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Colored organic matter increases CO_2 in meso-eutrophic lake water through altered light climate and acidity

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Abstract

Many surface waters across the boreal region are browning due to increased concentrations of colored allochthonous dissolved organic carbon (DOC). Browning may stimulate heterotrophic metabolism, may have a shading effect constraining primary production, and may acidify the water leading to decreased pH with a subsequent shift in the carbonate system. All these effects are expected to result in increased lake water carbon dioxide (CO_2) concentrations. We tested here these expectations by assessing the effects of both altered allochthonous DOC input and light conditions through shading on lake water CO_2 concentrations. We used two mesocosm experiments with water from the meso-eutrophic Lake Erken, Sweden, to determine the relative importance of bacterial activities, primary production, and shifts in the carbonate system on CO_2 concentrations. We found that DOC addition and shading resulted in a significant increase in partial pressure of CO_2 (pCO_2) in all mesocosms. Surprisingly, there was no relationship between bacterial activities and pCO_2. Instead the experimental reduction of light by DOC and/or shading decreased the photosynthesis to respiration ratio leading to increased pCO_2. Another driving force behind the observed pCO_2 increase was a significant decrease in pH, caused by a decline in photosynthesis and the input of acidic DOC. Considering that colored allochthonous DOC may increase in a warmer and wetter climate, our results could also apply for whole lake ecosystems and pCO_2 may increase in many lakes through a reduction in the rate of photosynthesis and decreased pH.

Lakes play an essential role in the global carbon cycle as they are active sites for carbon transformations (Cole et al. 2007; Battin et al. 2009; Tranvik et al. 2009). Much of the organic and inorganic carbon processed in lakes originates from the surrounding terrestrial ecosystems (i.e., allochthonous). Dissolved organic carbon (DOC) and surface water partial pressure of carbon dioxide (pCO_2) are, on a spatial scale, positively correlated, which has been suggested to be due to in-lake mineralization of DOC (Hope et al. 1996; Sobek et al. 2003; Lapierre and del Giorgio 2012). The external carbon inputs and their mineralization in lakes largely contribute to the widespread supersaturation of carbon dioxide (CO_2) in lake surface waters and to its subsequent evasion to the atmosphere (Jonsson et al. 2007; Lapierre and del Giorgio 2012). However, there are also other processes, such as import of inorganic carbon (Weyhenmeyer et al. 2015), primary production (Balmer and Downing 2011), and distributions within the carbonate system (Lazzarino et al. 2009), which can affect CO_2 concentrations in surface waters.

Over the past two decades, increasing DOC concentrations, mostly derived from the terrestrial environment, have been observed in surface waters across large parts of the boreal region (Evans et al. 2005; Monteith et al. 2007; Filella and Rodriguez-Murillo 2014). Increasing DOC inputs can have, at least, three effects on pCO_2 (Fig. 1). First, allochthonous DOC may be readily available and degraded by microorganisms and subsequently converted into CO_2 in freshwaters. Hence, increased DOC input may stimulate CO_2 production by heterotrophs (Lennon 2004; McCallister and del Giorgio 2012; Guillemette et al. 2013). Indeed, studies have shown that bacterial respiration of allochthonous DOC is one of the key drivers of net heterotrophy in high-DOC lakes (Tranvik 1992; del Giorgio and Peters 1994). Also, heterotrophic bacteria may be more efficient at taking up nutrients than phytoplankton under high-DOC conditions leading to repressed phytoplankton production and subsequent decrease in phytoplankton CO_2 uptake (Jansson et al. 2007; Ask et al. 2009).

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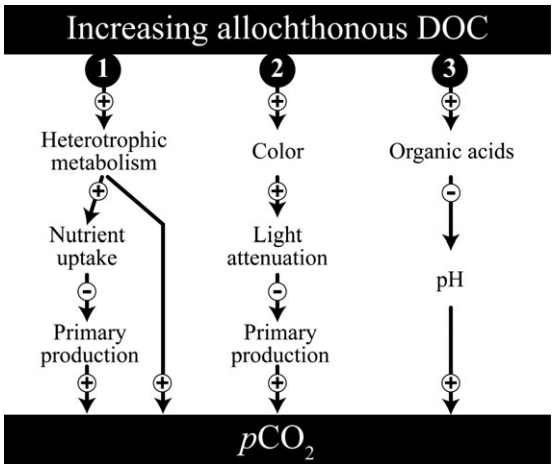

Fig. 1. Conceptual model of three possible main effects of increased allochthonous DOC on pCO_2 concentrations in lake water discussed in this study. Plus indicates a positive effect, and minus indicates a negative effect.

Second, allochthonous DOC generally contains large proportions of humic-like components with high amounts of aromatic structures, which give water a brownish color. These chromophoric aromatic structures are effective at absorbing photosynthetically active radiation (PAR); hence, allochthonous DOC may have a strong positive effect on light attenuation (Jones 1992; Pace and Cole 2002). This increased light attenuation can constrain primary production as a large fraction of PAR is absorbed by the DOC rather than by the photoautotrophs (Jones 1992). For instance, Thrane et al. (2014) found that chromophoric DOC absorbed, on average, more than 50% of PAR in the majority of their 75 Northern European study lakes, which spanned a large DOC range. Consequently, shading may further enhance net heterotrophy of high-DOC lakes (Cole et al. 2000).

Third, allochthonous DOC is partly composed of organic acids; hence, DOC can have an acidifying effect and lower the pH. A decreased pH could subsequently lead to an increase in free CO_2 as the distribution within the carbonate system shifts and the proportion among free CO_2, bicarbonate (HCO_3^-), and carbonate (CO_3^{2-}) changes. To underline the importance of DOC as a regulator of pH, increased DOC is one of the major factors contributing to spring flood pH decline in boreal streams of northern Sweden (Laudon and Bishop 1999; Laudon et al. 2001). Conversely, elevated pCO_2 levels due to enhanced bacterial respiration or decreased primary production could also lead to decreased pH. Decreasing pH trends were observed in clear water lakes in northern Wisconsin during winter, when primary production was low, and it was suggested that this was due to build-up of under ice pCO_2 levels (Kratz et al. 1987).

