LETTER

Contrasting impacts of warming and browning on periphyton

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Scientific Significance Statement

Northern lakes are becoming warmer and browner due to increasing colored dissolved organic matter (cDOM) concentrations that affect algal growth through light inhibition and nutrient supplementation. Still, attached algae (periphyton) are often neglected in field and mesocosm studies despite their tendency to outgrow algae in the water column (phytoplankton). Consequently, impacts of browning and warming on periphyton are understudied, and it is unclear to what extent periphyton utilizes pelagic nutrients. This study shows that periphyton growing on nutrient-poor substrates efficiently utilizes pelagic nutrients associated with browning. However, while periphyton accrual increased with increasing cDOM and associated nutrients, the accrual decreased with warming, likely due to thermal compensation mechanisms and/or community shifts. Browning and warming thus strongly affect periphyton accrual, but in contrasting ways.

Abstract

We tested interactive effects of warming ($+2°C$) and browning on periphyton accrual and pigment composition when grown on a synthetic substrate (plastic strips) in the euphotic zone of 16 experimental ponds. We found that increased colored dissolved organic matter (cDOM) and associated nutrients alone, or in combination with warming, resulted in a substantially enhanced biomass accrual of periphyton, and a comparatively smaller increase in phytoplankton. This illustrates that periphyton is capable of using nutrients associated with cDOM, and by this may affect nutrient availability for phytoplankton. However, warming weakened the positive impact of browning on periphyton accrual, possibly by thermal compensation inferred from altered pigment composition, and/or changes in community composition. Our results illustrate multiple impacts of climate change on algal growth, which could have implications for productivity and consumer resource use, especially in shallow areas in northern lakes.

Over the last decades, surface waters throughout the boreal northern hemisphere have been rapidly warming (O'Reilly et al. 2015) and receiving increasing concentrations of colored dissolved organic matter (cDOM) resulting in the browning of waters (Evans et al. 2005; Roulet and Moore 2006). The warming and browning of surface waters affect aquatic algae in contrasting ways. Although increasing temperatures may directly increase algal growth rates (Lürling et al. 2013; Hood et al. 2018), algae may employ compensation mechanisms for suboptimal warmer temperatures (Barton and Yvon-Durocher 2019; Liu et al. 2022), that on the longer term may result in a reduction of algal accrual. Increasing cDOM concentrations supplement dissolved organic carbon (DOC) and nutrients that stimulate algal growth but also cause light limitation resulting in a unimodal relationship of algal growth with cDOM in lakes (Jones and Lennon 2015; Seekell et al. 2015; Bergström and Karlsson 2019). In addition, nutrients, light, and temperature combined have interactive effects on aquatic algal growth (De Senerpont Domis et al. 2014; Endo et al. 2017; Burrows et al. 2021). For instance, algae become more efficient at utilizing nutrients at higher temperatures, resulting in a synergetic effect on algal growth (Rhee and Gotham 1981; Hamdan et al. 2021) and biomass accrual (Bergström et al. 2013), where the net responses are further constrained by light. Free-floating algae (phytoplankton) are restricted to utilizing nutrients from the water and can position themselves at optimal light conditions, whereas periphytic (attached) algae are stationary and thus restricted to use the light reaching their location. Because of this contrasting mobility, periphyton and phytoplankton are two distinct algal communities. Periphyton is often more abundant compared to phytoplankton, especially in shallow areas of lakes or in lakes with clear water, but particularly understudied (Vadeboncoeur et al. 2002; Vander Zanden and Vadeboncoeur 2020). Hence, separate and/or interactive effects of light, temperature and nutrients caused by warming and browning on biomass accrual of periphyton are unresolved.

Periphyton includes algae growing on submerged surfaces with nutrient contents ranging from nutrient-poor rocks (epilithic algae) to nutrient-rich sediments (epipelic algae) from which it derives nutrients for its growth (Vadeboncoeur and Lodge 2000). Because of the relatively nutrient-rich sediment conditions, epipelic algae in northern lakes are primarily light- and not nutrient-limited (Vadeboncoeur et al. 2003; Ask et al. 2009; Puts et al. 2022). However, positive responses to in situ inorganic nutrient (Myrstener et al. 2018; Fork et al. 2020) and nutrients combined with cDOM additions (Vinebrooke and Leavitt 1998), as well as field studies (Hansson 1992), indicate that epipelic algae can also be nutrient limited, especially in nutrient-poor systems. Conversely, epilithic algae are constrained to utilize pelagic nutrients, and are often nutrient- and/or light-limited (Vinebrooke and Leavitt 1998; Fork et al. 2020; Myrstener et al. 2020). Thus, among periphyton in general and phytoplankton the differences in growth-strategies are both nutrient- and light-related, and among epipelic and epilithic algae these differences are mainly nutrient-related. Therefore, it is unclear if ongoing warming and browning of lakes influence pelagic nutrient utilization among periphyton, which is plausible especially when considering periphyton growing on nutrient-poor substrate.

