https://doi.org/10.5194/egusphere-2026-2970
Preprint. Discussion started: 24 June 2026

Warming vs. browning: a dual mechanism behind net ecosystem production of shallow brown aquatic systems

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Abstract

Northern lakes are warming and browning due to increased cDOM inputs, altering water column C:N:P ratios and temperatures that regulate photoautotrophic and heterotrophic production. Yet the relative importance of warming versus browning, and their combined effects on nutrient stoichiometry and microbial dynamics remain unclear. We experimentally manipulated boreal ponds along a cDOM gradient under ambient and +2 °C warming to quantify impacts on nutrient conditions and microbial production. Browning shifted C:N:P stoichiometry in both total and dissolved pools, with increased C:N and C:P ratios in the total nutrient pools, while reducing relative inorganic nutrient availability. Despite declining N:P ratios in both total and dissolved pools, both pools were strongly carbon-saturated and depleted in both N and P. Nutrient-addition assays confirmed NP co-limitation. Browning increased bacterial production, while browning and warming combined reduced primary production, lowering PP:BP ratios, although net heterotrophy didn't occur, likely because the ponds were so shallow. We show that browning -not warming- drives boreal freshwaters toward carbon-rich, N- and P- colimited conditions, while warming and browning together modulate microbial production by amplifying DOC-driven heterotrophy and suppressing photoautotrophic production. Our study reveals a dual mechanism behind the shifting of brown systems toward net heterotrophy. As browning and warming are pervasive trends across Northern Hemisphere lakes, these findings have wide applicability and advance general understanding of how freshwater ecosystems respond to ongoing environmental change.

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1. Introduction

Widespread browning of lakes and rivers due to increased mobilization of terrestrial dissolved organic matter and carbon (DOM and DOC) from forest soils, wetlands, and thawing permafrost is transforming northern freshwaters. This terrestrially derived DOM is typically humic, highly colored, and often released together with associated substances including iron (Kritzberg and Ekström 2012; Weyhenmeyer et al. 2014) and nutrients (Jones 1992; Evans et al. 2005; Stetler et al. 2021). These co-mobilized constituents further darken the water and modify the availability and stoichiometry ("ratios") of carbon, nitrogen, and phosphorus (Berggren et al. 2014; Isles et al. 2018, 2020), thereby shaping microbial activity and altering biogeochemical functioning in lakes (Solomon et al. 2015; Creed et al. 2018).

Browning interacts strongly with warming. High DOC concentrations frequently occur in warmer, lower-latitude boreal regions, and the shallow morphology typical of boreal lakes makes them prone to strong and long thermal stratification, and disproportionally increasing surface water temperatures (Pilla et al. 2018; Puts et al. 2023a). The surface water (epilimnion) of stratified boreal lakes is mainly influenced by external inputs of elements from catchments. Hence in addition to disproportional warming, intensified and prolonged stratification during summer can increase DOM and nutrient concentrations in surface waters (Bergström and Jansson 2000; Puts et al. 2025), compounding the effects of browning on metabolic balance and resource availability especially in surface layers.

Both browning and warming influence microbial production, yet their effects differ between photoautotrophic and bacterial phytoplankton. Moderately increased DOM (approx. DOC < 11 mg/L) can stimulate algal production through nutrient supplementation, whereas high DOM (DOC > 11 mg / L) suppresses algal growth via light limitation (Hanson et al. 2003; Jones and Lennon 2015). Warming may enhance algal growth rates but can also reduce standing biomass when cells compensate for thermal stress (Filiz et al. 2020; Liu et al. 2022). Indeed, phytoplankton shows complex responses to browning and warming over large scales (Paltsev et al. 2024). In contrast, bacterial production typically continues to increase along the DOM gradient, as well as with higher temperatures (Del Giorgio and Cole 1998; Berggren et al. 2010b; a), resulting in a trend toward greater heterotrophy at higher levels of browning and warming. Growing evidence therefore points to interactive effects of DOM and warming that together may shift lake ecosystems from algal-dominated to bacterial-dominated production regimes, with implications for CO_2 dynamics and lake metabolism (Jonsson et al. 2003; Duarte and Prairie 2005; Ask et al. 2012; Lapierre et al. 2013; Bogard and del Giorgio 2016).