All three effects of increasing allochthonous DOC inputs could result in increased CO_2 concentrations (Fig. 1), yet the relative importance of these three effects are unknown. The knowledge gap is particularly apparent for eutrophic lakes, as most studies on carbon processing in inland waters have been performed in boreal oligotrophic lakes (Sobek et al. 2003; Alin and Johnson 2007; Ask et al. 2009). However, in eutrophic lakes, CO_2 sources may be outweighed by fixation of CO_2 by primary production, making them net autotrophic and CO_2 sinks, particularly during summer (Balmer and Downing 2011). Increased DOC input could potentially switch a eutrophic lake from being a net sink to become a net source of atmospheric CO_2. In oligotrophic lakes, it has been shown that moderate DOC input can have a positive effect on primary production due to enhanced nutrient availability (Seekell et al. 2015*a*). However, since eutrophic lakes are not as nutrient limited, the negative effect of allochthonous DOC input on biological CO_2 uptake due to increased light attenuation may be greater than the potential positive effect due to increased nutrient availability in eutrophic lakes, warranting further investigations in those ecosystems. Here, we used two mesocosm experiments with water from the meso-eutrophic Lake Erken, Sweden, to test the effect of allochthonous DOC input and altered light conditions through shading on CO_2 production. Furthermore, we aimed to determine the relative importance of bacterial activities, primary production, and shifts in the carbonate system on lake water CO_2 concentrations.

We propose that increased allochthonous DOC input will lead to enhanced CO_2 concentrations, which, in meso-eutrophic lakes, will result in a reversal from net uptake to net release of CO_2 (Fig. 1). We tested three hypotheses: (1) allochthonous DOC input stimulates bacterial activities, thus resulting in increased CO_2 concentrations; (2) increased light attenuation by allochthonous DOC hampers CO_2 uptake by autotrophs; and (3) an increase in allochthonous DOC decreases pH, causing a shift in the carbonate system, leading to increased CO_2 concentrations.

Methods

Field site and experimental mesocosms

We conducted two mesocosm experiments with water from Lake Erken (59°51′N, 18°36′E), a meso-eutrophic dimictic lake in eastern Sweden, with a lake surface area of 24.2 km^2, a mean depth of 9 m, and maximum depth of 21 m (Pettersson 1990). The mesocosms consisted of high-density polyethylene, white opaque, open top cylinders, 2 m deep with a diameter of 0.92–1.01 m. A total of 20 mesocosms were held on a fixed and floating wooden jetty, and positioned 10–20 m from the shore sitting approximately 0.6 m above the lake bottom (Fig. 2).

Experimental designs

The mesocosm experiments were part of a larger study which aimed to investigate the influence of addition of allochthonous DOC and shading on biogeochemical processes and the food web. Two crossed full factorial design experiments

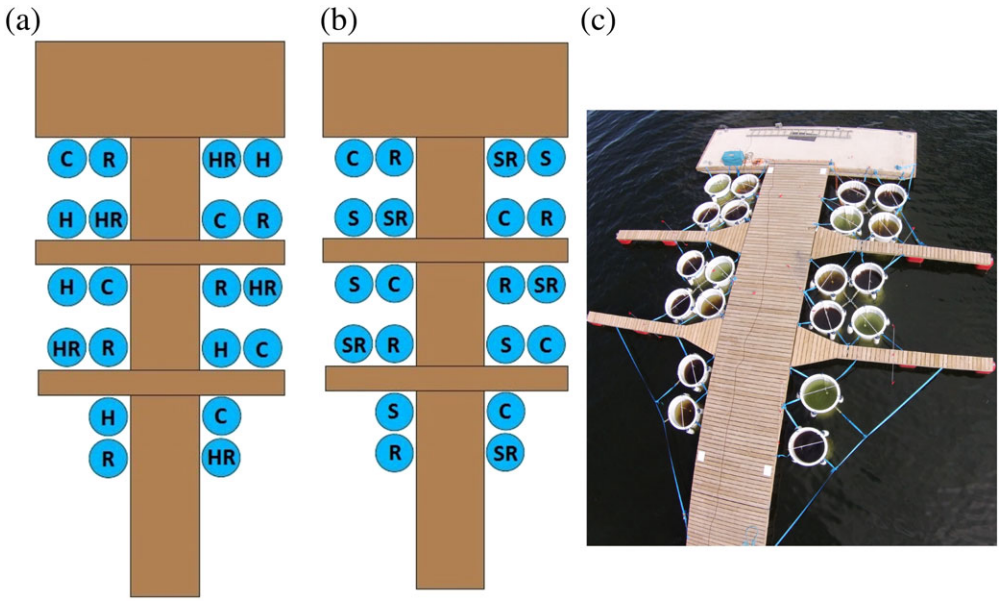

Fig. 2. Schematic of the experimental setup for **(a)** Experiment A and **(b)** Experiment B and **(c)** an aerial photograph of the mesocosm Experiment A. The circles in **(a)** and **(b)** represent the mesocosms and illustrate the different treatments. Treatments for Experiment A: (1) addition of reverse osmosis concentrate of DOC from humic stream water (R); (2) DOC from HuminFeed® (H); (3) a mix of DOC from reverse osmosis and HuminFeed (HR); and (4) no addition of DOC (C). Treatments for Experiment B: (1) addition of reverse osmosis concentrate of DOC from humic stream water (R); (2) covering of the outside of the mesocosms using black polyethylene film and on top using black nylon chiffon fabric, to generate a shading effect (S); (3) addition of DOC and shading (SR); and (4) no addition of DOC or shading (C).

with five replicates of four treatments were run between 15 June and 13 July (Experiment A) and between 10 August and 07 September (Experiment B) 2016. The mesocosms were filled up, at the commencement of the experimental periods (i.e., Day 0), to 1.65 m with filtered (through 200 μm to remove large plankton, algal colonies, and fish) lake water, leading to volumes between 1000 and 1300 liters. The mesocosms were cleaned between the two experiments and refilled with lake water for Experiment B. Additional zooplankton were added from assembled open-water zooplankton tows collected in Lake Erken for controlled studies of the effect of DOC addition on the food web (separate study). In the first experiment (A), we added approximately 14 individuals of large zooplankton (large Cladocerans including Daphnia and Copepods) from natural zooplankton communities of Lake Erken per liter of lake water to all mesocosms. In the second experiment (B), approximately 34 individuals of large zooplankton (Cyclopids and Cladocerans) per liter were added. The difference in zooplankton addition was due to the difference in zooplankton catch for the same effort, thus the increased zooplankton density in Experiment B mimics natural lake conditions later in summer. During the period between the two experiments, there was also high phytoplankton production in Lake Erken, likely due to increased water temperature, leading to lower pCO_2 levels and higher chlorophyll *a* (Chl *a*) concentrations at the commencement of Experiment B. All mesocosms were mixed manually twice daily throughout the duration of both experiments to avoid stratification, using a disc mounted on a shaft, a method shown to minimize impact on plankton biomass (Striebel et al. 2013).