Aquatic algae have developed a variety of photosynthetic pigments in order to adapt to a wide range of light conditions, mostly to protect against harmful radiation causing oxidative stress but also to optimize light harvesting at different intensities and wavelengths. Chlorophyll α (Chl *a*) is often used as a biomarker for biomass in plants including algae (Wetzel and Likens 1991). Chlorophyll c (Chl *c*) occurs in mixed algae groups (i.e., diatoms, chrysomonads and brown algae; Jeffrey and Humphrey 1975) and is particularly rich in the carotenoid fucoxanthin (accessory pigment related to photo-protection; Young and Britton 1993). Although not perfect proxies, algal pigment compositions can be used as markers for algal communities, and especially carotenoids are successful taxonomic markers (Young and Britton 1993). Accordingly, pigment composition is often used to assess algal responses to varying light intensities both in experimental (Ehling-Schulz et al. 1997) and natural settings, for instance reflected by lake depth or turbidity (water color; Vinebrooke and Leavitt 1998; Hodgson et al. 2004). Yet, interactive effects of warming, nutrient supplementation, and changes in water color by cDOM additions on periphyton biomass and pigment composition in a natural setting remains understudied.

Here, we test interactive effects of warming ($+2°C$) and nutrient supplementation associated with cDOM on biomass of phytoplankton and periphyton growing on a nutrient-poor synthetic substrate in 16 experimental ponds. In addition, we test the response in pigment composition (Chl *a*, Chl *c*, and fucoxanthin) of periphyton to browning and warming. We hypothesize that:

1. Periphyton and phytoplankton accrual increase with cDOM and associated nutrients (browning) alone, or in combination with warming.
2. Periphyton growing on nutrient-poor substrates can efficiently utilize pelagic nutrients associated with cDOM for its growth.
3. Periphytic pigment composition (Chl *a*, Chl *c*, and fucoxanthin) responds to both browning and warming.

Methodology and approach

Experimental design and monitoring

Using 20 (16 + 4 buffer ponds) experimental ponds at the Experimental Ecosystem Facility (EXEF), we tested periphyton and phytoplankton biomass accrual over a cDOM and associated nutrients gradient combined with warming. We used dissolved organic carbon (DOC) as proxy for cDOM, and measured associated (total and inorganic) nutrients and water color (Table 1) to assess the nutrient-supplementing and light-reducing effect of the supplemented cDOM (Jones 1992;

Bergström and Karlsson 2019). The experiment was carried out between 15 June 2018 and 30 August 2018, which was an exceptionally warm summer for this area (Blunden and Arndt 2019). The EXEF ponds are naturally functioning ecosystems and contain a soft benthic habitat and naturally occurring primary producers and invertebrate consumers. The ponds were separated by impermeable dark-green polyvinyl-chloride (PVC) sheets, are each 11.5 m long, 6.7 m wide, and on average 1.5 m deep, and contain a 6.7-m-long natural shoreline (Fig. 1A). We established a 4×2 factorial design that included four increasing cDOM categories (duplicates) and warming (+2°C; Table 1; Fig. 1) and used the remaining four ponds to separate the warmed ponds from the ambient ponds. We choose a warming of 2°C because the Intergovernmental Panel on Climate Change (IPCC) formulated that a global warming of 2°C goes accompanied with heat extremes where ecosystems will more often reach critical tolerance thresholds (IPCC, AR6; Ch1, 2021). We created the cDOM gradient by combining continuously provided input of cDOM-rich water, collected biweekly from the naturally cDOM-rich small boreal river Hörneån located 45 km northwest of EXEF, with tap water derived from the Umeå municipality that uses ground water as source (control treatment; Table 1 for water chemistry for tap and river water). The water of each individual pond was warmed by a land-based individual heat exchanger and circulated through a filter cube (10 PPI) in each pond (*see* Capo et al. 2021; Hamdan et al. 2021 for specifics). The ambient ponds were subjected to the same circulation process without heat exchangers. The treatments commenced in autumn 2017, and the ponds were fish-free until 11 May 2018, when we introduced 43 adult sticklebacks (total biomass 38.5 g) into each pond as part of another study, and to make sure top-down control (predation) by chironomids and zooplankton on the algae was minimal (Mahdy et al. 2015; Carpenter et al. 2022).

We measured DOC, total nitrogen and phosphorus (TN and TP), dissolved inorganic nitrogen and phosphorus (DIN [$NO_3^- + NO_2$] and PO_4^{3-}) and pH, every 3 weeks, starting 28 May (2 weeks before installing periphyton strips). We took water samples from the surface with a 0.6-m-long water sampler and stored them dark in 1-L bottles with minimum air. We immediately processed the samples in the lab, and we measured pH directly after all other sampling in the lab. We filtered water for DOC, DIN, and PO_4^{3-} analyses through a 0.45-μm filter (Sarstedt) before storing. We acidified the DOC samples with HCl to an end concentration of 12 mM and stored them in a refrigerator before analyzing. We kept DIN and PO_4^{3-} (filtered), and TN and TP (unfiltered) samples frozen until analysis. We retrieved incoming photosynthetically active radiation (PAR) from stations installed next to the ponds, and continuously monitored water temperatures in each pond in situ at 15-min intervals. Because of interference by a nest-building bisam rat causing high turbidity, we excluded one of the control ponds

subjected to ambient temperature and miss temperature data just before the last sampling. Light attenuation coefficients (Kd) were calculated as the absolute slope of natural logarithmically transformed PAR against depth.

