:	W-shape, ∩-shape or increasing trends: (/ -shape),
Acton	spring	not incl.		∖		∖	
	summer-fall	not incl.		∖		ns	
Chascomús	winter-spring	/		∖		ns	
	summer-fall	/			W		/
Kamusigaura	winter-spring		U	∖		∖	
	summer-fall	/			W	∖	
Peipsi	spring		M	∖			W
	summer-fall		∖	∖		∖	
Vörtsjärv	spring	/		∖		ns	
	summer-fall	ns		∖		ns	
Waahi	winter-spring	/		∖		∖	
	summer-fall	/		∖		∖	
Waikare	winter-spring		∖	∖		ns	
	summer-fall	ns		∖		ns	
Whangape	winter-spring	/		∖		ns	
	summer-fall	/		ns		W	

Table 3

Summary of trends showing responses of chlorophyll *a* concentration when the explanatory variables lake depth and incident irradiance were considered after adjusting GAMs to the multi-year monitoring data from eight eutrophic lakes located at the northern (USA, Estonia, Japan, Russia) and the southern (New Zealand, Argentina) hemispheres. The trends correspond to the best fitting models across two seasonal periods: winter-spring and summer-fall. GAMs: Generalized Additive Models. ns: not significant, not incl.: not included.

Lake	Seasonal period	Water level	Irradiance
Acton	Spring	not incl.	/
	summer-fall	not incl.	∩
Chascomús	winter-spring	∖	∩
	summer-fall	∖	ns
Kamusigaura	winter-spring	M	∩
	summer-fall	∩	∩
Peipsi	Spring	ns	S
	summer-fall	W	S
Vörtsjärv	Spring	∩	/
	summer-fall	∖	∖
Waahi	winter-spring	∖	ns
	summer-fall	ns	/
Waikare	winter-spring	ns	/
	summer-fall	∖	ns
Whangape	winter-spring	ns	/
	summer-fall	∖	ns

for variables: i) lake depth and ii) total irradiance. For the eight lakes, the best fitted models corresponded to the analyses by seasons. For Chascomús (winter-spring), Kasumigaura (both periods), Peipsi (summer-fall), and Vörtsjärv (both periods) the full model, including lake depth and irradiance, explained the highest percentage of the deviance. Different Chla concentration trends with depth and irradiance were observed. See Table 3 for a summary of significant trends.

In order to identify conditions of potential CO₂ limitation, we examined the partial effect of total irradiance i) on Chla concentration, and ii) on the availability of carbon (CO₂ concentration). The partial effect of total incident irradiance (i.e., after statistically removing the partial effect of lake depth) on Chla displayed differ-

ent trends. The adjusted trends were monotonic positive (for example Acton spring, Fig. 3), parabolic (Fig. 4) or zigzagging (for example Peipsi, Fig. S2_Chlorophyll). In four cases (Chascomús in winter-spring, and Acton, Kasumigaura and Vörtsjärv in summer-fall), the Chla concentration initially displayed an increasing trend with irradiance, but the trend reversed at higher irradiance levels (~15 MJ/m²). In other words, the relationship resembled an open downward parabola with maxima around 10–20 MJ/m² (Fig. 4). The parabolic trends of Chla vs irradiance occurred over the same range of incident irradiance in which the concentration of CO₂ was observed to decline or remain low (Fig. 4 lower panels). Moreover, the adjusted trend and its simultaneous interval often fell far below saturation levels (~15 μmol/l) in Kasumigaura (at irradiance levels of ~15 MJ/m²) or close to this concentration (in the rest of the lakes) (Fig. 4). Whenever Chla increased with irradiance, there was a corresponding decrease in CO₂ concentration, except in Kasumigaura that showed a multimodal pattern.