In Experiment A, we manipulated the amount of allochthonous DOC. Four treatments were set up: (1) addition of DOC concentrated from a humic stream draining a forested wetland (59°92′N, 17°34′E), with a DOC concentration of 37.7 (± 0.49 SE) mg L^{-1}; the stream water was filtered (0.2 μm) with a submersible pump through 10-in. filter cartridges, and DOC was concentrated by reverse osmosis using a Real Soft PROS/2S unit as described by Serkiz and Perdue (1990), to a final concentration of approximately 800 mg L^{-1} (i.e., reverse osmosis); (2) DOC from HuminFeed® (Humintech, GmbH), an alkaline extract of Leonardite, which has previously been used as a humic matter source in aquatic studies (Heinze et al. 2012; Rasconi et al. 2015) (i.e., HuminFeed); (3) a mix of reverse osmosis concentrate and HuminFeed adding equal amounts of DOC from the two sources (i.e., mixed); and (4) no addition of DOC (i.e., control) (Fig. 2a). For initial DOC concentrations after DOC manipulations, see Table 1. The reverse osmosis concentrate was stored at dark at 4°C until the beginning of the experiment (for 21–77 d). The reverse osmosis concentrate and the HuminFeed (dissolved in MilliQ before addition on site) were added to the mesocosms to increase the in situ DOC concentration by about 5 mg L^{-1} in the reverse osmosis and the HuminFeed treatments and by about 10 mg L^{-1} in the mixed treatment. These DOC concentrations were chosen to represent natural DOC levels in boreal lakes, while also have a notable effect on pCO_2 (Sobek et al. 2003) as well as being

Table 1. Concentrations of DOC, BCP, Chl *a*, pH, total nitrogen (N), and total phosphorous (P) on the first sampling day (the day after DOC additions) of two mesocosm experiments with DOC additions and/or changed light climate through shading. For all variables, data are presented as mean values ± standard error (SE). Treatments for Experiment A were: no addition of DOC (control), addition of DOC from concentrated humic stream water using reverse osmosis (reverse osmosis), addition of DOC from HuminFeed (HuminFeed), and a mix of DOC from reverse osmosis concentrate and HuminFeed (mixed). Treatments for Experiment B were: no addition of DOC (control), addition of DOC from concentrated humic stream water using reverse osmosis (reverse osmosis), increased light attenuation via shading using shading cloth (shading), and both DOC addition from reverse osmosis concentrate and shading (DOC-shading).

	Treatments			
Experiment A	**Control**	**Reverse osmosis**	**HuminFeed**	**Mixed**
DOC (mg L^{-1})	13.0±0.05	18.1±0.10	18.4±0.06	23.5±0.05
BCP (μg C L^{-1} h^{-1})	0.50±0.08	0.52±0.05	0.48±0.09	0.47±0.07
Chl *a* (μg L^{-1})	2.21±0.82	2.45±1.56	1.83±0.97	3.06±1.14
pH	8.28±0.07	8.18±0.07	8.29±0.03	8.17±0.06
Total N (μg L^{-1})	613±11.80	743±7.70	860±90.90	905±31.80
Total P (μg L^{-1})	14.5±0.51	15.5±0.46	17.1±1.88	17.2±0.76
Experiment B	**Control**	**Reverse osmosis**	**Shading**	**DOC-shading**
DOC (mg L^{-1})	12.0±0.01	16.4±0.08	12.0±0.05	16.4±0.04
BCP (μg C L^{-1} h^{-1})	0.92±0.13	1.13±0.13	0.95±0.15	0.93±0.13
Chl *a* (μg L^{-1})	13.7±4.68	15.6±2.89	14.5±2.69	14.6±2.58
pH	8.69±0.05	8.59±0.04	8.64±0.02	8.53±0.05
Total N (μg L^{-1})	711±35.26	811±4.86	692±4.94	798±28.60
Total P (μg L^{-1})	21.5±1.14	22.9±1.20	22.8±1.26	22.4±1.02

logistically feasible. We expected a subsidy effect (in terms of energy release through oxidation of terrestrial organic material) from the reverse osmosis concentrate whereas the HuminFeed was assumed to predominantly affect the light climate. HuminFeed has previously been used to reduce light climate in a mesocosm experiment testing the effect of changed light climate on community resilience and stability (Hillebrand et al. 2017). The reverse osmosis, HuminFeed, and mixed treatments increased water color by 300%, 1400%, and 1700%, respectively, compared to the control. Water color was measured as absorbance at 420 nm in a 5-cm quartz cuvette using a Lambda 40 UV/VIS spectrophotometer (Perkin Elmer) after filtering through a glass microfiber filter (approximately 1.2μm pore size, Grade GF/C, Whatman^{TF}, GE Healthcare).

In Experiment B, we again manipulated the DOC input by addition of reverse osmosis concentrate. However, we covered the top of mesocosms with a black mesh and the outside with black plastic to alter and maintain natural DOC concentrations, rather than using HuminFeed. Again, four treatments were set up (for initial DOC concentrations after DOC manipulations see Table 1): (1) addition of reverse osmosis concentrate of DOC from the same humic stream water as in Experiment A (i.e., reverse osmosis); (2) covering of the outside of the mesocosms using black polyethylene film and on top using black nylon chiffon fabric, to generate a shading effect (i.e., shading); (3) addition of reverse osmosis concentrate of DOC and shading (i.e., DOC-shading); and (4) no DOC addition or shading (i.e., control) (Fig. 2b). In the treatments with added DOC, that is, the reverse osmosis and DOC-shading treatments, water color increased by 220% and 210%, respectively, compared to the control. For both experiments, DOC was added only once, at Day 0, and the first measurements were conducted the following day. The black nylon chiffon fabric reduced PAR at the water surface by 60.7% (± 0.8 SE). After 1 week, three young-of-the-year perch (*Perca fluviatilis L.*) of approximately the same biomass (4.37 g ± 0.14 SE) were added to each of the mesocosms in Experiment B, to investigate the effect of DOC addition on the food web (separate study). The fish were caught by seine netting from Lake Erken (permit C59/15, authorized by the Uppsala board of animal ethics).

Starting DOC concentrations ranged between 13.0 and 23.5 mg L^{-1} for Experiment A and between 12.0 and 16.4 mg L^{-1} for Experiment B (Table 1). Chemical conditions for the lake water observed through the monitoring in Lake Erken are equivalent to conditions in the control treatment at the commencement of the experiments (as reported in Table 1).