Algal biomass accrual and pigments

We measured Chl *a* as proxy for both periphyton and phytoplankton algal biomass, on three and four sampling occasions, respectively (Fig. 1B; Table 1). Due to the presence of sticklebacks (fish), the abundance of pelagic zooplankton was low indicating low grazing pressure by zooplankton on both phytoplankton and periphyton (Hamdan 2021). In addition, although present in the ponds, snails (Lymnaeidae sp.), and chironomids were rare on the strips (Koizumi et al. in rev.) and thus grazing on the periphyton strips was minimal. We grew the periphyton on vertical synthetic (polycarbonate) strips that were 60 cm long, 10 cm wide, and 0.75 mm thick. On 15 June, we deployed six strips per pond attached to small floaters to ensure the strips were always at the water surface, and we excluded the algae growing on the top 10 cm (Fig. 2C). Periphyton biomass accrual was measured over time by harvesting two strips on two sides (i.e., quadruplicate measurements per pond) into separate plastic containers using a plastic scraper and stored dark and frozen (−20°C) before further analysis. We estimated the overall biomass accrual based on the periphyton and phytoplankton measurements taken on the last date of the experiment, that is, periphyton that has grown on the strips during the whole experiment, and the Chl *a* from the water column during the last sampling (Fig. 1C). We freeze-dried the periphyton samples before extraction and used the whole sample for analysis. Periphytic Chl *a* (sensu Steinman et al. 2017), Chl c^{1+2} (here: Chl *c*; sensu Jeffrey and Humphrey 1975) and fucoxanthins (sensu Seely et al. 1972) were extracted in 90% acetone and estimated spectrophotometrically, measuring the full absorbance spectra with 1-nm band intervals. We took pelagic Chl *a* every 3 weeks from the same water used to measure the water chemistry, by filtering 100 mL onto Whatman GF/F filters and extracted for 24 h in the dark in 95% ethanol before measuring with a spectrofluorometer (Perkin Elmer LS-55) with 433 nm as excitation and 673 nm as emission wavelength.

Data interpretation

We expressed the response of periphytic and phytoplankton biomass accrual to cDOM and associated nutrients, and warming, compared to the control treatment as log-transformed response ratios (RR_x) on each sampling date (Fig. 1B). We combined the measurements from duplicate ponds and included all values to get a mean seasonal RR_x (Table 1). We performed two-way ANOVAs for the accrual, RR_x, and pigment composition, and tested interactive effects (Table 2) and established 95% confidence intervals to assess if the responses to cDOM and warming treatments differed. To compare periphytic and phytoplankton accrual (measured in mg cm^{-2} and mg m^{-3},

Table 1. Seasonal average water physio chemistry (including the tap water and cDOM-rich river source water used to create treatments; mean ± standard deviation) and accrual per treatment. Periphyton and phytoplankton accrual (mg Chl a m^{-2}) represent values measured on the last sampling.

| | | Source water | | cDOM and temperature treatment | | | | | | | |
| | | | | Control | | Low | | Mid | | High | |
	Seasonal n	Tap	River	Ambient ($n=2$)	+2°C ($n=1$)	Ambient ($n=2$)	+2°C ($n=2$)	Ambient ($n=2$)	+2°C ($n=2$)	Ambient ($n=2$)	+2°C ($n=2$)
DOC (mg L^{-1})	6	1.4 ± 0.3	7.96*	4.6 ± 2.9	5.0 ± 1.7	4.5 ± 2.4	5.1 ± 1.9	6.6 ± 4.8	6.6 ± 2.5	9.4 ± 2.9	11.3 ± 4.4
TN (µg L^{-1})	6	74 ± 10	440†	159 ± 24	250 ± 16	217 ± 225	279 ± 97	235 ± 42	310 ± 70	476 ± 41	419 ± 87
TP (µg L^{-1})	6	5.4 ± 3.3	38 ± 9†	9.2 ± 2.2	15.8 ± 3.1	12.7 ± 4	15.1 ± 4	13.4 ± 2	17.3 ± 3	31.9 ± 13	37.9 ± 13
DIN (µg L^{-1})	6	31 ± 10		6 ± 3	7 ± 2	8 ± 3	8 ± 3	7 ± 3	8 ± 4	18 ± 17	10 ± 2
PO$_4^{3-}$ (µg L^{-1})	6	0.2 ± 0.4		0.6 ± 0.4	0.8 ± 0.7	0.6 ± 0.6	0.9 ± 0.6	3.7 ± 9.6	1.4 ± 1.7	3.5 ± 3.4	4.2 ± 2.8
DIN : TP	6	0.3 ± 0.3		0.4 ± 0.3	0.4 ± 0.2	0.4 ± 0.3	0.3 ± 0.2	0.3 ± 0.2	0.3 ± 0.2	0.2 ± 0.1	0.2 ± 0.1
Kd (m^{-1})	6			0.9 ± 1.0	0.8 ± 0.3	0.8 ± 0.4	1.0 ± 0.2	1.3 ± 0.6	1.2 ± 0.6	2.7 ± 1.1	1.7 ± 1.2
pH	6	8.0 ± 0.3		9.4 ± 0.3	8.3 ± 0.7	9.2 ± 0.7	8.7 ± 0.3	9.2 ± 0.2	8.1 ± 0.4	8.7 ± 0.9	7.6 ± 0.3
Periphyton accrual	1			38.0 ± 3.1	10.2 ± 8.3	6.8 ± 3.5	1.7 ± 0.8	109.7 ± 36.0	16.0 ± 15.1	250.5 ± 27.9	235.7 ± 24.7
Phytoplankton accrual	1			1.4	1.1 ± 0.3	1.9 ± 0.6	2.4 ± 0.4	2.4 ± 0.1	1.7 ± 0.8	6.8 ± 3.5	13.3 ± 0.0

