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4 **Coupling DOM, CO₂ and productivity in boreal lakes**
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Summary

1. Lakes world-wide are in rapid change due to direct or indirect climate impacts. In boreal areas, the increased concentrations of dissolved organic matter (DOM) are profoundly affecting light climate and productivity in multiple ways. Photochemical and microbial mineralization of DOM are major sources of CO₂ in these lakes. It has been suggested that this CO₂ could potentially promote primary production and offset negative impacts of increased light attenuation.
2. A synoptic survey of 76 Scandinavian lakes along gradients of DOM and total phosphorus (TP) revealed a major negative impact of DOM on primary production and algal biomass primarily related to light attenuation, while a strong positive impact of TP. The negative impact of DOM on lake productivity is thus partly offset by DOM-associated P,
3. Concentrations of CO₂ did not relate significantly to either production, biomass or seston stoichiometry, and thus while elevated CO₂ may promote primary production in more productive lakes, it does not in these CO₂-supersaturated boreal lakes.
4. Zooplankton biomass correlated strongly with TP and total algal biomass, less so with primary production, and was negatively related to DOM, likely reflecting the autotroph responses to DOM.

Introduction

For most boreal freshwater systems as well as some coastal regions influenced by forested catchments, there is a current trend with increased export of terrestrially fixed carbon (C), causing elevated concentrations of colored dissolved organic matter (cDOM) (Monteith *et al.*, 2007; Garmo *et al.*, 2014; Finstad *et al.*, 2016). This “browning” will play a multitude of partly contrasting roles for primary and secondary productivity in lakes (Hessen, 1988; Solomon *et al.*, 2015; Williamson *et al.*, 2015). For nutrient poor lakes allochthonous DOM may be a major source not only of colored, organic carbon, but also a source of phosphorus (P) (Meili, 1992), nitrogen (N) (Dillon & Molot, 2005) and iron (Fe) (Kritzberg & Ekström, 2012). Colored organic matter also has a strong effect on light attenuation which will impact primary production negatively (Karlsson *et al.*, 2009; Thrane, Hessen & Andersen, 2014).

Colored DOM also promotes CO₂ production by microbial mineralization and photo-oxidation, making terrestrially derived DOM the major source of CO₂ in boreal lakes (Sobek *et al.*, 2003; Humborg *et al.*, 2010; Larsen *et al.*, 2011). This renders these lakes net heterotrophic, serving as major conduits of CO₂ to the atmosphere (Hessen, Andersen & Lyche 1990; Cole *et al.*, 1994; Sobek *et al.*, 2003). Increased DOM implies both increased net heterotrophy and increased CO₂ concentration. While the positive role of DOM as a mediator of nutrients and its negative role via light shading is rather straightforward and well settled for primary producers, the impact of increased CO₂ concentration on the quantity and quality of autotroph production is less obvious.

The ability of CO₂ to enhance plant biomass is considered an important regulator of atmospheric CO₂ (Arneeth *et al.*, 2010). In aquatic systems, elevated CO₂ has experimentally been shown to promote a short-term increase in algal biomass (Urabe, Togari J & Elser, 2003; Beardall, Roberts & Raven, 2005; Spijkerman *et al.*, 2010), which then implies a quality cost from a grazer’s perspective due to elevated C:N and C:P ratios (Urabe, Togari & Elser, 2003). The long-term impacts of increased CO₂ for phytoplankton productivity remains unsettled for aquatic ecosystems. In a study of northern lakes, Jansson, Karlsson & Jonsson (2012) reported that CO₂ strongly promoted primary productivity and autotroph biomass, challenging the generally held belief that

autotrophs in nutrient-poor systems are bottom-up controlled by nutrients (and light) alone. If mineralization of DOM may offset the negative impacts caused by DOM-mediated light limitation, this would have large implications for assessing the impacts of browning in lakes.

CO₂ could also affect autotroph stoichiometry. As with terrestrial systems, a growth-promoting effect of CO₂ may be constrained by nutrient scarcity, but unlike terrestrial autotrophs, phytoplankton cannot compensate for nutrient scarcity by root activity enhancing weathering rates. Autotrophs have rather flexible elemental ratios (Sterner & Elser, 2002), but although algal monocultures can be manipulated to increase their C:P-ratios more than tenfold (Sterner & Elser, 2002; Hessen, Færøvig & Andersen, 2002), the natural range of seston, and then also algae as part of the seston, varies over a considerably narrower range (Sterner *et al.*, 2008). The higher C : nutrient ratio observed in both lakes and oceans in more recent studies relative to Redfield ratio (Sterner *et al.*, 2008) could, however, hint at a systematic C-enrichment. This may not necessarily reflect increased CO₂-levels, but could also be promoted by increased stratification, meaning reduced access to nutrients in the mixed layer which, together with elevated light (due to shallower mixing depths) would pull towards elevated C:P-ratios. On the other hand, increased DOM-mediated attenuation could also promote reduced C-fixation and thus reduced C:P (Kelly *et al.*, 2016),

Since the C to nutrient ratio is an important aspect of food quality for zooplankton grazers, the impact of DOM (and light), P and CO₂ on seston stoichiometry will have bearings on the zooplankton (Andersen and Hessen 1991; Jones *et al.* 2012). Finally, zooplankton responses will feed back on the phytoplankton both via recycling and grazing (Kissman *et al.*, 2016), further complicating the net ecosystem responses to variations in DOM. Although increased DOM currently is reported over vast boreal areas, temporal or spatial drought may in fact reduce DOM (Yan *et al.*, 1996; Schindler *et al.*, 1997; Williamson *et al.*, 2016) with an anticipated concomitant reduction in CO₂. Given the current changes in DOM, and its many and partly contrasting roles for the aquatic biota, it is important to assess the overall, net effects on ecosystem productivity.