Despite these advances, the mechanistic links between browning, warming, and changes in water-column stoichiometry (C:N:P) and how these stoichiometric shifts regulate primary production (PP) and bacterial production (BP) remain poorly resolved. Stoichiometric constraints are expected to be particularly important during the ice-off period, when nutrient availability and microbial demand change rapidly. Moreover, in organic-rich systems that are abundant at northern latitudes, high concentrations of organic nutrients are also expected, which bioavailability may depend on the composition of DOM (Berggren et al. 2014, 2025; Rulli et al. 2022). Yet this pool cannot be measured directly, and the bioavailability therefore remains largely unquantified. Indeed, although water stoichiometry gives insights into patterns across systems, it is not a powerful predictor for phytoplankton nutritional constraints (Ptacnik et al. 2010). Therefore, to understand true phytoplankton nutritional constraints, classic nutrient limitation assays (Harpole et al. 2011), where inorganic nutrients are added to brown waters may provide a better understanding of true nutrient limitation in brown systems than assessing water stoichiometry and microbial production alone.

Here, we experimentally test how browning and warming shape water stoichiometry and microbial production during the ice-off season. We manipulated a natural colored DOM (cDOM) gradient in boreal ponds under ambient and +2 °C warming to quantify effects on (1) total and dissolved inorganic C:N:P stoichiometry of the water, (2) phytoplankton responses in inorganic nutrient-addition assays and (3) net ecosystem production by measuring PP, BP, and their ratio (PP:BP) with a lower ratio indicating increased heterotrophy. Our approach allows us to disentangle the individual and combined effects of browning and 2°C warming on early-season microbial dynamics (photoautotrophic vs heterotrophic production) in boreal freshwaters.

2. Material & Methodology

2.1. Experimental approach and setup

We experimentally established a colored dissolved organic matter (cDOM) gradient under ambient and +2 °C warmed conditions. We monitored effects on water chemistry (dissolved and total nutrients, and dissolved organic carbon (DOC)) in seven samplings. We additionally monitored bacterial production, primary production, dissolved inorganic carbon (DIC), as well as phytoplankton responses to nutrient amendments (nitrogen (N), phosphorus (P), N+P) in three sampling events. The experimental treatments began in autumn 2017, and monitoring ran from 7 May 2018 to 3 September 2018, which was an exceptionally warm summer (Blunden and Arndt 2019). The experiment was conducted at the Experimental Ecosystem Facility (EXEF) in Umeå (Sweden) using 16 treatment ponds and 4 buffer ponds to separate temperature treatments. We aimed for a 4x2 factorial design, with 4 DOC treatments in duplicate (DOC categories “Control”, “Low”, “Mid” and “High”) and 2 temperature treatments. We interpret actual DOC concentrations rather than categorical DOC treatments.

The EXEF ponds (11.5 × 6.7 m and ~1.5 m deep) are naturally functioning ecosystems with soft sediments, primary producers, and invertebrate consumers and a natural shoreline. The cDOM gradient was created by continuously mixing cDOM-rich water from the nearby Hörneån river with low-cDOM municipal tap water. The seasonal average (± SD) DOC concentrations ranged from 1.4 to 21.3 mg L^{-1}.

DOC served as an effective proxy for cDOM, and the warming treatment was successfully established with an average temperature difference of 1.8 °C between warmed and ambient ponds (Puts et al. 2023b). Ponds were fish-free until 11 May 2018, when 43 sticklebacks were added to each pond, and zooplankton biomass was low while periphytic biomass was relatively high compared to phytoplankton production. One ambient control pond was excluded due to turbidity caused by a muskrat. Specifics about the experimental water used, and management of the experimental treatments are available in (Capo et al. 2021; Koizumi et al. 2023; Puts et al. 2023b).