*DOC values estimated based on the linear correlation between TN and TDN ($n=175$; $R^2=0.81$, $p<0.05$), and TDN and DOC ($n=183$; $R^2=0.46$, $p<0.05$).

†Average values ($n=4$) from Jonsson and Byström (unpubl. data).

respectively) we calculated a pond average for phytoplankton Chl a (in mg m^{-2}) by multiplying the measured Chl a values with the pond volume between 0 and 0.6 m depth and then dividing by the pond surface area. We expressed the pigment compositions Chl c and fucoxanthin in relation to biomass of Chl a (g g^{-1} and mg g^{-1}, respectively) and fucoxanthin to the biomass of Chl c (g g^{-1}), to remove the effect of biomass increase. We removed one erroneous measurement (one of quadruplicate periphyton measurements likely due to dilution error) and performed analyses in R and SPSS (Supporting Information Data S1).

Results and discussion

Effects of cDOM and warming on water physiochemistry

We successfully created cDOM categories that formed a DOC (4.5–11.5 mg L^{-1}) and nutrient (TN 159–476 µg L^{-1}, TP 9.2–37.9 µg L^{-1}) gradient, and a warming treatment with an average of $+1.9 \pm 0.3$°C compared to the control (Fig. 1; Table 1). Indeed, the cDOM-rich river water had significantly ($p < 0.05$) higher DOC, TN, and TP concentrations compared to the tap water, with decreased light conditions (i.e., higher Kd) along the cDOM gradient (Table 1), illustrating the dual impact of cDOM on algal development, that is, by supplementing nutrients and constraining light (Jones 1992; Bergström and Karlsson 2019). The inorganic nutrient (DIN, and PO$_4^{3-}$) concentrations were low in each CDOM category (Table 1), typically like pristine browned water bodies in northern Sweden (Bergström and Jansson 2000; Deininger et al. 2017). Concentrations of TN, TP, and DOC in the highest cDOM category were similar to those found in the river (Table 1; Jonsson and Byström unpubl. data). The DIN : TP ratios were further low (mass < 3), suggesting N-limited conditions in all treatment ponds (Liess et al. 2009; Bergström 2010). TP and PO$_4^{3-}$ concentrations increased more than DIN along the cDOM gradient (except for the ambient high cDOM treatment: Table 1), indicating a tendency of declining DIN : TP and intensified N-limited conditions with increasing cDOM, similar to what has been reported for northern Swedish lakes with browning (Jansson et al. 2001; Isles et al. 2018). Although we did not find a (significant) effect of the temperature treatment on total or dissolved nutrients, it is possible that temperature increased microbial activity and cDOM mineralization (Hall et al. 2008; Gudasz et al. 2010) and nutrient availability for autotrophic algae (Stepanauskas 1999a; Stepanauskas et al. 1999b). The possible increased microbial activity with warming may also have promoted competition for nutrients between periphyton and bacteria (Carr et al. 2005; Li et al. 2017).