In this study we thus assess the links between DOM, CO₂ and autotroph responses. We further investigate how these key parameters affect seston stoichiometry (C:P-ratio)

and subsequently zooplankton biomass. While several studies have addressed the role of DOM for phyto- and zooplankton, this study is unique we believe by being based on a synoptic survey in 76 boreal lakes along a latitudinal transect from the Swedish Baltic coast to the Norwegian western coast. Lakes were deliberately chosen to let DOM and TP vary as independently as possible, constrained by the general covariation of these two parameters. Both phytoplankton biomass and production as well as zooplankton biomass was related to CO₂ and other lake-specific parameters to reveal the net effect of DOM and CO₂ on the biota.

Material and Methods

Lakes for this survey were selected from existing data on Norwegian and Swedish lakes from the “Rebecca” dataset (Solheim *et al.*, 2008) and the “Nordic lake survey 1995” dataset (Henriksen *et al.*, 1998). The datasets were subset to lakes with latitude 57-64 degrees N, < 600 meters above sea level, area > 1 km² (1.09 – 140 km²) pH > 5 (5.41 – 7.84), TP < 30 µg L⁻¹ (0.5 – 27.5 µg L⁻¹) and total organic carbon (TOC) < 15 mg L⁻¹ (0.34 – 12.9 mg L⁻¹) yielding a set of similarly large lakes within a narrow latitudinal and altitudinal range that spanned wide and rather orthogonal gradients in TOC and TP, but avoiding acidic, eutrophic, and highly dystrophic lakes (S1 Table and S2 Fig). These lakes spread out over a geographical gradient from western Norway to eastern Sweden (Fig 1). For sampling of the lakes, field permits were granted by local authorities for all localities upon request.

Composite samples (15 L in total) from 0-5 m (above thermocline) were taken with an integrating water sampler (Hydro-BIOS, Germany) in the central part of each lake during daytime. For control, the concentrations of TP, TOC, and total nitrogen (TN) were measured both at the accredited lab at the Norwegian institute for water research (NIVA) and at the University of Oslo (UiO). In general the results from both labs were in good accordance, and we thus applied the means from both labs in the analysis.. DOC was calculated as the difference between total organic carbon (TOC) and particulate organic carbon (POC). TOC was measured by infrared CO₂ detection after catalytic high temperature combustion (Shimadzu TOC-VWP analyzer (UiO) or Phoenix 8000 TOC-TC analyzer (NIVA)). On the average > 95 % of TOC was on dissolved form (DOC),

which also constitute the key component of DOM in terms of mass. DOC is a major source of CO₂ by mineralization processes, and also constitute the chromophoric fraction of DOM that, along with Fe, determines most of the light attenuation (Thrane, Hessen & Andersen 2014). Thus while the terrestrial input to lakes is labeled DOM (comprising more than C), it is quantified by its dominant component DOC, and hence in the result is referred to as DOC.

Particulate C and N were measured on an elemental analyzer (Flash EA 1112 NC, Thermo Fisher Scientific) through rapid combustion in pure oxygen of a pre-combusted GF/C filter with particulates. TN was measured on unfiltered samples by detecting nitrogen monoxide by chemiluminescence using a TNM-1 unit attached to the Shimadzu TOC-VWP analyzer (UiO), or detection of nitrate after wet oxidation with peroxydisulfate in a segmented flow autoanalyzer (NIVA). TP was measured on an auto-analyzer as phosphate after wet oxidation with peroxodisulfate in both laboratories. Particulate P was measured on acid-washed GF/C-filters (similar procedures as for C and N), and then analysed following the protocol for total P above. PO₄ was analysed, but was below detection limits in most localities and thus not included in the analysis.

For pCO₂-analysis, water from the integrated water sample (same depth as for the other parameters) was gently let into 120 ml glass serum-vials without bubbling, fixed with 0.1 % HgCl and sealed with gas-tight butyl rubber stoppers (see (Yang *et al.*, 2015) for details). The bottles were stored dark and cold (4°C) prior to analysis. 20-30 ml of headspace was created by applying a gentle pressure (needle valve) through a needle to the top of the bottle volume. After headspace preparation, all bottles were shaken horizontally at 150 rpm for 2 hrs at room temperature to equilibrate liquid and gas phase. Headspace concentrations of CO₂ were determined by automated GC analysis with back-flushing of H₂O. Sample pH, measured immediately after opening the bottles by a 'sure-flow' electrode while gently stirring, was used to calculate total inorganic carbon (TIC) using analytical expressions for temperature dependencies of carbonate equilibrium (Apello & Postema, 1993).

Vertical profiles of scalar irradiance in the PAR region (400-700 nm; E_0) were measured using a spherical irradiance sensor (BioSpherical instruments) attached to a 10-channel CTD profiler (XRX-620, RBR Ltd., Canada). The sensor was deployed at ~20

cm s⁻¹ with a sampling rate of six times s⁻¹. To avoid shading from the hydroplane, we always lowered the CTD at the side facing the sun. We estimated the vertical attenuation coefficient for scalar PAR (K₀PAR) by taking the median of the distribution of slopes obtained from regressing natural log-transformed E_0 against depth (z) for ten and ten sampling points (i.e., a sliding window). This was done to reduce the influence of changes in incoming irradiance (e.g. from changes in cloud cover) during the haul.

Chlorophyll *a* (chl *a*), was analyzed both by high performance liquid chromatography (HPLC) following (Schagerl & Donabbaum, 2003), and by fluorescence after extraction in 96% ethanol. The average chl *a* concentration from the two methods was used in the analysis. Area-specific primary production (PP_A) was calculated using a bio-optical model based on phytoplankton absorption coefficients, *in situ* irradiance, and the light dependent quantum yield of photosystem II measured by a Pulse Amplitude Modulated (PAM) fluorometer (AquaPen, PSI Czech Republic) (see Thrane, Hessen & Andersen, 2014 for details). This method is based on “first principles” for photon flux and absorbance, and serves not only as a robust estimate of primary production (Kromkamp *et al.*, 2008), but also the only feasible tool for assessment of primary production in such synoptic surveys in a large number of sites. It also avoids many of the pitfalls by ¹⁴C-bottle incubation. As a measure of photosynthetic efficiency, we also determined the maximum quantum yield of photosystem II (F_v/F_m) using the same PAM on unfiltered water samples left to dark-acclimate for 1 h.