Measurements

Manual sampling of pCO_2 was conducted weekly, between 10:00 and 14:00 (Central European Summer Time [CEST]), using the headspace equilibrium method (described in Sobek et al. [2003]) as modified by Kokic et al. (2015). From each mesocosm, 30 mL of water was taken with a syringe right below the surface followed by adding 30 mL of ambient air to

create a headspace. Initially, triplicates were taken to test the reproducibility of the measurements. They varied on average by 1.5%; hence, single sample was sufficient to provide a good estimate of the pCO_2 in the surface water of each mesocosm. Equilibrated gas samples were analyzed on a portable infrared gas analyzer (IRGA, EGM-4) within 5 min of sampling. The pCO_2 was calculated according to Weiss (1974) using the appropriate Henry's constant after correcting for temperature, atmospheric pressure, and the amount of ambient air CO_2 added. Sampling for pCO_2 was performed first at each sampling occasion to avoid outgassing due to turbulence and disturbance from water sampling. Water samples for dissolved inorganic carbon (DIC) analyses were taken directly after sampling for pCO_2 at each sampling occasion. Aliquots of 17 mL were injected into gas tight glass vials leaving no headspace and later analyzed on a Sievers 900 TOC analyzer (GE Analytical Instruments).

Water samples of 15–18 liters per mesocosm were collected weekly, between 11:00 and 15:00 (CEST), using a tube sampler (1.5 m long, ~ 3 liter volume). Water was sampled from five to six different places throughout the water column in the mesocosms and pooled for subsampling to minimize stochasticity. Aliquots of 50 mL were filtered (approximately 0.7 μm effective pore size, grade GF/F, Whatman^{TF}, GE Healthcare) and analyzed for DOC concentration using a Sievers M9 TOC analyzer (GE Analytical Instruments).

Four aliquots of 1.7 mL (three replicates and one blank) of pooled water from each mesocosm were used to determine bacterial carbon production (BCP) via incorporation of ^{3}H-leucine into the protein fraction using the protocol of Smith and Azam (1992). The samples were incubated at in situ temperatures at a final leucine concentration of 100 μmol L^{-1} for 1 h in the dark. Additionally, 580 mL of water was taken from the pooled samples for Chl *a* analysis. Samples were vacuum filtrated through a glass microfiber filter (approximately 1.2 μm effective pore size, Grade GF/C, Whatman^{TF}, GE Healthcare), frozen at −20°C in the dark until further analysis. Following ethanol extraction (95%), samples were analyzed on a Lambda 40 UV/VIS spectrophotometer (Perkin Elmer) in a 1-cm cuvette at the wavelengths of 665 and 750 nm following the ISO 10260 standard technique (e.g., Strombeck and Pierson 2001; Kutser et al. 2005). Furthermore, total nitrogen (N) and total phosphorous (P) were analyzed on unfiltered pooled water samples on a SEAL AutoAnalyzer 3HR (Seal Analytical).

We measured pH between 15:00 and 17:00 (CEST) directly in the mesocosms on a weekly basis using a YSI multiprobe (EXO2 Multiparameter Sonde, YSI). As it is difficult to disentangle whether pH drives CO_2 or CO_2 drives pH, we quantified a CO_2 effect on pH by accounting for potential pH changes through bacterial mineralization and primary production. In addition to pH, light was measured weekly at seven depths in each mesocosm using a handheld light meter (Li-Cor LI-A, LI-COR) equipped with a light sensor (Li-Cor, LI-192 SA Underwater Quantum, LI-COR). Based on the light measurements, we calculated the vertical light attenuation coefficient for PAR (K_d) for each mesocosm. Using K_d, we could calculate the average light availability (meanPAR) throughout the entire 1.65 m water column for each mesocosm using an equation modified from Minor et al. (2016)

$$\text{meanPAR} = \left(\text{PAR}_{(Z=0)}\right)\left(K_d z\right)^{-1}\left(1-e^{-K_d z}\right)$$

where $PAR_{(Z = 0)}$ is the light intensity immediately at the surface at the depth of 0 m, K_d is the vertical light attenuation coefficient for PAR and z is the depth of the mesocosms (i.e., 1.65 m). The $PAR_{(Z = 0)}$ was set to 100% for all mesocosms without shading cloth, whereas for the shading and DOC-shading treatments $PAR_{(Z = 0)}$ was set to 39.3% to account for the reduction of incoming PAR at the water surface due to the black fabric covering those treatments.

Statistics

We performed the mixed-effect model repeated measures analyses of variances (RM-ANOVA) to test for differences in pCO_2, BCP, Chl *a*, pH, and light climate between treatments, with mesocosm ID as a random factor. Additionally, we performed one-way ANOVAs on the measurements of pCO_2, BCP, Chl *a*, and pH taken on the first day of the experiments to evaluate the direct effect of DOC on these parameters. Where significant differences were detected, multiple comparisons of means within treatment groups were performed using the post hoc Tukey Test. Statistical analyses were performed in the software package JMP version 13.0.0 (SAS Institute 2013) or R Version 1.0.136 (R-Development-Core-Team 2010). Significance was set at an alpha level of 0.05 for all tests. Data were tested for normality using the Shapiro–Wilk test and for homogeneity of variance using Bartlett's test.

Results

Changes in light climate

In Experiment A, additions of DOC significantly reduced the light throughout the water column in the mesocosms, with the DOC from HuminFeed having a stronger effect on light attenuation than DOC from the reverse osmosis concentrate (Table 2). However, there was no significant difference in K_d or meanPAR between the HuminFeed and mixed treatments (Table 2). Approximately 35% of incoming light was available for photosynthesis throughout the water columns of the control treatments, whereas in the HuminFeed and mixed treatments, the light availability was reduced to merely 12% of incoming PAR (Table 2).

In Experiment B, addition of black mesh fabric on top of the mesocosms and black plastic around the outside resulted in significantly less light being available for photosynthesis throughout the water column compared to the control and the reverse osmosis treatments (Table 2). Only 10% and 11%

Table 2. Vertical light attenuation coefficient for PAR (K_d) ± SE and the average PAR (meanPAR) ± SE throughout the water columns in two mesocosm experiments. Letters in parentheses refer to results from Tukey's post hoc test following one-way ANOVAs. Treatments not connected by the same letter are significant different at 0.05. See legend of Table 1 for explanation of treatments.