Effects of cDOM and warming on algal accrual

Periphyton and phytoplankton biomass accrual increased with cDOM in both ambient and warmed treatments, with interactive effects between temperature and cDOM for periphyton only, and with significantly higher accrual of
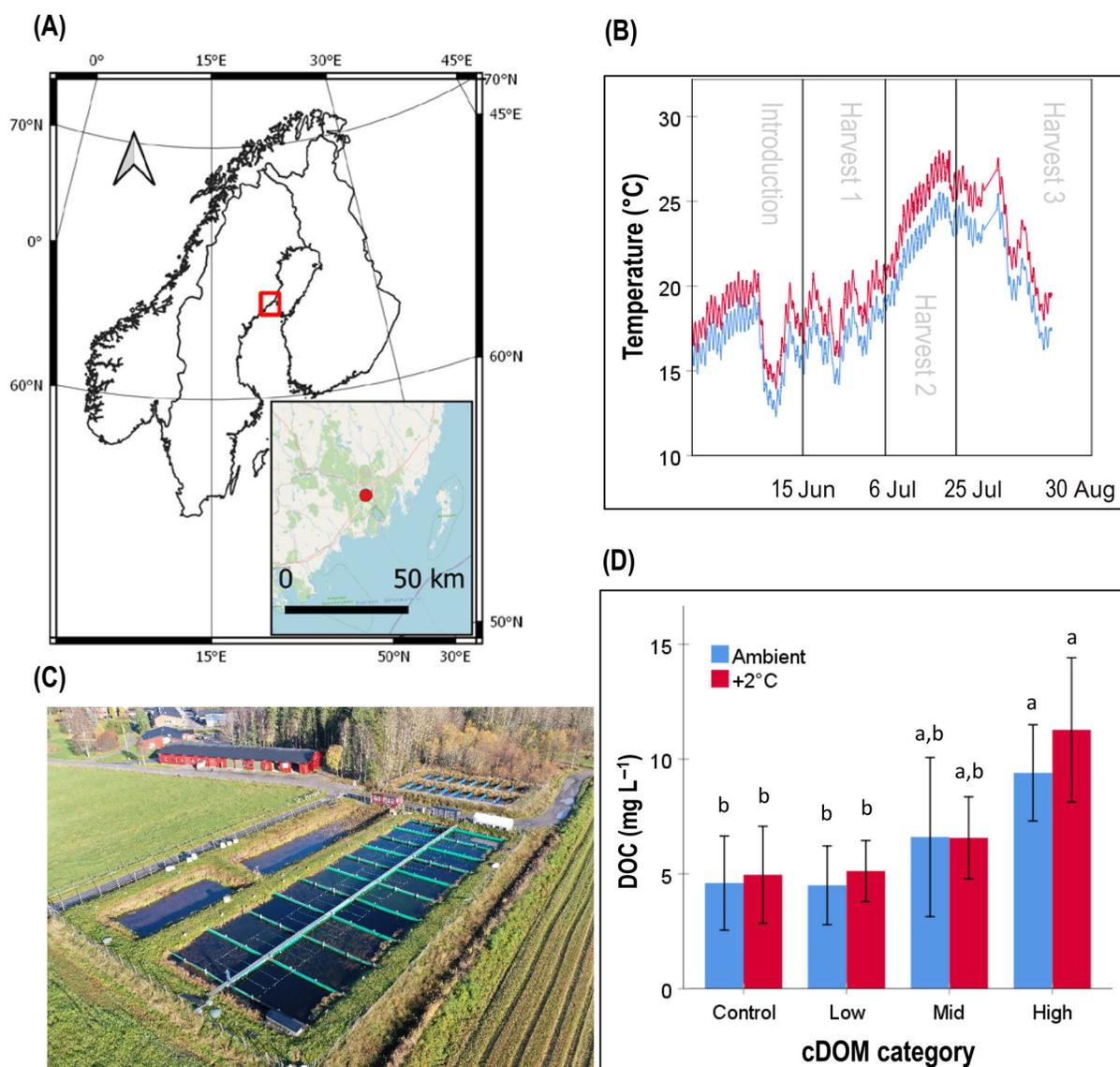
Fig. 1. (**A**) Location of EXEF within Umeå, Sweden; (**B**) average temperature of the treatments (blue = ambient, red = warmed) during the experiment and periphyton introduction and harvest dates; (**C**) drone picture of the EXEF ponds; (**D**) average seasonal DOC concentrations per temperature treatment (blue = ambient, red = warmed). Bars represent 95% confidence intervals, and comparative levels of significance are additionally indicated with letters.

periphyton relative to phytoplankton (Fig. 2A–C; Table 2). Our results thus confirm interactive effects of nutrients, temperature and to some extent light on algal growth (De Senerpont Domis et al. 2014; Endo et al. 2017; Burrows et al. 2021), and possibly also on microbial growth (Carr et al. 2005; Li et al. 2017), here induced by warming and browning. Periphyton accrual (73–98% of total periphyton and phytoplankton accrual) well exceeded phytoplankton accrual, except for the warmed low-cDOM pond (40%), indicating a much higher nutrient uptake from the water by periphyton compared to phytoplankton (Fig. 2B). Since chironomid and

zooplankton biomass was low in all ponds because of fish predation (Koizumi et al. in rev.), effects of zooplankton grazing on phytoplankton and periphyton biomass accrual is negligible compared to the effect of the treatments. Nonetheless, in natural systems, the relative nutrient uptake of periphyton vs phytoplankton depends on the relative availability of surface (periphyton habitat) vs. water volume (phytoplankton habitat) and should therefore be higher in small ponds and lakes with increased lake surface to volume ratio.