Phytoplankton was obtained from the integrated water sample where subsamples of 250 ml were fixed by acid Lugol, and counted by inverted microscope and an Utermöhl chamber. Biovolume for each species or taxon was measured in an inverted microscope and total biovolume (mm³ L⁻¹ = mg wet weight (ww) L⁻¹) calculated and used as a proxy for biomass. To obtain a rough proxy the fraction of algal and non-algal C in POC, we multiplied the biovolume by a factor 0.14 mg C : mg ww (Hessen *et al.*, 2003). As another proxy for the amount of non-algal particles in the seston, we used the average absorption coefficient (m⁻¹) of non-algal particles in the PAR region. This parameter was measured by first treating particulate glass fibre filters with sodium hypochlorite to oxidise algal pigments (following Thrane, Hessen & Andersen, 2014). Then, the filter – presumably only holding non-algal pigments – was placed in an integrating sphere (ISR

2200, Shimadzu scientific instruments, Columbia, Maryland, USA) attached to a double beam Shimadzu UV-2550 spectrophotometer, and the absorption spectrum of the filter from 400-700 nm measured against a water-saturated blank filter (see Thrane, Hessen & Andersen, 2014; Tassan & Ferrari, 1995 for method details).

Vertical zooplankton net hauls were taken from just above the bottom to the surface at the same site as samples for phytoplankton and chemistry, using a standard zooplankton net with 90 μm mesh size and a diameter of 40 cm. The reason for including the entire vertical profile was to include species and individuals that would be missing from the 0 - 5 m depth due to diurnal, vertical migration. The sample was screened on pre weighted 40 mm GF/C filters and frozen for determination of total zooplankton biomass. Before analysis the filters were thawed and dried at 60 $^{\circ}\text{C}$ for ca 24 h, or until there was no further weight loss. The volume-specific dry-weight (DW) was then divided by the estimated total volume filtered by the net (which clearly represent a minimum estimate of total mass). DW was converted to C by multiplying with 0.46 (Andersen & Hessen, 1991).

Statistical analysis

All multiple regressions and most univariate regressions were done with log-transformed data (noted as log-log below) to reduce heteroscedasticity and obtain normally distributed residuals. Chl *a* and phytoplankton biovolume were highly correlated ($R^2 = 0.83$, $p < 0.001$, log-log), and we used phytoplankton biovolume as the response variable in the further analyses of phytoplankton biomass. Model selection was done by backwards selection, removing non-significant variables until all were significant at the level of $p < 0.05$. In the multiple regressions of phytoplankton biovolume and PP, we estimated the relative contribution of each significant predictor variable to the total R^2 using the R-package *relaimpo* and the algorithm *lmg* (Grömping, 2006). The method is based on sequential R^2 s, but accounts for the order of the variable in the model by averaging over orderings. We also present standardized regression coefficients, i.e., the coefficients from a multiple regression where all variables (log-transformed) have been centered and standardized before analysis. The coefficients thus refer to how many standard deviations

the logarithm of the response variable will change when the logarithm of the predictor is changed by one standard deviation.

Results

The significant correlations between DOC and $K_0\text{PAR}$, total P, and CO_2 ($p < 0.001$; $R^2 = 0.57$, 0.20 and 0.14 , respectively) reflect the importance of DOM as a contributor to light, nutrients and CO_2 in these lakes. Omitting the three most eutrophic lakes that received additional P from agricultural activities improved these correlations. DOC also strongly correlated (univariate) with organic N ($R^2 = 0.38$, $p < 0.001$), with Fe ($R^2 = 0.50$, $p < 0.001$) and SiO_2 ($R^2 = 0.34$, $p < 0.001$).

The 2 orders of magnitude variation in CO_2 (from 0.82 to $133 \mu\text{M}$, with mean and median of 46.7 and $38.9 \mu\text{M}$) is much larger than the variability caused by changes in atmospheric concentrations. There was also a strong supersaturation of CO_2 in most lakes, with a mean $\text{CO}_2 > 2$ times atmospheric equilibrium (887 ppm). CO_2 was positively correlated with DOC ($p < 0.001$) yet with a pronounced scatter ($R^2 = 0.23$, $p < 0.001$). CO_2 contributed on average to 27% of TIC (mole CO_2 : mole TIC; 1st quartile = 0.15 , 3rd quartile = 0.34), while TIC was only weakly positively correlated with DOC ($R^2 = 0.056$, $p = 0.044$). For a full scatter-plot matrix of the included parameters, see S2).

Neither the area-specific primary production ($p = 0.42$), nor algal biovolume ($p = 0.74$, one outlier removed) correlated significantly with CO_2 alone (log-log). This would imply that primary production was not boosted by CO_2 , and also that primary production did not constitute a major regulator of CO_2 relative to DOC in these (mostly) low productivity lakes. The ratio of CO_2 : DOC decreased with increasing phytoplankton biovolume ($R^2 = 0.1$, $p < 0.01$, log-log) (or TP), which after all indicates that algae exert a detectable uptake of CO_2 . A positive correlation with TIC was found for phytoplankton biovolume ($R^2 = 0.34$, $p < 0.001$, log-log) and for PP_A ($R^2 = 0.11$, $p < 0.01$, log-log). TIC was positively correlated with TP however ($R^2 = 0.52$, $p < 0.001$, log-log), and including both TP and TIC as predictors, TIC became non-significant ($p > 0.05$) in multiple regressions for both phytoplankton biovolume and PP_A .