4. Discussion

4.1. Frequency of CO₂ undersaturation in agriculturally eutrophic Pampean lakes

The first objective of our study was to evaluate how frequent is CO₂ undersaturation in shallow lakes within the Pampean region of Argentina. The collection of Pampean lakes included in this study are typically eutrophic to hypertrophic. They tend to display higher nutrients (TP, TN) and Chla concentrations, and shallower Secchi depths than other shallow lakes at similar latitude (Diovisalvi et al., 2015). The ranges of P and N span over three orders of magnitude and cover ranges similar to those reported by Balmer and Downing (2011) for 131 lakes in the mid-western United States, one of the most intensive agricultural areas in the world. The median pH in Pampean lakes was 9 (range 6.8–10.7) suggesting a scenario of high photosynthetic rates and CO₂ demand. Accordingly, the percentage of samples undersaturated with CO₂ (i.e., pCO₂ < atmospheric equilibrium) was 34%, i.e., 2.6 times higher than the percentage reported for less impacted lakes (~13%) (Cole et al., 1994; Lazzarino et al., 2009), but somewhat lower than the percentage reported for midwestern USA

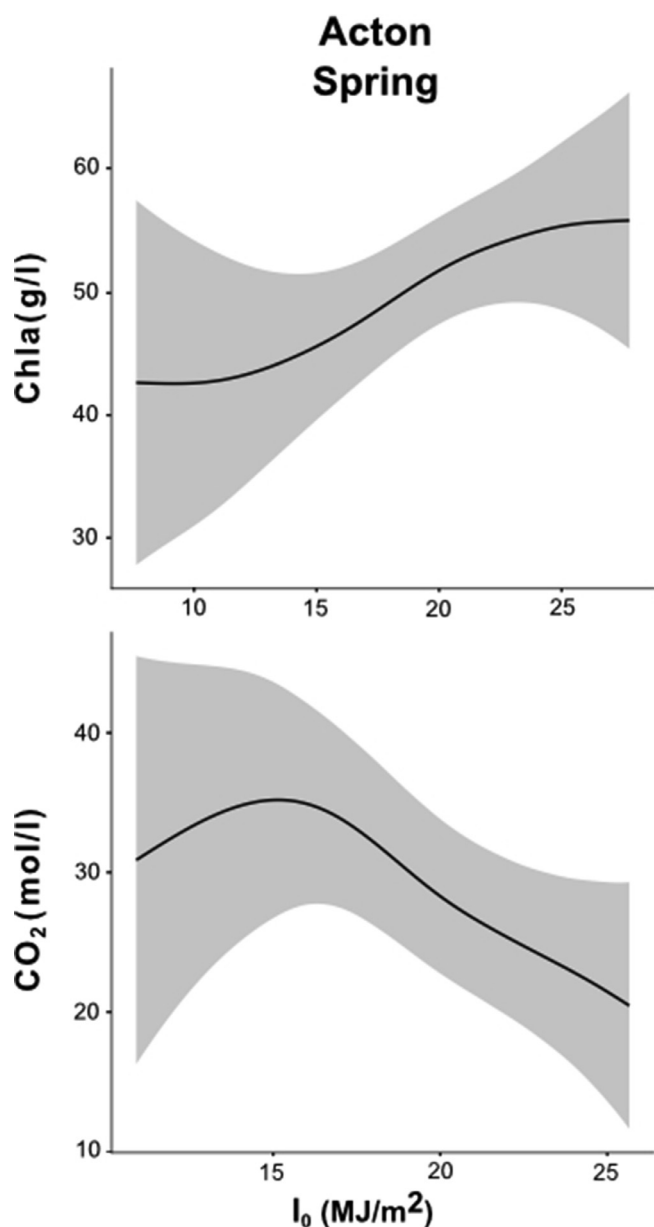


Fig. 3. Example of a scenario of light limitation shown by the relation between chlorophyll *a* concentration and total irradiance (upper panel), and CO₂ concentration and total irradiance (lower panel) on Lake Acton during spring. Trends were fitted using Generalized Additive Models (GAMs). Graphs show the fitted trend, and its simultaneous interval (95%) generated from the posterior distribution of the fitted GAM. Plots are partial plots of the smooth term in the model, and the y axis is the intercept plus the partial effect of the individual smooth. Total irradiance corresponds to the seven-day average, previous to the sampling date for each lake.