Seasonal average response ratios of phytoplankton biomass accrual increased with cDOM and associated nutrients,
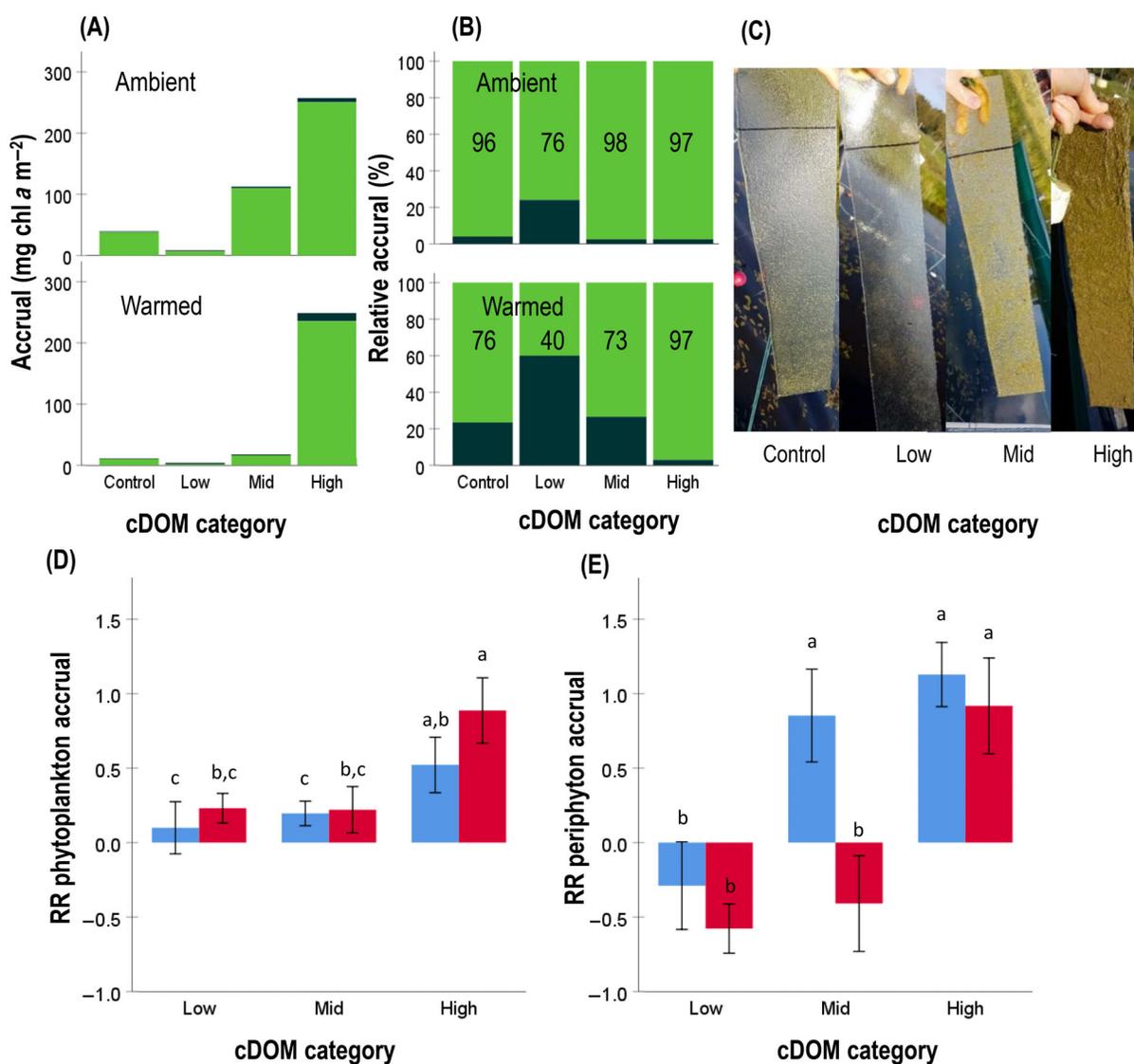
Fig. 2. (**A–C**) Accrual of periphyton (light green) and phytoplankton (dark green) for the ambient (top panels) and warmed (bottom panels) treatments, measured on the last sampling occasion expressed as (**A**) average per m², (**B**) the relative accrual of periphyton and phytoplankton (%, expressed as relative amount of periphyton), and (**C**) picture of the periphyton strips exposed to the four cDOM treatments from the ambient ponds. (**D,E**) Seasonal average response ratios (RR) of biomass accrual per cDOM and temperature (ambient = blue, warmed = red) treatment of (**D**) phytoplankton and (**E**) periphyton. RR = log transformed response ratio; error bars represent 95% confidence intervals, and comparative levels of significance are additionally indicated with letters.

and there was a trend of increased accrual with warming (Fig. 2D; Table 2). These results indicate that enhanced phytoplankton accrual in response to additions of limiting nutrients by cDOM (Table 1; Seekell et al. 2014; Thrane et al. 2014) can be enhanced by higher water temperatures (Myrstener et al. 2018; Bergström et al. 2013). In contrast, warming weakened periphyton biomass accrual, with consistently lower biomass accrual in warmed compared to ambient treatments (Fig. 2D; Table 2). A possible explanation for this difference could be that the phytoplankton community was better adapted to higher water temperatures

compared to the periphyton community due to different optimum temperatures (Lürling et al. 2013; Liu et al. 2022), or altered competition between microbes and periphyton (Li et al. 2017). Yet, our results clearly illustrate that periphyton indeed utilize nutrients supplemented by cDOM when growing on nutrient-poor, hard substrates (here plastic strips) under light satisfactory conditions. Indeed, our results of higher accrual of periphyton than phytoplankton at all cDOM levels suggest that periphyton may be capable of constraining nutrient resources coupled to cDOM for phytoplankton, with slight modification by warming.