K₀PAR was strongly positively correlated with DOC ($R^2 = 0.57$, $p < 0.001$, log-log), and significantly, but less strong, with phytoplankton biovolume ($R^2 = 0.19$, $p < 0.001$, log-log). Combining the two variables explained 69 % of the variation in K₀PAR.

In a full regression model for PP_A with CO₂, TP, TN and DOC as predictors (Table 1, Fig. 2, panels A and C), CO₂ was not significant ($p = 0.82$). Both TP ($p < 0.0001$) and TN ($p < 0.01$) had significant positive slopes, while DOC had a significantly negative slope ($p < 0.0001$). The corresponding regression model for phytoplankton biovolume (Table 1 and Fig. 2, panels B and D) had TP as the by far most significant predictor ($p < 0.0001$). TN also contributed positively ($p = 0.02$), while DOC showed a negative relationship ($p = 0.01$). There was also a significant contribution from CO₂ ($p < 0.01$), but with a negative slope likely representing the uptake of CO₂ by phytoplankton. Note that while the upper panels in Fig. 2 (a and b) display standardized regression coefficients, the lower panels (c and d) provide the average contribution of each variable to the total explained variation (the R^2) of the multiple regression.

CO₂ concentration could not explain any of the variation in the maximum quantum yield of PSII (F_v/F_m) between lakes (Table 1). Nutrients and DOC, however, explained 41% of the variation in a multiple regression, with positive slopes for TP ($p < 0.0001$) and TN ($p < 0.01$), and a negative for DOC ($p < 0.001$).

A multiple regression with zooplankton biomass modelled as a function of TP, TN, CO₂, DOC, and phytoplankton biovolume rendered only TP ($p < 0.0001$) and DOC ($p < 0.001$) as significant predictors with positive and negative signs, respectively ($R^2 = 0.26$). Seston C:P (POC:POP, atomic ratio) ranged from 49 to 544 (mean 190). We found no clear relationship between C:P and the potential predictors CO₂, TP, and K₀PAR ($p > 0.05$ for all predictors in a multiple regression). Median molar TIC:TP and CO₂:P were 1213 and 313 respectively, but again seston C:P were not significantly correlated to any of these ratios. Neither the ratio of phytoplankton C (calculated on the basis of biovolume) to POP showed any significant relationship with these predictors. There was however a significant negative relationship between the phytoplankton fraction of seston C (phytoplankton C : POC) and DOC ($p < 0.01$, $R^2 = 0.11$, y-variable log-transformed; Fig. 3, upper panel), and an even stronger relationship between the light absorption by non algal particles and DOC ($p < 0.001$, $R^2 = 0.56$, y-variable log-transformed; Fig. 3,

lower panel). This clearly shows the increasing role of detritus with increased allochthonous inputs.

Discussion

Our study confirmed coloured DOM as a major determinant of light attenuation, nutrient concentrations and CO₂ in these fairly low-productive, boreal lakes. P exerted a strong positive impact on phytoplankton as well as zooplankton biomass, but still there was a net negative effect of increased DOM on lake productivity, mediated by the attenuation of photosynthetic active radiation (cf. Karlsson et al 2009; Thrane et al. 2014). While the negative impacts of DOM is mechanistically straight forward, a potential growth-promoting role of elevated CO₂ on lake productivity is controversial.

Lakes are generally supersaturated with CO₂ (Cole, Caraco & Kling 1994), and the degree of net heterotrophy typically increases with increased allochthonous inputs of DOM (Larssen et al. 2011). This partly reflects high rates of photo-oxidation but also high rates of mineralization by microbial heterotrophs (Tranvik et al. 2009; Larsen, Andersen & Hessen, 2011). The pronounced scatter in the CO₂ vs. DOC-regression nevertheless point to other determinants of CO₂. For more productive lakes, clearly autotroph production is a major determinant of CO₂, and also groundwater input could serve as an important source of inorganic C in lakes (Humborg *et al.*, 2010).

The negative impact of higher DOM was not offset by elevated levels of CO₂, i.e. we could not identify any net positive effect of CO₂ on phytoplankton biovolume or primary production (cf. Fig. 2). TIC did however give a positive correlation with both primary production and algal mass, but the positive correlation was likely linked to TP, which correlated quite strongly with TIC.

The minor impact of CO₂ on autotroph biomass and production in temperate lakes is in support of the classical works from Schindler et al. (1997) and on, but apparently runs counter to a number of experimental studies that have demonstrated strong, positive phytoplankton responses to CO₂. E.g. by keeping nutrients constant, Urabe, Togari & Elser (2003) were able to increase algal biomass almost 10-fold by increasing CO₂ from 360 ppm to 3500 ppm, which on the other hand increased cellular C:P correspondingly with adverse effect on *Daphnia* grazers. Similar effects have been found in marine

systems (Schoo, 2013), but the responses both in terms of biomass and stoichiometry is highly species dependant (Gervais & Riebesell, 2001; Urabe & Waki, 2009). Moreover experiments with immediate exposure to very high levels of CO₂ might yield transient responses, and clearly also eutrophic systems are more likely to face temporal CO₂-limitation (Urabe & Waki, 2009; Verspagen *et al.*, 2014) than low-productive sites with CO₂-supersaturation.

Some lake studies indicate an unimodal response of pelagic primary production to DOM with an initial stimulation of DOM which likely reflects that DOM is a major contributor of P, N, and Fe in oligotrophic boreal lakes, but with a turning point around 5 mg C l⁻¹ above which the negative effects of increased light attenuation becomes dominant (Karlsson *et al.*, 2009; Thrane, Hessen & Andersen, 2014; Seekell *et al.*, 2015). A similar unimodal response to DOM was also found for fish (Finstad *et al.*, 2014), which partly reflects reduced overall productivity at the base of the food web, probably in concert with reduced visibility of prey. We did not reveal any such inflection point around 5 mg C l⁻¹ related to CO₂, however.