2.2. Water physico chemistry

We collected water just below the surface using a 0.6 m long sampler and stored the samples in 1 L bottles with minimal headspace. Samples were kept dark and processed immediately in the laboratory on the same day. We monitored total and dissolved N and P (TN, TP, DIN (NO_x + NH_4^+), and PO_4^{3-}), DOC, and pH every three weeks starting 7 May during seven samplings, and DIC in three samplings (concerted with primary production). Water for DOC, DIN, and PO_4^{3-} was filtered (0.45 µm, Sarstedt Filtropur) and DOC samples were acidified to 12 mM HCl and refrigerated, and TN, TP, DIN, and PO_4^{3-} frozen. DOC was and analyzed through high-temperature combustion using a Formacs HT-I analyzer (Skalar), and nutrients on a segmented flow analyzer (QuAAtro, SEAL Analytical GmbH, Norderstedt, Germany). DIC samples (used for primary production measurements) were prepared by injecting 4 mL air-free water into N_2-flushed, tightly sealed 18 mL glass vials containing 0.1 mL 1.2 M HCl, and analyzed on a Clarus 500 gas chromatograph (Perkin Elmer) using N_2 as carrier gas.

2.3. Bacterial and primary production, and nutrient amendment assays.

Pelagic primary- and bacterial production (PP and BP) were measured using isotopic tracers from water acquired in the same way as water for physico chemistry. The BP was measured every sampling (in total seven samplings) by incubating the water in triplicates with an isotopically labeled tracer (^{3}H, leucine) in the laboratory as soon as possible after the water sample was acquired. The water was incubated for approximately 1 hour at in situ temperature in the dark, according to Smith & Azam (1992). We measured PP during 3 sampling events, using a ^{14}C isotopic tracer as described by (Schindler et al. 1972). We filled transparent glass bottles in triplicate, and one dark bottle, with pond water. Bottles were incubated in-situ at 0.5m for 4h during midday, and the production was corrected for light factor which was calculated using continuous PAR measurements. Reported values represent the average of the three light incubations minus the dark incubation.

We tested the responses of chlorophyll-a (chla) to nutrient additions in assays where we added 500 mL pond water to transparent polyethylene bottles, with control (no added nutrients), +N, +P, and +NP treatments, each with triplicate incubations. Nitrogen and phosphorus were added as NH_4NO_3 and KH_2PO_4 in amounts that increased bottle concentrations by 10 µg P L^{-1} and 100 µg N L^{-1}, respectively. We did these assays only in the control, mid, and high DOC treatment ponds for each temperature treatment (no duplicate ponds: specifically, pond ID 15, 19 ,16; vs 3, 2 ,7, for the ambient vs warmed treatments, and control, mid, high DOC treatments). Bottles were incubated for approximately 72 hours. We did not filter water for zooplankton, but the biomass was very low due to high predation pressure (Koizumi et al. 2023). We measured chla as endpoint, by filtering 100 mL on GF/F Whatman filters and storing them in the freezer. We extracted chla in 95% ethanol for 24 h and measured on a LS-55 luminescence spectrophotometer with excitation and emission wavelengths set to 433 and 673 nm, respectively.

2.4. Data analysis

We assessed whether the browning and temperature treatments independently ("Base model") or additively ("interactive") explained variation in nutrient stoichiometry and production. We fitted linear mixed-effects regression models that accounted for the hierarchical structure of the experiment, including repeated measurements from the same experimental ponds. Response variables included primary and bacterial production and their ratio (PP, BP, and PP:BP, respectively), and nutrient stoichiometric ratios of total nutrient pools (DOC:TP, DOC:TN and TN:TP) and dissolved inorganic pools (DIC:PO_4^{3-}, DIN:PO_4^{3-}, and DIC:DIN). For each response variable, we fitted two models: 1) a base model assessing whether the browning and warming independently better explained variation in nutrient stoichiometry and production (warming + browning), and 2) an interaction model including a term where browning and warming exert independent, additive effects on predictors (warming + browning + warming*browning). Pond identity was included as a random intercept to account for repeated observations and among-pond variability. To assess seasonality, sampling occasion (Sampling) was included as a categorical fixed effect in all models, allowing responses to vary among sampling periods without assuming a linear temporal trend. All response variables were log-transformed (^{10}log) prior to analysis (Isles 2020). Models were fitted using the *lme4* package in R (Bates et al. 2015). Statistical significance of fixed effects was assessed using Satterthwaite's approximation for degrees of freedom as implemented in the *lmerTest* package (Kuznetsova et al. 2017).