Treatments	K_d (m^{-1})	MeanPAR (μmol photons m^{-2} s^{-1})
Experiment A		
Control	1.68±0.09 (A)	35.1±1.49 (A)
Reverse osmosis	2.40±0.11 (B)	25.8±1.07 (B)
HuminFeed	4.90±0.12 (C)	12.54±0.31 (C)
Mixed	4.99±0.15 (C)	12.46±0.45 (C)
Experiment B		
Control	1.70±0.10 (A)	35.9±2.75 (A)
Reverse osmosis	2.15±0.11 (AB)	28.6±1.35 (A)
Shading	2.20±0.13 (AB)	11.4±0.62 (B)
DOC-shading	2.56±0.15 (B)	10.0±0.67 (B)

of incoming PAR was available to phytoplankton in the shading and DOC-shading treatments, respectively (Table 2). Contrary to what was observed in Experiment A, there was no difference in meanPAR between the control and the reverse osmosis treatments in Experiment B (Table 2).

Effects of DOC input and changed light climate on pCO_2

In Experiment A, addition of DOC from reverse osmosis concentrate resulted in a rapid increase in pCO_2 with significantly higher pCO_2 in the reverse osmosis and mixed treatments than in the control and HuminFeed treatments ($F_{3,16} = 29.6$, $p < 0.0001$). In the control, pCO_2 appeared to steadily decrease throughout the experiment, and by Week 2, the system had switched from being oversaturated to being undersaturated in CO_2 relative to the atmosphere (Fig. 3a). In the mixed treatment, pCO_2 appeared to increase considerably during the first week, but after 2 weeks, pCO_2 started to decline (Fig. 3a). From Week 1 onward, the highest pCO_2 was observed when both HuminFeed and reverse osmosis concentrate were added (mixed treatment), while there was no difference in pCO_2 between the reverse osmosis and HuminFeed treatments (Fig. 3a; Tables 3, 4).

On Day 1 in Experiment B, there were already significantly higher pCO_2 in the DOC addition treatments (i.e., reverse osmosis and DOC-shading) relative to the control ($F_{3,16} = 13.6$, $p = 0.0001$). During the first 2 weeks of the experiment, pCO_2 in the DOC-shading treatment appeared to increase steadily, and after 2 weeks, the system had switched from being a sink of CO_2 to being a source of CO_2 (Fig. 3b). However, by Week 3, pCO_2 levels were again below atmospheric concentrations, while all other treatments were CO_2 sinks during the entire Experiment B. Throughout the experiment, highest pCO_2 was observed in the DOC-shading combined treatment, which had significantly higher pCO_2 than all other treatments (Tables 3, 4). No difference in pCO_2 was observed between the reverse osmosis and the shading treatments; however, these treatments had significantly higher pCO_2 than the control (Tables 3, 4).

Effects of DOC input and changed light climate on BCP, Chl *a*, and pH

In Experiment A, BCP ranged between 0.50 and 0.96 μg C L^{-1} h^{-1}; however, there was no significant difference between treatments on Day 1 ($F_{3,16} = 0.48$, $p = 0.703$) or throughout the duration of the experiment (Fig. 3c; Table 3). As with BCP, there was no difference in Chl *a* between treatments on Day 1 ($F_{3,16} = 0.99$, $p = 0.423$). Throughout the duration of the experiment, we observed significant differences in Chl *a* between treatments, although this was not affected by time (Tables 3, 4). Chl *a* concentrations varied between 0.7 and 3.5 μg L^{-1} and were significantly higher in the HuminFeed and mixed treatments than in the control and reverse osmosis treatments (Fig. 3e; Table 4). On Day 1, there was already significantly lower pH in the reverse osmosis and mixed treatments compared to the control and HuminFeed treatments ($F_{3,16} = 4.04$, $p = 0.026$). The pH ranged from 8.0 to 8.4 throughout the experiment and differed significantly between treatments; however, this was dependent on time (Fig. 3g; Table 3). The control treatment had significantly higher pH than all other treatments and lowest pH was observed in the mixed treatment (Tables 3, 4).

In Experiment B, there was no difference in BCP between treatments on Day 1 ($F_{3,16} = 2.64$, $p = 0.085$). BCP ranged from 0.67 to 0.88 μg C L^{-1} h^{-1}, and as in Experiment A, there was no significant difference in BCP between treatments (Fig. 3d; Table 3). Again, there was no difference in Chl *a* between treatments on Day 1 ($F_{3,16} = 0.26$, $p = 0.854$); however, throughout the experiment, significant differences in Chl *a* between treatments were observed (Table 3). Chl *a* concentrations ranged between 5.7 and 20.0 μg L^{-1}, and contrary to our expectations, we found highest Chl *a* in the DOC-shading treatment (Fig. 3f; Table 4). As in Experiment A, there was a significant difference in pH already on Day 1 in Experiment B with significantly lower pH in the reverse osmosis and the DOC-shading treatments (i.e., all treatments with reverse osmosis DOC addition) compared to the other two treatments ($F_{3,16} = 13.3$, $p = 0.0001$). The pH was generally higher in Experiment B, ranging from 8.4 to 8.9 (Fig. 3h). There was a significant difference in pH between treatments with the lowest pH observed in the DOC-shading treatment throughout the experiment (Tables 3, 4).

Discussion

Our study demonstrates that browning due to increased input of colored allochthonous DOC can increase CO_2