Experimental manipulations with CO₂-additions can no doubt promote primary production at constant nutrient concentrations via increasing the biomass by C-uptake at constant intracellular pools of N and P, or by stoichiometrically balanced growth when also nutrients are added along with CO₂. The lakes in our study were, as boreal or DOM-rich lakes in general, highly supersaturated with CO₂ which speaks against a general CO₂-limitation. The study of Jansson, Karlsson & Jonsson (2012) reported, however, a striking decline in primary production when shifting from supersaturation to equilibrium by aeration, and a partial recovery following re-supersaturation. Aeration procedures also cause an increase in the pH and hence the HCO₃⁻:CO₂ ratio as well an increase in the O₂-concentrations and the O₂: CO₂-ratio which both could have impacted the results (Jansson, Karlsson & Jonsson, 2012). They also found significant positive correlations between chl *a* and CO₂ combined with TP in natural lakes in support of a direct stimulus of CO₂ on primary production. Increased CO₂ could be expected to boost C-fixation by releasing rubisco from its CO₂-deficiency (Badger *et al.*, 1998), but phytoplankton taxa have evolved more or less efficient mechanisms for increasing the intracellular CO₂ concentration (Reinfelder, 2011). CO₂-concentrations may also affect community

composition owing to species-specific requirements and abilities to sequester CO₂ (Reinfelder, 2011; Low-Décarie, Fussmann & Bell, 2011), and while single-species cultures all responded positively to elevated CO₂, mixed cultures also experienced negative responses due to competitive responses (Reinfelder, 2011).

Short-term experiments either with monocultures or mixed cultures may not mimic long-term equilibrium responses across lakes, and whether a systematic stimulation of overall productivity at the ecosystem level takes place under permanent supersaturation like that of most boreal lakes is not obvious. E.g. Hein (1997) found no evidence of inorganic C limitation of photosynthesis in naturally CO₂ supersaturated lakes, while in productive lakes with medium or low DIC-concentrations photosynthesis may indeed be C-limited (Verspagen *et al.*, 2014). An inherent problem with pCO₂ is, however, that it represents a snapshot of net balance between production (respiration, photo-oxidation) and algal uptake, as well as the net exchange with atmosphere. The fact that pCO₂ correlates strongly with DOC, combined the low biomass of pelagic autotrophs in these oligotrophic lakes, do however strongly suggest that CO₂-production strongly exceeds algal uptake. Hence it is very unlikely that the negative correlation between pCO₂ and phytoplankton biovolume can be accredited autotroph uptake, but rather is a spurious correlation.

If extra CO₂ yields growth by unbalanced uptake of C, the resulting increase in biomass would also imply increased C:P (or C:N) just as in the case of unbalanced light : nutrient ratio (Urabe & Sterner, 1997; Hessen, Færøvig & Andersen, 2002). We did not find any relationship between seston C:P and CO₂ or DIC, however. In principle, a stoichiometric response in the algal fraction with increased DOC (and CO₂) could be masked by an increased fraction of non-algal particles in the seston along the same gradient. Phytoplankton contributed in general less than 20% to seston C, and there was a significant negative relationship between the fraction of phytoplankton C to seston C. This clearly demonstrates that the fraction of C-rich detritus increases with increased degree of allochthony (Hessen *et al.* 2003, cf. also Fig. 2) which could override the impacts of K₀PAR and CO₂ on phytoplankton C:P. Less light and more nutrients would favour heterotrophic bacteria over phytoplankton, and would also favour a mixotroph strategy among the protists (Hessen, Andersen & Lyche 1990).

We did not detect any interactive effect between CO₂ and TP in our study, despite the fact that phosphate availability may affect CO₂-acquisition (Beardall, Roberts & Raven, 2005). From a stoichiometric point of view a C-limitation is unlikely with a mean TIC of almost 1800 µg C l⁻¹, compared to 1.3 µg for PO₄ l⁻¹ (and most sites around or below detection limits), and 5.7 µg l⁻¹ for TP, of which a major fraction would be organic and hardly bioavailable. This does not exclude the possibility of CO₂ boosting biomass, however, partly due the aforementioned stoichiometrically unbalanced growth and also because the CO₂-saturation for rubisco machinery is less than half-saturated at atmospheric equilibrium (Badger *et al.*, 1998).

The potential dichotomy related to light attenuation and CO₂ is but one of the potential contrasting impacts of allochthonous DOC on autotrophs. The increased light attenuation caused by DOC will be most prominent in the UV-region, hence DOC also offers photoprotection for autotrophs in the upper layers. Since also bacterial and fungal parasites and competitors, as well as grazers, may benefit from reduced UV, the net outcome of reduced UV on primary producers is not settled. Also DOM is a carrier of Fe that may stimulate autotroph production (Vrede & Tranvik, 2006). Hence the balance and net outcome of these partly contrasting drivers and effects with regard to autotroph productivity in lakes is thus not obvious.

Likewise the role of DOC for heterotrophs could work in opposite directions. Negative effects on primary production will affect the entire trophic ladder to zoobenthos, zooplankton and fish (Carpenter *et al.*, 2015), but DOC also provides a subsidiary source of allochthonous energy that could stimulate heterotrophic production (Hessen, Andersen & Lyche, 1990; Grey, Jones & Sleep, 2001; Cole *et al.*, 2011). TP turned up as the most significant predictor of zooplankton biomass, but with an additional negative effect of DOC. The TP effect is hardly surprising, since an impact on zooplankton production necessarily must act indirectly via food availability. Substituting TP with algal biovolume or chl *a* thus yielded almost exactly the same results.