We compared Akaike Information Criterion (AIC) to evaluate the relative explanatory importance of the base vs interaction model ($\Delta AIC_{Int\text{-}Base}$). This approach does not test whether either driver is statistically significant, but instead evaluates which driver explains more variation in the response variable. To facilitate comparison of the relative roles of browning and temperature treatments alone and their combined impacts across response variables, we visualized each relationship as scatter plots of the response variable against DOC.

We then evaluated if water stoichiometry met algal nutritional requirements in a ternary diagram, which shows the relative proportions of C:N:P indexed by the algal nutrient requirement ratio (Redfield ratio). We used molar concentrations of DIC, DIN, PO_4^{3-}, as well as DOC, TN, and TP, which we normalized relative to the Redfield ratio using the equations (Andersen et al. 2025). DIC was measured during only three sampling events. Across these samplings, the standard deviation of DIC was low (15% across all ponds), indicating limited variability. We therefore used the overall mean DIC when calculating Redfield-normalized values for the ternary diagram. As we found that browning (not warming) is the most dominant driver of total and dissolved C:N:P ratios, we do not distinguish between the temperature treatments when evaluating water stoichiometry. Ternary diagrams were generated using the R package *Ternary* (Smith, 2017).

For the nutrient-amendment assays, we calculated the natural logarithmic response ratio (RR_x) for each triplicate per treatment (Control, N, P, or +NP) by dividing the chla concentration in each replicate per treatment ($chla_{replicate,\ treatment}$) by the average chla concentration of the control of each treatment ($Chla_{control\ average,\ treatment}$): so that $\ln RR_x = Ln\ (chl\ \ chla_{replicate,\ treatment} / Chla_{control\ average,\ treatment})$.

3. Results & discussion

3.1. Warming vs browning: nutrient stoichiometry

Browning (DOC concentration) was a strong predictor of the stoichiometry of the total nutrient pools across all sampling dates, and warming did not alter these relationships along the DOC gradient (Fig. 1; Table 1). In linear mixed-effects models, the estimate (Est.) indicates the direction and magnitude of the relationship, i.e., the expected change in the response for a one-unit increase in the predictor. DOC strongly increased DOC:TN (Est. = 0.74, $p < 0.001$) and DOC:TP (Est. = 0.75, $p < 0.001$), indicating progressive C enrichment relative to N and P with browning (Fig 1A-B). This pattern is uniform across browning systems across the northern hemisphere (Creed et al. 2018; Isles et al. 2020). Although declines in TN:TP along the DOC gradient suggested increasing nutrient imbalance and stronger N limitation, this relationship was weak (Fig 1C). Nonetheless, this effect may be underestimated, as DIN:TP are shown to be better estimators for phytoplankton nutrient limitation (Bergström 2010).

In the dissolved nutrient pools, DOC was also an important predictor of stoichiometry (Table 1). DOC marginally decreased DIC:DIN (Est. = -0.34, $p = 0.04$), strongly decreased DIC: PO_4^{3-} (Est. = -2.38, p = <0.001) and DIN: PO_4^{3-} (Est. = -1.14, p=0.0002), indicating decreasing carbon enrichment relative to dissolved nutrients along the DOC gradient (Fig1D-F). This pattern, of increasing importance of C in the total nutrient pools but decreasing importance of carbon (hence increasing importance of dissolved nutrients) in the inorganic pools, reflects the increasing importance of organic nutrients along the DOC gradient (Corman et al. 2018; Stetler et al. 2021; Andersen et al. 2025). The ternary plots with Redfield normalized C:N:P ratios, confirm that ponds are consistently C-rich, and therefore P and N- poor relative to Redfield proportions in both dissolved (DIC–DIN– PO_4^{3-}) and total (DOC–TN–TP) nutrient pools throughout the sampling season (Fig. 2B-C; Fig. S1). The PO_4^{3-} and TP pools are around 0-15% and 0-10% of algal nutrient requirements, respectively, while the DIN and TN pools are around 0-35%, and 10-30%, respectively. Most observations fell within the P-depleted and co N&P-depleted regions of conceptual stoichiometric space (Fig. 2A). Stoichiometric imbalances were strongest in the dissolved fraction, which showed greater carbon dominance and more pronounced seasonal variability than total pools.