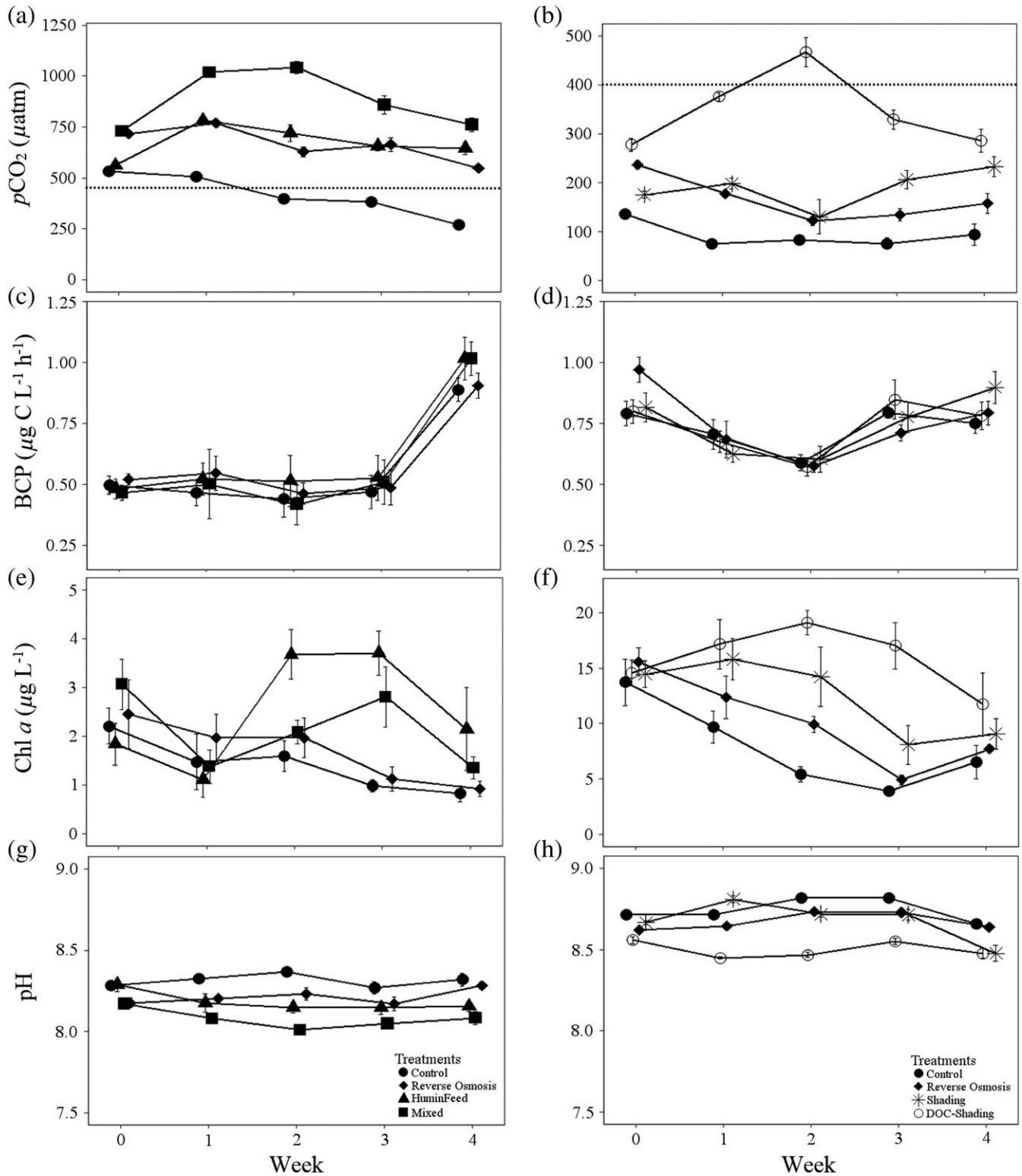

Fig. 3. Weekly variations in water chemistry under four treatments (see legends in figure) during two 4-week mesocosm experiments with DOC additions and/or changed light climate through shading. The left panel shows the results of Experiment A and the right panel of Experiment B. Panels **(a and b)** refers to the pCO_2, panels **(c and d)** to the BCP, panels **(e and f)** to Chl *a*, and panels **(g and h)** to pH. Values are mean (± SE, $n = 5$). Error bars are sometimes so small that they are hidden within the symbols. The dotted line in panels **(a and b)** represents the partitioning of oversaturation and undersaturation of CO_2 relative to the atmosphere.

Table 3. F-statistics and *p* values from mixed-effect model RM-ANOVA to test for differences in pCO_2, BCP, Chl *a*, and pH observed in two 4-week mesocosm experiments.

	F				*p*			
	pCO_2	BCP	Chl *a*	pH	pCO_2	BCP	Chl *a*	pH
Experiment A								
Treatment	36.2	0.2	5.5	32.0	<0.0001*	0.8906	0.0086*	<0.0001*
Time	17.7	125.3	3.1	0.5	0.0007*	<0.0001*	0.0965	0.5013
Treatment × time	5.5	1.5	3.0	25.8	0.0087*	0.2573	0.0606	<0.0001*
Experiment B								
Treatment	80.1	0.2	20.0	35.6	0.8311	<0.0001*	<0.0001*	<0.0001*
Time	2.2	0.1	39.6	1.3	<0.0001*	<0.0001*	0.0009*	<0.0001*
Treatment × time	3.8	1.4	2.0	2.7	0.2521	0.0049	0.0477	0.0016

*Significant at $p > 0.05$.

Table 4. Results from Tukey's post hoc analyses following mixed-effect model RM-ANOVA, where significant differences were detected. Treatments not connected by the same letter are significantly different at a significance level of 0.05. See legend of Table 1 for explanation of treatments.

	pCO_2	Chl *a*	pH
Experiment A			
Control	A	A	A
Reverse osmosis	B	AB	B
HuminFeed	B	B	B
Mixed	C	B	C
Experiment B			
Control	A	A	A
Reverse osmosis	B	AB	A
Shading	B	B	A
DOC-shading	C	C	B

concentrations in lake water, which is in agreement with previous research (Lennon 2004; Guillemette et al. 2013). Surprisingly, there was no difference in BCP between treatments in either of the experiments; hence, we found no support for our Hypothesis 1 (Fig. 1) that increased allochthonous DOC input stimulates bacterial activities (Table 3; Fig. 3). In Experiment A, average BCP was 0.55 and 0.58 μg C L^{-1} h^{-1} for the control and the mixed treatment, respectively. This is equivalent to an average of 0.42 to 0.45 g carbon assimilated by bacteria per mesocosm in the control and mixed treatments, respectively, for the entire experiment (based on mean values for each treatment multiplied by experimental time and volume of mesocosms). A bacterial growth efficiency of 30% would correspond to a bacterial mineralization of, on average, 1.84 μg C L^{-1} h^{-1} for the control treatment and 1.94 μg C L^{-1} h^{-1} for the mixed treatment. We calculated a bacterial carbon respiration of 1.00 and 1.05 g per mesocosm, for the control and mixed treatments, respectively, for the entire experiment. Corresponding bacterial respiration for Experiment B were 1.31 and 1.33 g carbon per mesocosm for the control and DOC-shading treatments, respectively. However, in Experiment A, we saw a loss in DOC of 0.52 g in the control and of 2.36 g in the mixed treatment, while in Experiment B, DOC loss was 0.46 and 1.00 g carbon per mesocosm in the control and DOC-shading treatments, respectively. Although a part of the added DOC may have been consumed by the bacteria and accounted for some of the observed pCO_2 increase, carbon consumptions were equal in all treatments. Consequently, part of the carbon consumed by bacteria must have been sustained by autochthonous sources (i.e., primary production).