The negative impact of DOC on zooplankton thus likely reflects that DOC negatively impact algal productivity and biomass. Allochthonous C may in itself serve as a source of energy for methanogenic and metanotrophic or heterotrophic bacteria that again may support zooplankton (Hessen, Andersen & Lyche, 1990; Tanentzap *et al.*,

2014). Moreover, increased K_0 PAR (caused by DOC) will limit the search field for visible predators like fish, potentially offering a larger low-light refugium for zooplankton. Although these mechanisms could dampen or partly offset the bottom-up impacts of reduced primary production, the net negative impact of elevated DOC on zooplankton was still negative. The median zooplankton : phytoplankton biomass ratio (C:C) was 0.21 (SD = 0.37), but did not vary with TOC or K_0 PAR, however, which could be interpreted as a negligible subsidy of other energy sources with increased allochthony. There are however some obvious pitfall in judging this based on biomass, rather than productivity ratios, and studies based both on radiotracers (Hessen, Andersen & Lyche, 1990) and stable isotopes (Grey, Jones & Sleep, 2001; Cole *et al.*, 2011) have demonstrated that there may be a significant flux of C from DOM to metazoans.

The ecosystem responses to different (or increasing) levels of DOM or DOC in lakes will clearly be both site-specific and also differ between short-term and long-term responses. An inherent problem related to these (and most other) ecosystem responses is the confounding effect of DOM-related drivers and that DOM yields a multitude of potential contrasting responses. The role of DOM for light attenuation and photosynthesis boils, however, down to physics and first principles, and overrides most of other responses, including that of CO₂. The old term “dystrophic” (misfed or bad fed) for colored, humic lakes may thus be valid for the consumers, but the overall negative impact of low light in these systems suggests *dysphotic* or *oligophotic* as a more appropriate term.

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References

- Ågren A., Berggren M., Laudon H. & Jansson M. (2008) Terrestrial export of highly bioavailable carbon from small boreal catchments in spring floods. *Freshwater Biology*, **53**, 964–972.
- Andersen T. & Hessen DO. (1991) Carbon, nitrogen, and phosphorus content of freshwater zooplankton. *Limnology and Oceanography*, **36**, 807–814.
- Apello C.A.J. & Postma D. (1993) *Geochemistry, groundwater and pollution*. 2nd ed. Rotterdam: A. A. Balkema Publishers..
- Arnell A., Harrison S.P., Zaehle S., Tsigaridis K., Menon S., Bartlein P.J., *et al.* (2010) Terrestrial biogeochemical feedbacks in the climate system. *Nature Geoscience*, **3**, 525–532.
- Badger M.R., Andrews T.J., Whitney S.M., Ludwig M., Yellowlees D.C., Leggat W., *et al.* (1998) The diversity and coevolution of Rubisco, plastids, pyrenoids, and chloroplast-based CO₂-concentrating mechanisms in algae. *Canadian Journal of Botany*, **76**, 1052–1071.
- Beardall J., Roberts S., & Raven J.A. (2005) Regulation of inorganic carbon acquisition by phosphorus limitation in the green alga *Chlorella emersonii*. *Canadian Journal of Botany*, **83**, 859–864.
- Carpenter S.R., Cole J.J., Pace M.L. & Wilkinson G.M. (2015) Response of plankton to nutrients, planktivory and terrestrial organic matter: A model analysis of whole-lake experiments. *Ecology Letters*, **19**, 230–239.
- Cole J.J., Caraco N.F. & Kling GW (1994) Carbon Dioxide Supersaturation in the Surface Waters of Lakes. *Science*, **265**, 1568–1570.
- Cole J.J., Carpenter S.R., Kitchell J., Pace M.L., Solomon, C.T. & Weidel B. (2011) Strong evidence for terrestrial support of zooplankton in small lakes based on stable isotopes of carbon, nitrogen, and hydrogen. *Proceedings of the National Academy of Sciences*, **108**, 1975–80.
- Dillon P.J. & Molot L.A. (2005) Long-term trends in catchment export and lake retention of dissolved organic carbon, dissolved organic nitrogen, total iron, and total phosphorus: The Dorset, Ontario, study, 1978–1998. *Biogeosciences*, DOI: 10.1029/2004JG000003

- Finstad A.G., Andersen T., Larsen S., Tominaga K., Blumentrath S., de Wit H.A., *et al.* (2016) From greening to browning: Catchment vegetation development and reduced S-deposition promote organic carbon load on decadal time scales in Nordic lakes. *Scientific Reports*, **6**, DOI: 10.1038/srep31944
- Finstad A.G., Helland I.P., Ugedal O., Hesthagen T. & Hessen D.O. (2014) Unimodal response of fish yield to dissolved organic carbon. *Ecology Letters*, **17**, 36–43.
- Garmo O.A., Skjelkvale B.L., De Wit H.A., Colombo L., Curtis C., Fölster J. *et al.* (2014) Trends in surface water chemistry in acidified areas in Europe and North America from 1990 to 2008. *Water Air and Soil Pollution*, **225**, doi:10.1007/s11270-014-1880-6
- Gervais F. & Riebesell U. (2001) Effect of phosphorus limitation on elemental composition and stable carbon isotope fractionation in a marine diatom growing under different CO₂ concentrations. *Limnology and Oceanography*, **46**, 497–504.
- Grey J., Jones R.I. & Sleep D. (2001) Seasonal changes in the importance of the source of organic matter to the diet of zooplankton in Loch Ness, as indicated by stable isotope analysis. *Limnology and Oceanography*, **46**, 505–513.
- Grömping U. (2006) Relative importance for linear regression in R: the package relaimpo. *Journal of Statistical Software*, **17**, 139–147.
- Hein M. (1997) Inorganic carbon limitation of photosynthesis in lake phytoplankton. *Freshwater Biology*, **37**, 545–552.
- Henriksen A *et al.* (1998) Northern European lake survey, 1995: Finland, Norway, Sweden, Denmark, Russian Kola, Russian Karelia, Scotland and Wales. *Ambio*, **27**, 80–91.
- Hessen D.O. (1988). Food webs and carbon cycling in humic lakes. pp. 285–315 in: Hessen, D.O. & Tranvik, L.J. *Aquatic humic substances*. Berlin: Springer-Verlag.
- Hessen D.O., Andersen T., Brettum P. & Faafeng B.A. (2003) Phytoplankton contribution to sestonic mass and elemental ratios in lakes: Implications for zooplankton nutrition. *Limnology and Oceanography*, **48**, 1289–1296.
- Hessen D.O., Andersen T. & Lyche A. (1990) Carbon metabolism in a humic lake: Pool sizes and cycling through zooplankton. *Limnology and Oceanography*, **35**, 84–99.
- Hessen D.O., Færøvig P. & Andersen T. (2002) Light, nutrients, and P: C ratios in algae:

- grazer performance related to food quality and quantity. *Ecology*, **83**, 1886–1898.
- Humborg C., Mörtz C.M., Sundbom M., Borg H., Blenckner T., Giesler R., *et al.* (2010) CO₂ supersaturation along the aquatic conduit in Swedish watersheds as constrained by terrestrial respiration, aquatic respiration and weathering. *Global Change Biology*, **16**, 1966–1978.
- Jansson M., Karlsson J., Jonsson A. (2012) Carbon dioxide supersaturation promotes primary production in lakes. *Ecology Letters*, **15**, 527–532. doi:10.1111/j.1461-0248.2012.01762.x
- Karlsson J, Byström P, Ask J, Ask P, Persson L, & Jansson M. (2009) Light limitation of nutrient-poor lake ecosystems. *Nature*, **460**, doi:10.1038/nature08179
- Kelly P.T., Craig N., Solomon C.T., Weidel B.C., Zwart J.A. & Jones S.E. (2016) Experimental whole-lake increase of dissolved organic carbon concentration produces unexpected increase in crustacean zooplankton density. *Global Change Biology* **22**, 2766-2775
- Kissman C.E.H., Williamson C.E., Rose K.C. & Saros J.E. (2013) Response of phytoplankton in an alpine lake to inputs of dissolved organic matter through nutrient enrichment and trophic forcing. *Limnology and Oceanography* **58**, 867-880.
- Kritzberg E.S. & Ekström, S.M. (2012). Increasing iron concentrations in surface waters – a factor behind brownification? *Biogeosciences* **9**, 1465 – 1478.
- Kromkamp J.C., Dijkman N.A., Peene J., Simis S.G.H. & Gons H.J. (2008) Estimating phytoplankton primary production in Lake IJssel- meer (The Netherlands) using variable fluorescence (PAM- FRRF) and C-uptake techniques. *European Journal of Phycology*, **43**, 327–44.
- Larsen S., Andersen T. & Hessen D.O. (2011) The *p*CO₂ in boreal lakes: Organic carbon as a universal predictor. *Global Biogeochemical Cycles*, **25**, doi:10.1129/2010GBO03864
- Loladze I. (2002) Rising atmospheric CO₂ and human nutrition: Toward globally imbalanced plant stoichiometry? *Trends in Ecology and Evolution*, **17**, 457–461.
- Low-Décarie E., Fussmann G.F. & Bell, G. (2011) The effect of elevated CO₂ on growth and competition in experimental phytoplankton communities. *Global Change Biology*, **17**, 2525–2535.
- Meili M. (1992) Sources, concentrations and characteristics of organic matter in

- softwater lakes and streams of the Swedish forest region. *Hydrobiologia*, **229**, 23–41.
- Monteith D.T., Stoddard J.L., Evans C.D., de Wit H.A., Forsius M., Høgåsen T., *et al.* (2007) Dissolved organic carbon trends resulting from changes in atmospheric deposition chemistry. *Nature*, **450**, 537–540.
- Orr J.C., Fabry V.J., Aumont O., Bopp L., Feely R.A., Doney S.C., *et al.* (2005) Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. *Nature*, **437**, 681–686.
- Phillips J.C., McKinley G.A., Bennington V., Bootsma H.A., Pilcher D.J., Sterner R.W., *et al.* (2015) The potential for CO₂-induced acidification in freshwater: A Great Lakes case study. *Oceanography*, **28**, 136–145.
- Reinfelder J.R. (2011) Carbon concentrating mechanisms in eukaryotic marine phytoplankton. *Annual Review of Marine Sciences*, **3**, 291–315.
- Solomon C.T., Jones S.E., Weidel B.C., Buffam I., Fork M.L., Kalrsson J. *et al.* (2015) Ecosystem Consequences of Changing Inputs of Terrestrial Dissolved Organic Matter to Lakes: Current Knowledge and Future Challenges. *Ecosystems* **18**, 376–389
- Schagerl M. & Donabaum K. (2003) Patterns of major photosynthetic pigments in freshwater algae. 1. Cyanoprokaryota, Rhodophyta and Cryptophyta. *Annales Limnology – International Journal of Limnology*, **39**, 35–47.
- Schindler D.W., Curtis P.J., Bayley S.E., Parker B.R., Beaty K.G. & Stainton M.P. (1997) Climate-induced changes in the dissolved organic carbon budgets of boreal lakes. *Biogeochemistry*, **36**, 9–28.
- Schoo K.L, Malzahn A.M., Krause E. & Boersma M. (2013) Increased carbon dioxide availability alters phytoplankton stoichiometry and affects carbon cycling and growth of a marine planktonic herbivore. *Marine Biology*, **160**, 2145–2155.
- Seekell D.A., Lapierre J.-F., Ask J., Bergström A.-K., Deininger A., Rodríguez P., *et al.* (2015) The influence of dissolved organic carbon on primary production in northern lakes. *Limnology and Oceanography*, **60**, 1276–1285.
- Sobek S., Algesten G., Bergström A.-K., Jansson M. & Tranvik L.J. (2003) The catchment and climate regulation of pCO₂ in boreal lakes. *Global Change Biology*, **9**, 630–641.
- Solheim A.L., Rekolainen S., Moe S.J., Carvalho L., Phillips G., Ptacnik R., *et al.* (2008)