lakes (60%, Balmer and Downing (2011)). The study by Balmer and Downing (2011) was restricted to the summer period. The proportion of samples from Pampean lakes undersaturated with CO₂ in summer was 44%, i.e., somewhat higher than the yearly proportion, but still lower than that of midwestern USA lakes. In any event, our compilation of pCO₂ values from Pampean lakes confirm that conditions of CO₂ undersaturation in agriculturally impacted eutrophic lakes develop much more frequently than in deep, temperate lakes in forested watersheds (i.e., the subjects of most limnological research) (Sobek et al., 2005), and suggest that the drivers of [CO₂] variability in agriculturally eutrophic lakes deserve closer examination.

4.2. CO₂ patterns in agriculturally eutrophic lakes – analyses of multi-year monitoring datasets

For the second objective, we analyzed multi-year data of [CO₂] from 8 lakes in Estonia, Estonia-Russia, Japan, the US, Argentina, and New Zealand. All 8 lakes are eutrophic, due to high nutrient inputs. Taken together, they span a wide range of Chla concentration (<1 to >1000 µg/l) and water transparency (<0.05 to >4 m). In all 8 lakes, phytoplankton biomass is a major contributor of turbidity (Fig. 1). The proportion of samples displaying undersaturation with CO₂ ranges from 21% (L. Võrtsjärv) to 61.8% (L. Kasumigaura). Again, these percentages are higher than the world average, as could be expected for eutrophic and hypertrophic lakes.

In order to investigate the responses of individual lakes to changes in three potential drivers of [CO₂], which are also critical variables that control the functioning of shallow eutrophic systems, we used advanced modelling based on GAMs (Wiik et al., 2018). Two of these variables (lake depth, and total incident irradiance) can genuinely be considered external forcing variables, in the sense that they unidirectionally affect in-lake [CO₂]. A third variable was also evaluated (Chla), bearing in mind the important caveat that phytoplankton biomass may at times behave as a driver and at other times as a response variable of the in-lake [CO₂]. Depending on the lake and season, one, two, or three of the considered variables significantly explained variability in [CO₂]. This procedure allowed us to investigate the partial effect of each of these three drivers, after (statistically) removing the effect of the remaining two.

The best-fits were always obtained for models analyzed by seasons. We believe that this is related to the difference in the relative importance of autotrophic and heterotrophic processes between the periods of progressively increasing irradiance (winter-spring) vs. decreasing irradiance (summer-fall). Large residual variability was a common theme to all the modeled patterns. Most of the unexplained variability probably arises from the highly dynamic nature of the processes controlling CO₂ concentration in eutrophic systems (Raven et al., 2019). In fact, the photosynthetic activity of dense blooms can be as high as 12.5–50 mmol C L⁻¹ h⁻¹ (Hein, 1997), potentially depleting the CO₂ concentration in a lake within a few hours (Visser et al., 2016). Considering that most lakes were sampled during morning hours, the CO₂ concentrations could be expected to be higher and more variable than later in the day (Maberly and Gontero, 2017; Schindler and Fee, 1973). Thus, large sample size (i.e., many years of monitoring) was without doubts critical to reveal statistically significant trends.

In most cases, the concentration of CO₂ was significantly affected by lake depth and, as a general rule, CO₂ increased with lake depth. Areal fluxes of CO₂, as well as biological rates of CO₂ consumption and production, are relatively unaffected by lake depth. Depth integrated photosynthesis is controlled by the areal flux of solar radiation. On the other hand, a large percentage of total respiration takes place in the lake sediments. However, a larger lake volume implies a larger CO₂ storage capacity. The exchange of CO₂ at the air-water interface is a relatively slow process, but the CO₂ depletion by photosynthesis fixation can be very fast during blooms. Depletion can be expected to take longer as the ratio between lake depth / Secchi depth increases, because an increasing fraction of the water column would not be involved in CO₂ depletion. Therefore, CO₂ depletion is more likely to occur in shallow water (compared to deep water) and consequently, the direct positive relationship between CO₂ concentration and lake depth, which was obtained for most lakes, could be anticipated (Verspagen et al., 2014). Some lakes, however, displayed an inverse relationship. A notable example is illustrated by Waikare that showed a decreasing trend of [CO₂] with lake depth, during the winter-spring pe-