Closely related to our study, Lennon (2004) demonstrated in a mesocosm experiment that bacterial production increased significantly with DOC enrichment and argued that microbial metabolism of the terrestrial subsidy was responsible for the observed increase in CO_2. Although inorganic nutrients were positively correlated with subsidy supply in the study by Lennon (2004), they were not responsible for the increased CO_2 as inputs of N and P alone did not account for increasing CO_2, suggesting that CO_2 was less responsive to inorganic nutrients than organic material. Lennon (2004) did, however, increase the DOC concentration in the treatments by 153%, whereas we increased the DOC concentrations by 39–80% in Experiment A and by 36% in Experiment B. Perhaps we would have seen a response in bacterial production if we had added more DOC or if the experiment had been performed in a lake with lower initial DOC concentrations. Also, the mesocosm experiment by Lennon (2004) was only running for 10 d and we cannot rule out that we might have seen a change in bacterial activities if we had measured the bacterial activity more often during the first week. It has been shown that labile carbon can be quickly consumed by bacteria during the first week, resulting in an increase in pCO_2, and leaving the more recalcitrant DOC which in another experiment had caused pCO_2 to stabilize or decrease (Guillemette and del Giorgio 2011). Our reverse osmosis concentrate was stored for up to 77 d before being used for the experiments, and although it

was stored cold and dark, the most labile carbon may have already been consumed when the DOC was added to the mesocosms. Furthermore, Lapierre et al. (2013) showed that there was no relationship between colored organic matter and the bioavailability of DOC in their study waters as the DOC pool in browner waters were as biologically available as the DOC in clear water. Addition of reverse osmosis DOC in our study may not have increased the amount of bioavailable DOC enough in the mesocosms to show a response in bacterial activities. The lack of relationship between bacterial production and pCO_2 in our experiments suggests that there were other factors than heterotrophic respiration that mainly controlled CO_2 concentrations.

Another factor driving pCO_2 in our experiments might be primary production. We expected to see a negative relationship between Chl *a* and pCO_2, but surprisingly, in both experiments we found the highest Chl *a* concentrations in the darkest treatments, that is, mixed and DOC-shading. A possible explanation for the high Chl *a* concentrations in the darker treatments could be that the photoautotrophs produced more chlorophyll to compensate for the decreased light availability (Richardson et al. 1983). Phytoplankton increase their Chl *a* to biomass ratio when light availability decreases (Enberg et al. 2015). Accordingly, we found the highest Chl *a* per phytoplankton individual and highest Chl *a* per phytoplankton biovolume in the DOC-shading treatment (Supporting Information Fig. S4; Supporting Information Tables S1, S2). Additionally, in Experiment A, we found no difference in phytoplankton biovolume between treatments, and in Experiment B, biovolume was significantly lower in the DOC-shading than all other treatments (Supporting Information Fig. S4; Supporting Information Tables S1, S2). The reduction of light availability in the darker treatments (i.e., HuminFeed, mixed, shading, and DOC-shading) may have led to a reduction in the CO_2 bio-uptake by the phytoplankton which subsequently led to increased pCO_2 in these treatments, thus supporting our Hypothesis 2 (Fig. 1).

Increasing DOC may to some extent stimulate primary production due to nutrients associated with the allochthonous organic matter, but at higher concentrations, the shading effect of colored organic matter has been shown to dominate (Seekell et al. 2015*b*). Likewise, Kelly et al. (2018) generated a model to assess how simultaneous changes in DOC and nutrients could impact lake primary production and found that both gross primary production and algal biomass increased with increasing DOC up to a threshold. Upon addition of reverse osmosis concentrate, the total P and total N increased by 6% and 21%, respectively, in Experiment A. Corresponding numbers for HuminFeed were 17% and 40%, respectively. Nutrient enhancement due to DOC additions was almost twice as high in the HuminFeed compared to the reverse osmosis treatment, but this difference was not significant (Supporting Information Fig. S1; Supporting Information Table S1). This could potentially explain the higher Chl *a* concentrations in the HuminFeed treatment, yet this was not reflected in the pCO_2. Hence, a change in the light climate is a more likely explanation to the increased Chl *a* concentrations. Addition of reverse osmosis concentrate in Experiment B led to increased total P and total N concentrations by 7% and 14%, respectively. However, Chl *a* was higher in the shaded treatment where no DOC, hence no nutrients, had been added. Consequently, the DOC-mediated nutrient effect on primary production can be assumed to be of minor importance.

A third factor influencing pCO_2 in our experiments could be changes in pH. Overall, DOC addition decreased the pH, thus supporting our Hypothesis 3 (Fig. 1) that an acidifying effect of DOC can decrease pH and subsequently increase pCO_2. This pattern has been confirmed in a study investigating the pCO_2 in surface waters of more than 900 Florida lakes where pH was found to be the best predictor of pCO_2 (Lazzarino et al. 2009). The pH of surface waters is essentially controlled by the ratios of $CO_2 : HCO_3^- : CO_3^{2-}$, and if acids, such as the humic acids in DOC, are added to water, the equilibrium will be shifted leading to increased amounts of free CO_2 relative to HCO_3^- and CO_3^{2-} (Cole and Prairie 2009). There was a clear effect of the added DOC from the reverse osmosis concentrate, as all treatments with this DOC addition showed significantly lower pH on the first day of the experiments than the treatments without addition. The reverse osmosis concentrate had an initial pH of 3.4 and upon addition of this DOC, pH decreased with 0.1 unit in both experiments. This would theoretically, according to the carbonate equilibrium (Weiss 1974), correspond to an increase in pCO_2 for Experiment A of 177 μatm where pH decreased from 8.2 to 8.1. The theoretical value was close to the observed increase in pCO_2 of 186 μatm. The close correspondence between the theoretical and observed increase in pCO_2 for Experiment A together with the strong negative relationship between pCO_2 and pH (Supporting Information Fig. S2) suggests a carbonate equilibrium control of CO_2 concentrations. This is further supported as pCO_2 increased despite decreasing DIC concentrations in all DOC addition treatments (Supporting Information Fig. S1). The DIC pool in Lake Erken, and thus also in the mesocosms, is high (> 20 mg DIC L^{-1}). Although a small change in pH of just 0.1 unit would have a low effect on the relative redistribution within the carbonate system, it can cause a significant change in absolute pCO_2, given in general low pCO_2 being observed during the experiments. Furthermore, to change the pH by 0.1 unit merely through a change in CO_2 would require an increase of 87.3 and 32.7 μg C L^{-1} on average in Experiment A and B, respectively. This would require respiration rates of 3.55 and 1.36 μg C L^{-1} h^{-1}, whereas we only measured respiration rates of 1.09 and 2.18 μg C L^{-1} h^{-1} at the start of Experiments A and B, respectively. Consequently, production of CO_2 through bacterial mineralization of the added DOC could not have resulted in the decreased pH. The measured pCO_2 values for both

experiments were well in agreement (± 40 μatm on average) with the pCO_2 values calculated from measured pH and DIC according to Cai and Wang (1998), further confirming our findings (Supporting Information Fig. S3).