As the ponds are consistently carbon-saturated (Fig 2), it is particularly interesting to assess the N and P pools. The stoichiometric optimum that approximates the Redfield ratio (N:P = 16:1, molar) corresponds to a $\log_{10}$-transformed value of 1.2 for our molar concentrations (Figure 1). Ratios around this value indicate likely nitrogen–phosphorus co-limitation, whereas higher values suggest increasing phosphorus limitation and lower values indicate nitrogen limitation (Redfield 1960). In our experiment, N:P ratios spanned this critical range, from just below 1.0 to >1.5, thereby capturing conditions from nitrogen limitation to phosphorus limitation. Moreover, DOC quality is held constant as all ponds are treated with the same DOC source, but in natural systems DOC composition varies and strongly shapes its bioavailability and the resulting nutrient limitation of microbial production (Berggren et al. 2014, 2025; Soares et al. 2017).The mixed-effects model results indicate that in our experiment the stoichiometric optimum of 1.2 occurs at a DOC concentration of 7.2 mg L^{-1} for total pools, and 2.5 for dissolved pools (not accounting for the temperature treatments). These values are way below the proposed turning point of ~11 mg L^{-1} DOC, below which primary producers are primarily nutrient-limited and above which light limitation becomes more important (Hanson et al. 2003; Jones and Lennon 2015). Accordingly, the magnitude of the algal production peak near this transition (~11 mg L^{-1} DOC) is additionally driven by N:P nutrient stoichiometry (Kelly et al. 2018; Puts et al. 2023a). In our ponds, the optimal N:P ratio occurs well below 11 mg L^{-1} DOC, indicating that algal production is constrained by suboptimal nutrient ratios rather than by light availability. This explains the observed decline in primary production (Section 2.3), as light limitation in our system is unlikely (Section 2.3).

3.2.The effect of 2°C warming and seasonality on nutrient stoichiometry

For both total and dissolved nutrient pools, browning clearly dominated over warming, and warming did not modify the DOC effect (Table 1). Interaction models provided no evidence that warming altered DOC effects on total nutrient stoichiometry (with $\Delta AIC_{Int-Base}$ ranging from +0.03 to +1.45; Table 1). A notable exception was DIN: PO_4^{3-}, which was significantly affected by warming (Est. = 0.219, p = 0.038). Seasonal effects were similarly strong in both total and dissolved nutrient pools, with all pools showing pronounced temporal variation. This strong seasonal signal across both pools suggests that stoichiometric dynamics is not solely driven by the DOC loading within a waterbody but are also regulated by seasonal (moth-to-month) biogeochemical cycling (Kolzau et al. 2014). The ternary plots (Fig. 2) indicate phosphorus limitation early in the season, with nitrogen becoming relatively more limiting later. Seasonal differences in stoichiometry were limited in total pools, whereas dissolved pools showed greater variability, particularly in May when the relative contribution of nitrogen was higher than later in the season. Seasonal variability likely reflects synchronized changes in biological uptake, microbial processing, and hydrological inputs (e.g., spring run-off), which jointly restructure both total and dissolved nutrient pools.

Together, these results suggest that organic carbon enrichment was the primary driver of stoichiometric shifts in both pools, and 2°C warming does not modify these effects except for DIN: PO_4^{3-}. However, seasonal effects, where temperature is compounded, are observed. The observed effect of warming on DIN: PO_4^{3-} suggests that temperature mainly influences fast-cycling nitrogen processes. Other stoichiometric ratios from both the total and dissolved pools did not respond to warming because they may be buffered by slower turnover of total nutrient pools (Sinsabaugh and Follstad Shah 2012) or influenced by variation in microbial and phytoplankton community composition that can dampen the direct effects of temperature on nutrient cycling.