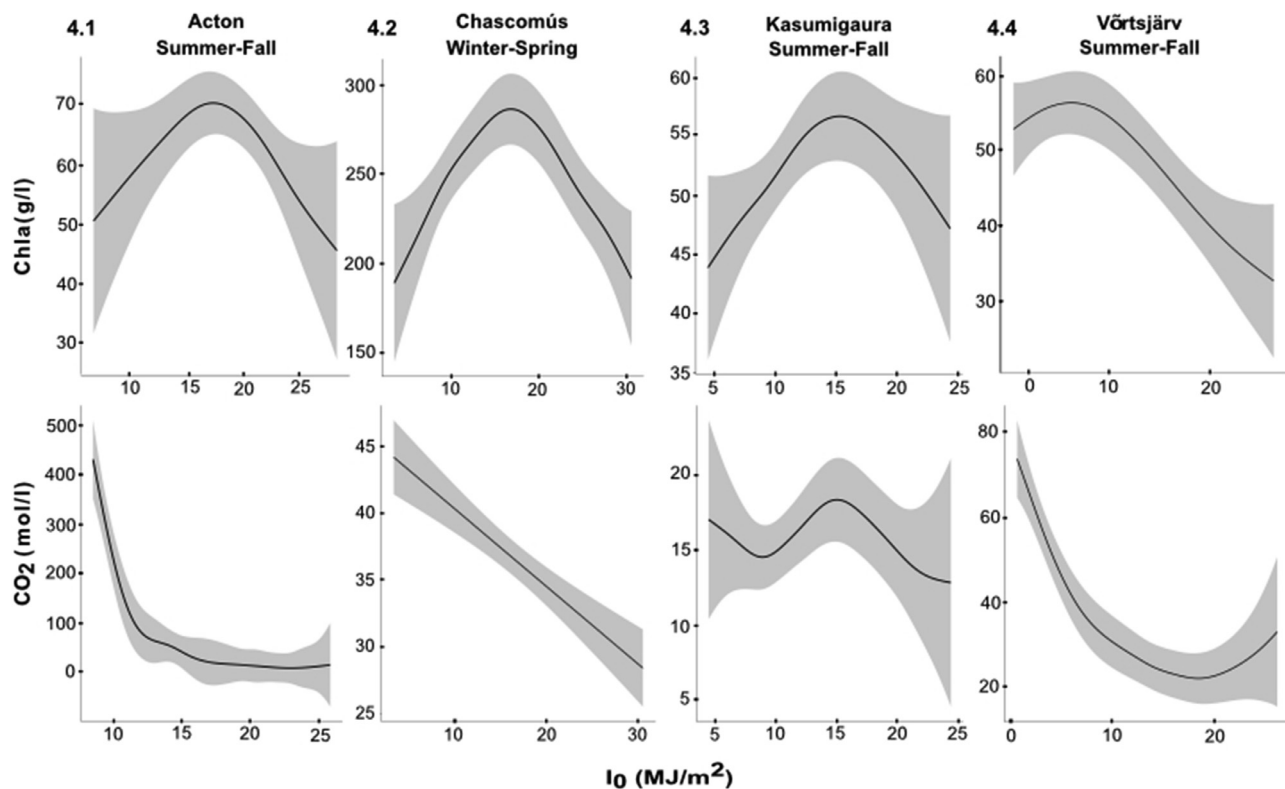


Fig. 4. Examples of carbon limitation on lakes Acton (4.1), Chacomús (4.2), Kasumigaura (4.3), Vörtsjärv (4.4). The partial effect between chlorophyll a concentration and total irradiance (upper panel), and CO_2 concentration and total irradiance (lower panel) is shown. Trends were fitted using Generalized Additive Models (GAMs). Graphs show the fitted trend, and its simultaneous interval (95%) (grey area) generated from the posterior distribution of the fitted GAM. Plots are partial plots of the smooth term in the model, and the y axis is the intercept plus the partial effect of the individual smooth. Total irradiance corresponds to the seven-day average, previous to the sampling date for each lake.

riod, probably related with changes in pH according with the pH-depth relationship found (suppl. mat., Fig. S3).