- Ecological threshold responses in European lakes and their applicability for the Water Framework Directive (WFD) implementation: Synthesis of lakes results from the REBECCA project. *Aquatic Ecology*, **42**, 317–334.
- Spijkerman E. (2010) High photosynthetic rates under a colimitation for inorganic phosphorus and carbon dioxide. *Journal of Phycology*, **46**, 658–664.
- Sterner R.W., Andersen T., Elser J.J., Hessen D.O., Hood J.M., McCauley E., *et al.* (2008) Scale-dependent carbon:nitrogen:phosphorus seston stoichiometry in marine and freshwaters. *Limnology and Oceanography*, **53**, 1169–1180.
- Sterner R.W. & Elser J.J. (2002) *Ecological Stoichiometry*. 1st ed. Princeton: Princeton University Press..
- Tanentzap A.J., Szkokan-Emilson E.J., Kielstra B.W., Arts M.T., Yan N.D. & Gunn J.M. (2014) Forests fuel fish growth in freshwater deltas. *Nature Communications*, **5**, 4077.
- Tassan S. & Ferrari G.M. (1995) An alternative approach to absorption measurements of aquatic particles retained on filters. *Limnology and Oceanography*, **40**, 1358–1368.
- Thrane J.-E., Hessen D.O. & Andersen T. (2014) The Absorption of Light in Lakes: Negative Impact of Dissolved Organic Carbon on Primary Productivity. *Ecosystems*, **17**, 1040–1052.
- Urabe J., Togari J. & Elser J.J. (2003) Stoichiometric impacts of increased carbon dioxide on a planktonic herbivore. *Global Change Biology*, **9**, 818–825.
- Urabe J. & Sterner R.W. (1996) Regulation of herbivore growth by the balance of light and nutrients. *Proceedings of the National Academy of Sciences*, **93**, 8465–9.
- Urabe J. & Waki N. (2009) Mitigation of adverse effects of rising CO₂ on a planktonic herbivore by mixed algal diets. *Global Change Biology*, **15**, 523–531.
- Verspagen J.M.H., Van De Waal D.B., Finke J.F., Visser P.M., Van Donk E. & Huisman J. (2014) Rising CO₂ levels will intensify phytoplankton blooms in eutrophic and hypertrophic lakes. *PLoS One*, **9**, doi:10.1371/journal.pone.0104325
- Vrede T. & Tranvik L.J. (2006) Iron constraints on planktonic primary production in oligotrophic lakes. *Ecosystems*, **9**, 1094–1105.
- Wang D., Heckathorn S.A., Wang X. & Philpott S.M. (2012) A meta-analysis of plant physiological and growth responses to temperature and elevated CO₂. *Oecologia*, **169**, 1–13.

Williamson C.E., Overholt E, Pilla R.M., Leach T.H., Brentrup J.A., Knoll L.B. *et al.*
 (2015) Ecological consequences of long-term browning in lakes. *Scientific Reports* **5**,
 doi: 10.1038/srep18666.

Williamson C.E., Overholt E, Brentrup J.A., Pilla R.M., Leach T.H., Schladow S.G. *et al.*
 (2016) Sentinel responses to droughts, wildfires, and floods: effects of UV radiation
 on lakes and their ecosystem services. *Frontiers in Ecology and the Environment* **14**,
 102-109.

Yan N.D., Keller W., Scully N.M., Lean D.R.S. & Dillon P.J. (1996) Increased UV-B
 penetration in a lake owing to drought-induced acidification. *Nature*, **6578**, 141–143.

Yang, H., Andersen T., Dörch P., Tominaga K., Thrane J-E. & Hessen D.O. (2015)
 Greenhouse gas metabolism in Nordic boreal lakes. *Biogeochemistry*, **125**, 211-225.

Table 1. Multiple regressions of phytoplankton biovolume (PBV), area specific primary productivity (PP_A), maximum quantum yield of photosystem II (F_v/F_m), and zooplankton dry weight (ZDW) as a function of environmental variables. The number in brackets behind the coefficient represents the standard error of the estimate.

Response	Intercept	log(TP)	log(TN)	log(CO ₂)	log(DOC)	R ²	df
log(PBV)	0.425	0.748*** (0.101)	0.276* (0.115)	-0.263** (0.084)	-0.313* (0.121)	0.61	69
log(PP _A)	6.49	0.375*** (0.09)	0.34** (0.103)	NS	-0.629*** (0.105)	0.46	68
log(F _v /F _m)	-1.12	0.171*** (0.04)	0.153 ** (0.047)	NS	-0.183 *** (0.048)	0.42	68
log(ZDW)	3.373	0.672*** (0.138)	NS	NS	-0.666*** (0.172)	0.26	70

*** p < 0.001, ** p < 0.01, * p < 0.05, NS = not significant.

Legend to figures

Fig. 1. Lakes included in the survey. Size of symbols scales with the concentration of total organic carbon (mg TOC l⁻¹) as indicated by number in legend.

Fig. 2. Relative importance of predictor variables for area-specific primary productivity, PP_A (mg C m⁻² d⁻¹; A, C) and phytoplankton bio-volume (mg ww L⁻¹; B, D). Panels A and B show the standardized regression coefficients, i.e., the coefficients from a multiple regression where all variables (log-transformed) have been centered and standardized before analysis. They thus refer to how many standard deviations the logarithm of the response variable will change when the logarithm of the predictor is changed by one standard deviation. Error bars represent a 95% CI for the coefficient estimates. Panel C and D show the average incremental contribution of each predictor to the total R^2 of the model (noted above the bars; see methods). Error bars in C and D represent 95% bootstrap intervals for the incremental contributions, obtained by sampling with replacement from the original dataset (see methods).

Fig. 3. The relationship between phytoplankton C : seston C (upper), and the mean non-algal particle absorption coefficient (lower) and DOC. Note the log-transformed y-axis.

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