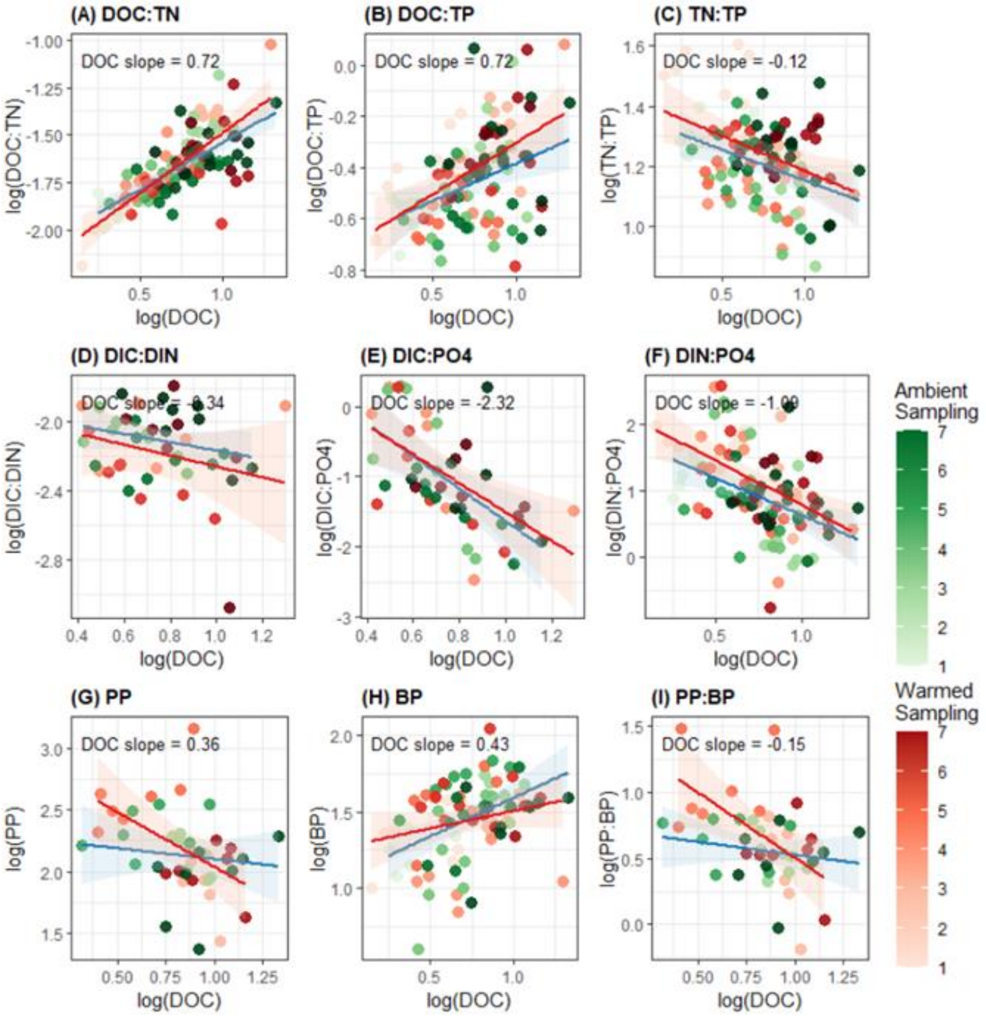

Figure 1: Relationships between dissolved organic carbon (DOC) and (A-C) stoichiometry of total nutrients pools, (D-F) stoichiometry of dissolved nutrient pools and (G-I) production metrics, derived from linear mixed-effects models. Panels show responses of (A) DOC:TN, (B) DOC:TP, (C) TN:TP, (D) DIC:DIN, (E) DIC: PO_4^{3-}, (F) DIN: PO_4^{3-}, (G) primary production (PP), (H) bacterial production (BP), and (I) PP:BP to DOC (all variables log-transformed on both axes).The colored lines are fitted trends from the mixed model (not raw regressions through all points). The slopes ("Est." in text) show average proportional response to browning, e.g., a 1% increase in DOC corresponds to a ~0.45% increase in BP after accounting for warming, sampling date and pond identity (random effect). Note that DOC concentrations in the calculated ratios are given on a molar basis, whereas the x-axis presents DOC in mg L^{-1}.

https://doi.org/10.5194/egusphere-2026-2970
Preprint. Discussion started: 24 June 2026