The response of $[\text{CO}_2]$ to increases in incident solar irradiance is mostly driven by photosynthesis. Therefore, a monotonically decreasing trend could be expected. In nature however, solar irradiance and temperature are highly correlated along the year. Temperature can be expected to affect $[\text{CO}_2]$ through different mechanisms. The solubility of CO_2 decreases with increasing water temperature, which other things being equal, would translate into a decreasing trend of $[\text{CO}_2]$ with increasing lake temperature (at a constant $p\text{CO}_2$ of $400 \mu\text{atm}$, $[\text{CO}_2]$ decreases by about 3%/degree C with increasing temperature). On the other hand, temperature can be expected to accelerate metabolic processes. Compared to photosynthesis rates, the metabolism of heterotrophic organisms is more sensitive to temperature (Winder and Sommer, 2012). The differential dependence of photosynthesis and respiration on temperature can be expected to switch the balance in favor of CO_2 production at higher temperature. Unfortunately, water temperature could not be included in our models of $[\text{CO}_2]$ given that it was used to calculate the carbonate system variables. Therefore, our analyses of the multi-year data sets are unable to separate the effects of these two variables.

Finally, Chla had most often a negative effect on the concentration of CO_2 . This agrees with similar results reported by Balmer and Downing (2011) and can be interpreted as the result of increased CO_2 demand or depletion under conditions of increased phytoplankton biomass. In addition, uncertainty in CO_2 concentration increased at higher Chla levels.

4.3. Diagnosing CO_2 limitation of phytoplankton biomass

As discussed above, raw estimates of CO_2 concentration by themselves are insufficient to unequivocally diagnose the nature of phytoplankton biomass limitation. Our criteria for identifying conditions of light- vs. carbon-limitation is based on the joint examination of Chla-irradiance and CO_2 irradiance patterns.

Light limitation: whenever light becomes the limiting resource of phytoplankton biomass, we may expect a direct positive relationship between biomass (as estimated by Chla) and the amount of incident solar irradiance (i.e., the assumed limiting resource). Presumably, the resulting higher phytoplankton biomass would tend to increase the demand for CO_2 , but (depending on a variety of factors, such as lake depth, alkalinity, and water temperature) this may or may not translate into a decreasing trend of CO_2 concentration with increasing I_0 . Development of light limitation in Acton Lake has been described previously (Kelly et al., 2019; Vanni et al., 2006), and the pattern observed during springtime in the present study (Fig. 3) provides a good example for such condition. In this example, Chla concentration increased monotonically with incident irradiance. Although for incident irradiance higher than $\sim 15 \text{ MJ/m}^2$, the concentration of CO_2 tends to decrease with irradiance, the concentration of Chla maintained an overall positive relationship with I_0 . Therefore, the observed increasing trend of Chla concentration with irradiance corresponds to the expected behavior of a lake in which the phytoplankton biomass is limited by light. Should the amount of incident light continue to increase, it is possible that a condition of carbon limitation would eventu-

ally develop. But with the observed range of incident light there are no signs of the onset of carbon limitation.

For other lakes or seasonal periods however, the initial increase in Chla concentration with incident irradiance eventually reached a plateau, or more frequently, it was followed by a decreasing trend at higher irradiance levels. The decrease in Chla at high irradiance contradicts expectations based on light being the limiting factor of phytoplankton biomass.