For Experiment B, the corresponding change in pCO_2 should theoretically be an increase by 56 μatm (when pH decreased from 8.7 to 8.6). Although we observed an increase in pCO_2 of 98 μatm, a strong negative relationship between pH and pCO_2 was still observed in Experiment B (Supporting Information Fig. S2). This increased pCO_2 and the observed decrease in pH in the shading treatment could potentially be due to a decrease in the photosynthesis to respiration ratio resulting from the increased light attenuation (del Giorgio and Peters 1994). Consequently, in Experiment B, the instant increase in pCO_2 in the treatments with added DOC could be explained by the acidifying effect of the reverse osmosis concentrate. Later in the experiment, biotic factors became more important and decreasing photosynthetic rates due to limited light availability in the shaded treatments could explain the increased pCO_2 and subsequently decreased pH. Due to the initial acidifying effect of the reverse osmosis concentrate followed by a reduction in photosynthesis the highest pCO_2 was observed in the DOC-shaded treatments. This is further emphasized in the treatments receiving HuminFeed in Experiment A. Addition of DOC from HuminFeed did not result in an initial drop in pH or an increase in pCO_2; however, after 1 week, pH had decreased and pCO_2 had increased. Again, the limited light availability led to decreased photosynthesis which increased pCO_2 and subsequently decreased pH. Similar to the DOC-shading, we found the highest response in pCO_2 in the mixed treatment in Experiment A.

The acidifying effect of the reverse osmosis DOC was a short-term driver of pCO_2, whereas changes in primary production due to altered light climate occurred over time in our mesocosms. However, the acidifying effect of DOC on lake water could potentially be more important in a natural system with continuous input of DOC. Lake Erken is alkaline during most of the year and is in that regard not a typical Swedish lake as the majority of lakes are nonalkaline boreal lakes with a pH < 7. The carbonate system may play a larger role in controlling CO_2 dynamics in more acidic oligotrophic lakes, commonly found in Sweden, as a change in pH in acidic water would have a greater effect on pCO_2 than in alkaline water. Due to the low alkalinity in these waters, addition of the acidic reverse osmosis concentrate would likely decrease the pH by more than 0.1, subsequently increasing the pCO_2 by more than what was seen in our study. Conversely, atmospheric emissions of sulfur dioxide (SO_2) have decreased considerably in the northern hemisphere since the 1970s (Vuorenmaa et al. 2006). Decreasing trends in SO_2 emissions have been suggested as an underlying driver for increased DOC concentrations in freshwater systems (Evans et al. 2005; Vuorenmaa et al. 2006). Recovery from acidification increases soil water pH and would, in theory, therefore increase pH of lake water. This could potentially be one explanation as to why there is lacking evidence of long-term trends in pCO_2, despite increasing surface water DOC concentrations (Seekell and Gudasz 2016; Nydahl et al. 2017). Perhaps these two processes, recovery from acidification and the acidifying effect of DOC, may to some extent balance each other out.

Another possible source of CO_2 is photochemical mineralization of DOC (Graneli et al. 1996). The monthly average photochemical production of CO_2 in Swedish lakes during the months of our experiments (June–September) was 686 mg C m^{-2} $month^{-1}$ (Koehler et al. 2014), which corresponds to 14.3 μg C L^{-1} d^{-1} in our mesocosms. This is in the range of the observed DOC loss and could potentially explain some of the observed CO_2 production. However, we would expect lower photochemical mineralization in the shading treatment compared to the control in Experiment B, yet we have higher pCO_2 in the shading treatment. Although we cannot rule out the effect of photochemical mineralization, it would not be high enough to explain the large increase in pCO_2 with allochthonous DOC addition.

Overall, only moderate effects of DOC additions and increased light attenuation on CO_2 dynamics were observed. The control treatments were undersaturated halfway through Experiment A and highly undersaturated at the start of Experiment B. Many temperate zone eutrophic lakes are undersaturated with CO_2, at least during the summer (Balmer and Downing 2011). Increased DOC input to these lakes could potentially switch these systems from being sinks to acting as sources of CO_2 to the atmosphere, particularly if the lakes are close to equilibrium with the atmospheric CO_2. This highlights the importance of considering trophic states and other lake characteristics in assessments of the contribution of inland waters to atmospheric CO_2. Accordingly, in a comparison of DOC budgets of 82 different water bodies, it was demonstrated that mesotrophic and eutrophic waters frequently accumulate rather than lose DOC over time, which may be an effect of DOC generation via indigenous primary production being higher than mineralization of DOC (Evans et al. 2017). It is likely that these ecosystems are also net sinks of CO_2. Agricultural eutrophication, a highly significant environmental problem (Carpenter et al. 1998; Charlton et al. 2018), is likely to continue to increase as the need for food production rises with global population. However, increased eutrophication may also result in an opposite switch where oligotrophic lakes become sinks of CO_2 rather than sources due to increased atmospheric carbon sequestration as sediment and DOC, further emphasizing the importance of eutrophic lake ecosystem research (Pacheco et al. 2013).

In conclusion, we found that increased allochthonous DOC input leads to enhanced CO_2 concentrations in meso-eutrophic lake water, which we attributed to a decreased photosynthesis to respiration ratio resulting from reduced light availability as well as to altered acidity. The allochthonous organic subsidy for bacterial mineralization was found to be

low and this could perhaps be due to poor bioavailability of the DOC. Input of allochthonous DOC from the reverse osmosis concentrate, which to a substantial extent contains organic acids, appeared to have shifted the carbonate system leading to a rapid decrease in pH and subsequently increased CO_2. However, this acidifying effect of DOC was more pronounced in the early stage of the experiments. Later, the change in light climate appeared to play the key role in controlling pCO_2. Decreased light availability may have led to an increased respiration to photosynthesis ratio, resulting in increased pCO_2 and a subsequent decrease in pH. Allochthonous DOC input resulted in a consistent increase in pCO_2 in treatments relative to the controls for both experiments. This may also be the case for whole lake ecosystems, particularly considering that changes in climate and land-use can affect the export of DOC and nutrients from terrestrial to aquatic ecosystems (Leavitt et al. 2009; Kritzberg et al. 2014). Given the paucity of studies of the CO_2 dynamics of mesotrophic and eutrophic lake ecosystems and the projected future increase in nutrient loads to lakes, the impact of increased allochthonous DOC input on CO_2 dynamics in eutrophic inland waters needs further attention.

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Conflict of Interest

None declared.

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