Table 2. Results of the two-way ANOVA per treatment (cDOM, temperature, and interaction between cDOM and temperature) for the different measures of accrual, RR_x, and pigment compositions.

Treatment	Measure	Response	n	df	Mean square	F	p-value
cDOM	Accrual	Periphyton	43	3	8.35	42.47	<0.01
		Phytoplankton	15	3	182.39	16.88	<0.01
	RR_x	$RR_{periphyton}$	121	3	14.64	49.77	<0.01
		$RR_{phytoplankton}$	75	3	1.71	37.52	<0.01
	Pigment	Chl c : Chl a	121	3	0.65	5.58	<0.01
		Fucoxanthin : Chl a	121	3	0.02	3.13	0.03
		Fucoxanthin : Chl c	121	3	1.87	4.44	0.01
Temperature	Accrual	Periphyton	43	1	0.00	0.00	0.98
		Phytoplankton	15	1	22.03	2.04	0.20
	RR_x	$RR_{periphyton}$	121	1	6.04	22.02	<0.01
		$RR_{phytoplankton}$	75	1	0.26	5.78	0.02
	Pigment	Chl c : Chl a	121	1	0.20	1.71	0.19
		Fucoxanthin : Chl a	121	1	0.12	19.12	<0.01
		Fucoxanthin : Chl c	121	1	5.30	12.62	0.01
Interaction	Accrual	Periphyton	43	3	1.34	6.80	<0.01
		Phytoplankton	15	3	31.07	2.88	0.11
	RR_x	$RR_{periphyton}$	121	3	2.44	8.87	<0.01
		$RR_{phytoplankton}$	75	3	0.14	3.08	0.03
	Pigment	Chl c : Chl a	121	3	0.10	0.86	0.47
		Fucoxanthin : Chl a	121	3	0.05	0.76	0.52
		Fucoxanthin : Chl c	121	3	0.43	1.03	0.38

n = sample size considered, SS (III) = sum of squares type III, df = degrees of freedom.

Periphyton pigment composition

We also tested if the browning and warming treatments resulted in altered pigment composition, using Chl c and fucoxanthin relative to Chl a, and to each other, as proxy (cf. Anning et al. 2001). Chl c was abundant in all treatments and amounts increased accordingly with Chl a (*see* publicly available data), and Chl c : Chl a decreased along the cDOM gradient but not with warming (Fig. 3A; Table 2). Fucoxanthin was detected at all treatments, and relative amounts (compared to Chl a and Chl c) responded to the cDOM treatments but showed a consistent and stronger increasing trend with warming, especially at low- and mid-cDOM categories (Fig. 3B,C; Table 2). Fucoxanthin : Chl c generally (insignificantly) decreased with the warmed treatment at all cDOM categories (Table 2). Such changes in pigment composition measured over a longer time may reflect changes in community composition, but they may also reflect changes related to growth mechanisms.