Carbon limitation: under this scenario, intense photosynthesis rates at high levels of incident irradiance would increase the demand for CO₂, resulting in low [CO₂], which would eventually prevent further increases of phytoplankton biomass with irradiance. As photosynthesis becomes limited by the supply of inorganic carbon, further increases in incident irradiance would not translate into higher photosynthesis rates. Increasing levels of incident light, decoupled from phytoplankton growth, would in turn translate into higher mean water column light availability. Interestingly, increased light availability implies that fewer Chla units are required to maintain a given photosynthetic carbon fixation rate (which is now being controlled by the supply of inorganic carbon, instead of light). In addition, the high photosynthetic rates would result in water basification and overall impoverishment of the water quality for algae growth. In summary, under nutrient sufficiency, high irradiance levels could eventually induce inorganic carbon limitation, resulting in reductions of phytoplankton biomass. Such sequence of events is well known to occur in outdoor mass culture systems (Kumar et al., 2015) in which the algae are grown under high nutrient supplies, high irradiance levels, and shallow depths. We recognize that a number of different bottom-up (e.g., limitation by nutrients other than inorganic carbon) and top-down (grazing, viral infection) processes, as well as thermal- or photo-inhibition at high levels of incident (visible and ultraviolet) radiation, may also lead to reductions of phytoplankton biomass in nature. However, in contrast to the situation just described the decrease in phytoplankton biomass in all these cases would not be driven by the depletion of inorganic carbon. Rather conversely, the lower phytoplankton biomass (resulting from grazing or photodamage) would lead to decreased photosynthesis rates and lowered CO₂ demand, which ultimately should result in increased CO₂ concentration. We argue that the joint examination of Chla-irradiance and CO₂-irradiance trends may be used to evaluate the likelihood of inorganic carbon limitation. The idea that phytoplankton biomass is limited by inorganic carbon availability implies that, as the incident irradiance increases, the photosynthetic CO₂ demand remains high, leading to the decrease in Chla, while [CO₂] remains low, or even decreases. A situation like this was observed in Acton, Kasumigaura and Vörtsjärv at higher incident irradiance levels (> ~15 MJ/m²) during the summer-fall period (Fig. 4.1, 4.3, 4.4), and in Chascomús, during the winter-spring period (Fig. 4.2). Previous studies of these 4 lakes have reported that the phytoplankton assemblage was dominated by cyanobacteria and that biomass was not limited by N or P (either continuously or during certain periods of the year): Acton (Vanni et al., 2006; Kelly et al., 2019), Chascomús (Llames et al., 2009; Torremorell et al., 2009; Torremorell et al., 2007), Kasumigaura (Matsuzaki et al., 2018; Tomioka et al., 2011), Vörtsjärv (Kisand et al., 1998; Nöges et al., 1997; Nöges et al., 2011). This does not mean that phytoplankton biomass in these four lakes is constantly limited by inorganic carbon. Rather our interpretation is that inorganic carbon limitation in these lakes develops frequently enough so as to symptoms of inorganic carbon limitation (decreases of chlorophyll concurrent with relatively low CO₂ concentration at high incident solar irradiance) can be captured by the statistical models. In the case of lakes Peipsi, Wahii, Waikare and Whangape, no clear signs of either light or CO₂ limitation were detected. Additional factors (e. g. grazing, nutrient ratios, photo-inhibition) could limit phytoplankton biomass, but our

analysis does not allow us to identify the nature of the controlling factor.

4.4. Ecological significance in the context of global change

Although conditions of CO₂ undersaturation develop frequently in agriculturally eutrophic lakes (Balmer and Downing, 2011; this study) and despite the fact that inorganic carbon limitation of phytoplankton biomass has been demonstrated experimentally (Kragh and Sand-Jensen, 2018; Shapiro, 1997), the paradigm of phytoplankton (productivity and biomass) limitation by nutrients and light availability is so pervasively established, that the lack of nutrient limitation (N and P) is ordinarily interpreted as sufficient evidence for the condition of light limitation, without considering the possibility of limitation by inorganic carbon. This paper builds on previous research (Balmer and Downing, 2011; Pacheco et al., 2013), demonstrating that agriculturally eutrophic lakes are frequently undersaturated with CO₂, which differs from the results of many previous studies on temperate boreal lakes. Moreover, we developed criteria for identifying conditions of inorganic carbon limitation of phytoplankton biomass (outlined above), which combined with the use of powerful statistical tools (GAM) for the analysis of multi-year datasets, allowed us for the first time, to produce whole-lake scale evidence of inorganic carbon limitation of phytoplankton biomass.

In a retrospective article, Schindler (2006) emphasized that the development of carbon limitation of algal production (following additions of N and P to Lake 227) was a response to eutrophication rather than the cause. While Schindler's conclusion is certainly valid over short time-scales (years), we suspect that over longer time-scales (decades), the increase of atmospheric CO₂ concentrations, from pre-industrial (~280 µatm) to present levels (~410 µatm), may have aggravated the trophic condition of lakes already eutrophied by excessive N and P. For example, for a lake with total alkalinity of 1000 µM and temperature of 20° C, the increase from ~280 µatm to ~410 µatm corresponds to a nearly 50% increase in [CO₂] from ~10.9 to ~16 µM. Moreover, we also suspect that water quality will continue to deteriorate, as atmospheric CO₂ concentrations continue to rise in the years to come. To illustrate this point, consider the three hypothetical scenarios in nutrient repleted lakes, depicted in Fig. 5. At low irradiance levels phytoplankton biomass is limited by incident light, and therefore changes in atmospheric CO₂ concentrations are deemed to be virtually inconsequential. However, once the algae become limited by inorganic carbon, they attain increasingly higher biomass as atmospheric CO₂ increases. Notice that the switch from light-to inorganic carbon-limitation is expected to take place around the same CO₂ concentration in the water.

Freshwater environments are being degraded globally through anthropogenic eutrophication as growing human populations increase demands for food, energy, and fiber that intensify agriculture. Increasing global population growth and agricultural industrialization have led to an increase in loads and concentrations of N and P in terrestrial environments, and ultimately in aquatic ecosystems (Moal et al., 2019). Mass proliferation of (potentially harmful) cyanobacteria is already a widespread environmental problem. Blue-green algae generally have better CO₂ kinetics than do other algae. The photosynthetic activity of dense blooms can be as high as 12.5–50 mmol C L⁻¹ h⁻¹ (Hein, 1997), potentially depleting the CO₂ concentration in a lake within a few hours (Visser et al., 2016). Moreover, although the initiation of the cyanobacteria blooms does not seem to depend upon conditions of low CO₂ concentration or high pH, once they become abundant they may ensure their dominance by reducing concentrations of CO₂ to levels available only to themselves (Shapiro, 1997).