Our observed decrease in Chl c : Chl a with browning is consistent with other studies, but the warming-induced decrease of fucoxanthin : Chl a and fucoxanthin : Chl c is more surprising, since fucoxanthin often is related to photo protection (Fig. 3; MacIntyre et al. 2002; Hodgson et al. 2004; Endo et al. 2017). Several enzymes involved in photosynthesis are temperature dependent, and have an optimum temperature, with declining accrual at lower or higher temperatures than the optimum temperatures (Schoolfield et al. 1981; Raven and Geider 1988; Lürling et al. 2013). There is evidence that the independence of Chl c : Chl a to temperature together with a decrease in fucoxanthin : Chl a ratio is characteristic for diatoms exposed at higher temperatures that may employ mechanisms to adapt to the high temperatures (Anning et al. 2001), as may be the case in our systems as well. Such "thermal compensation" mechanisms are debated and more often measured in experimental settings where optimal nutrient and light conditions are combined (Raven and Geider 1988), but are also observed in natural settings including community assemblages (Kingsolver 2009; Barton and Yvon-Durocher 2019; Liu et al. 2022). Our results therefore fit these patterns well, indicating that diatoms were likely abundant and caused the response to the additional warming on top of the extraordinarily warm temperatures during the experiment (*see* Blunden and Arndt 2019). In conclusion, the supplementing effect of nutrients by cDOM was dominant and caused an overall increase of periphyton accrual (Table 1; Fig. 2), which was likely impaired by thermal compensation, measured as decreased fucoxanthin : Chl a (and Chl c), especially for the mid-cDOM category (Fig. 3).
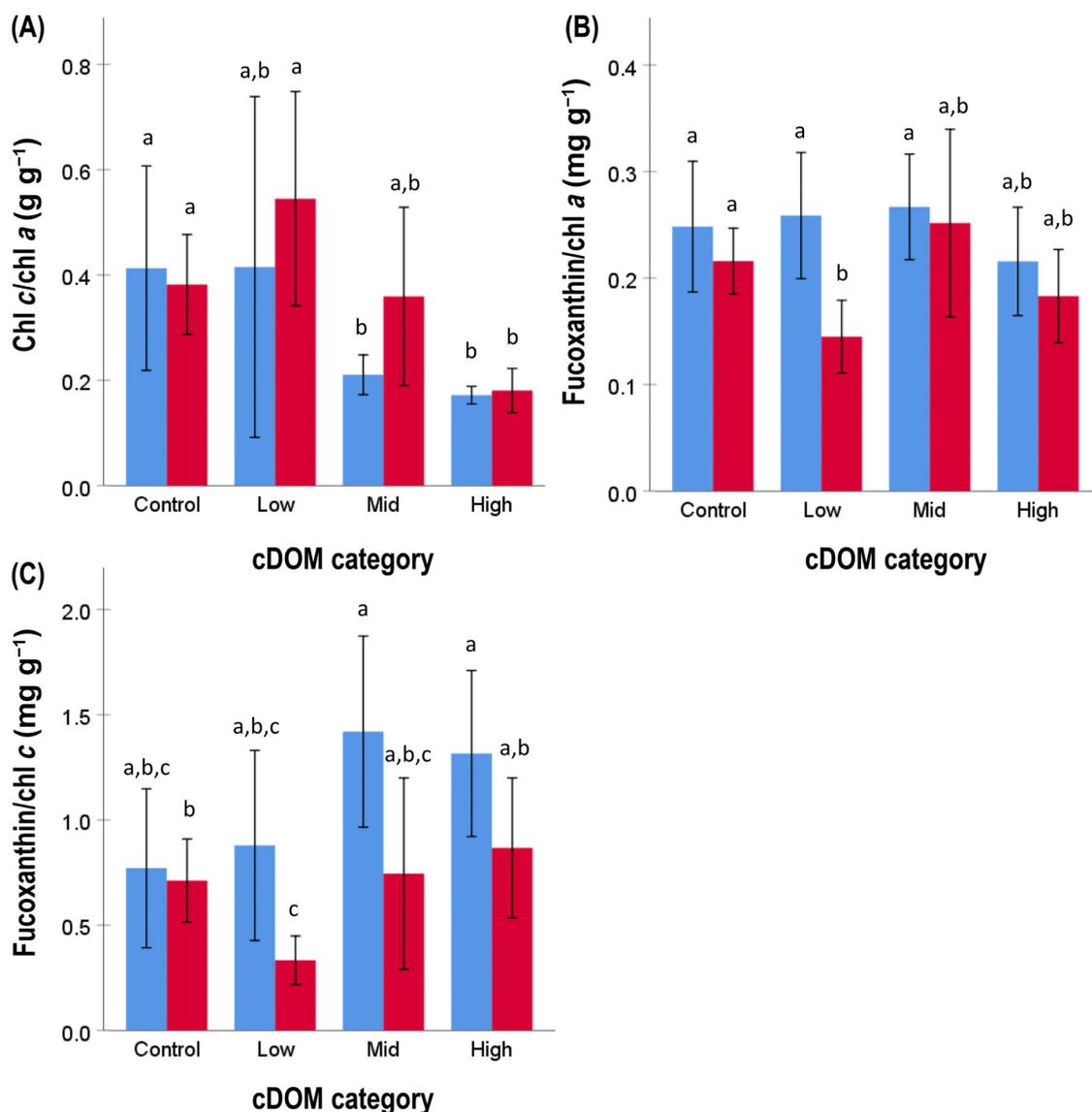
Fig. 3. Seasonal averages per cDOM and temperature (ambient = blue, warmed = red) treatment of (**A**) Chl *c* relative to Chl *a*, (**B**) fucoxanthin relative to Chl *a*, and (**C**) fucoxanthin relative to Chl *c*. Error bars represent 95% confidence intervals, and comparative levels of significance are additionally indicated with letters.

In summary, our results suggest that browning and warming affect periphyton accrual growing on nutrient-poor substrates in contrasting ways. While in natural systems, periphyton (growing on varying substrates) is often light limited due to water coloring by cDOM (Vinebrooke and Leavitt 1998), and additionally increases with temperature (Björk-Ramberg and Ånell 1985; Puts et al. 2022), here cDOM caused an increase in accrual due to nutrient supplementation, possibly due to the optimal position in the water column with respect to light and the nutrient-poor substrate. In addition, temperature had a negative effect on periphytic accrual (especially at the mid cDOM category),

likely by affecting periphytic algae communities and their growth strategies by thermal compensation mechanisms (occurring at all cDOM categories). Since most of the northern Swedish lakes have DOC levels up to 10.6 mg L^{-1} (Bergström and Karlsson 2019) which is similar to our highest cDOM category (corresponds to DOC concentrations up to 11.5 mg L^{-1}), warming may in addition to light (browning) constrain periphyton development in these ecosystems in the near future. Our results illustrate the complexity by which impacts of browning and warming manifest. Considering the importance of periphyton for the productivity of many lakes (Karlsson et al. 2009; Finstad

et al. 2014), the results in our study highlight the potency of browning and warming to change aquatic ecosystems.

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