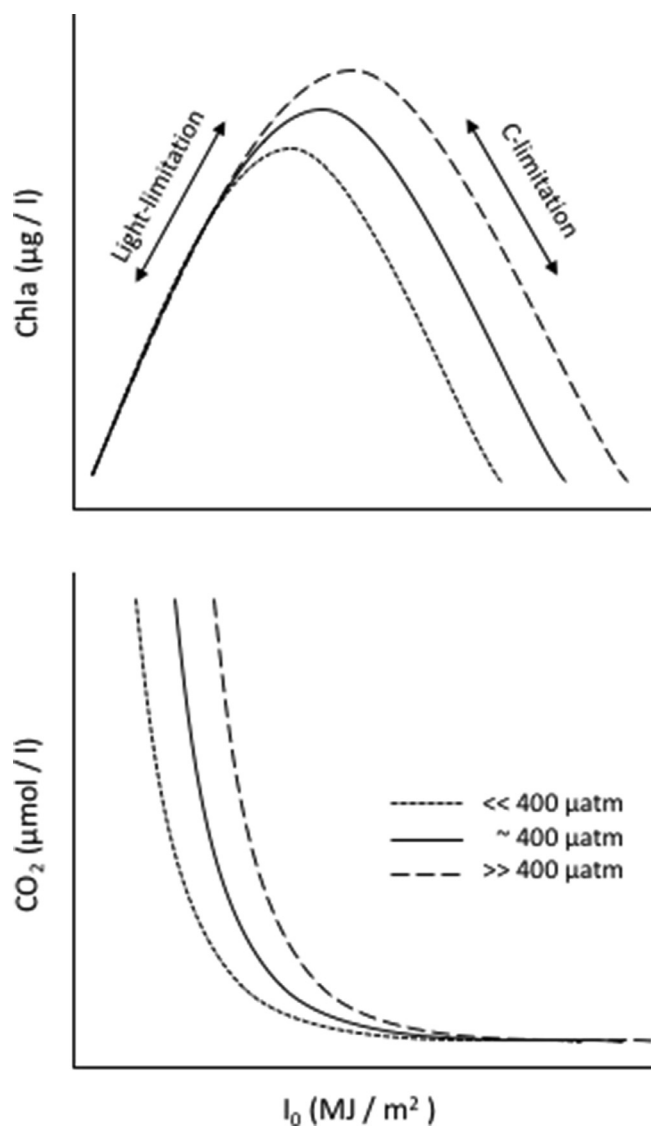


Fig. 5. Hypothetical trends of chlorophyll *a* concentration (upper panel) and CO_2 concentration (lower panel) vs incident irradiance (I_0), showing the transition between light- and CO_2 -limitation conditions as I_0 increases. The solid lines resemble to the present day, modelled response of Acton Lake during the summer-fall period; the dotted and dashed lines are hypothetical responses for much lower (for instance, preindustrial levels $\sim 280 \mu\text{atm}$) and much higher atmospheric CO_2 (for instance doubling of present levels) concentrations scenarios.

As pointed out by Pacheco and co-workers (Pacheco et al., 2013), geographic regions rich in surface waters are also prone to agricultural and urban development and so may become increasingly eutrophic as the population rises. Hence, while continued CO_2 emissions can be expected to exacerbate the consequences of eutrophication, ongoing and future agriculture developments would increase the geographic coverage of eutrophic systems with the obvious increase in the number of affected environments. Carbon emissions and eutrophication represent two major and converging environmental problems, whose interaction deserve further exploration and imaginative approaches.

5. Conclusion

CO_2 shortage is becoming increasingly frequent in agriculturally impacted, eutrophic lakes. Its intensity may range from slight undersaturation to severe depletion. CO_2 undersaturation within highly eutrofied Pampean (Argentine) lakes, as well as within se-

lected case-study lakes around the world, is much more frequent than the world average. The physical environment significantly affects inorganic carbon availability: high incident solar irradiance and shallow depth, both tend to decrease the concentration of CO_2 . There was also a trend of decreasing CO_2 concentration with increasing phytoplankton biomass (as estimated by chlorophyll *a* concentration), but the uncertainty (variability) in CO_2 estimates also increased with phytoplankton biomass.

It is known, from previous experimental studies, that severe CO_2 depletion may result in inorganic carbon limitation of phytoplankton biomass. However, the examination of chlorophyll and CO_2 concentration patterns along a gradient of naturally occurring incident solar radiation allowed us, for the first time, to provide non-manipulative, whole-lake scale evidence of phytoplankton biomass limitation by CO_2 in lakes. At least 4 lakes (Acton, Chascomús, Kasumigaura and Vörtsjärv) developed phytoplankton biomass limitation by inorganic carbon at the highest levels of incident irradiance. All in all, our findings reveal that agriculturally eutrophic lakes are prone to experience CO_2 undersaturation and inorganic carbon limitation of phytoplankton biomass. The shift from nutrients (N and P) to carbon limitation is an unforeseen consequence of recent human impacts. We also argue that increased atmospheric CO_2 has not contributed to alleviate CO_2 limitation in eutrophic lakes. Rather we believe that it has promoted increases in phytoplankton biomass.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:[10.1016/j.watres.2020.116715](https://doi.org/10.1016/j.watres.2020.116715).

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