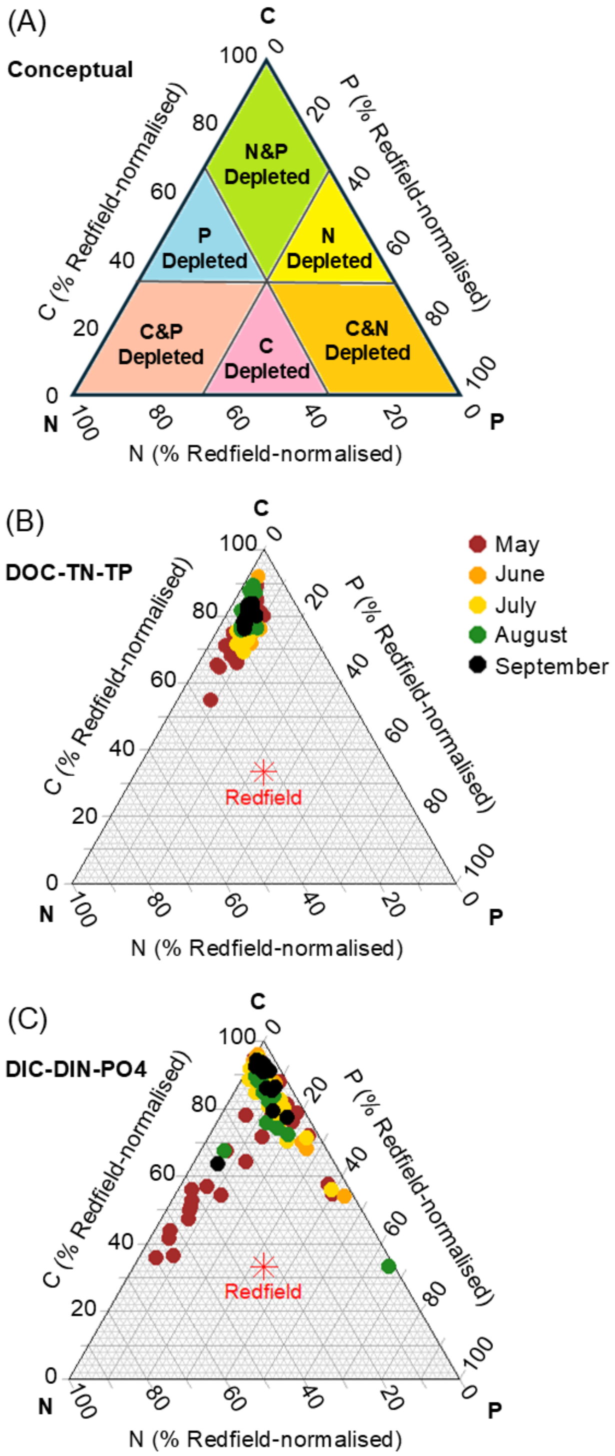

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Figure 2: Ternary diagrams illustrating carbon (C), nitrogen (N), and phosphorus (P) depletion relative to the Redfield ratio across treatments. (A) Conceptual diagram showing regions characterized by relative depletion of C, N, and P (generated ourselves, but following (Andersen et al. 2025)). (B–C) Redfield-normalized C:N:P ratios for total (unfiltered) nutrients (B) and dissolved nutrients (C), with points colored by sampling month.

3.3. Phytoplankton nutrient limitation assays

The nutrient limitation assay shows consistent N–P co-limitation across all DOC treatments, with (chlorophyll-α; chla) responses strongest when nitrogen and phosphorus were added together. Responses to P in the highest DOC treatment increased significantly compared to the control with low DOC (Fig. 3A). Responses to N and P alone were low to moderate, with slight increases at higher DOC concentrations. Our results are in line with large spatial studies across Sweden showing phytoplankton in southern lakes are co-limited by N and P, while phytoplankton in carbon-poor northern lakes were mainly N-limited (Bergström et al. 2008; Bergström 2010). DOC enrichment (browning) therefore shifts the system toward stronger nutrient stress for phytoplanktonic algal (chla) production, confirming that browning leads to less favorable nutritional requirements for algal production (section 2.1). Temperature had a minimal and insignificant effect (Fig. 3B). Nonetheless, the highest RR_{chla} values were observed under Ambient temperature conditions, and warming generally reduced response, particularly in Mid and High DOC treatments, although this response was not significant. Overall, higher DOC enhanced nutrient-driven responses, particularly under NP additions, while warming tended to dampen these effects, consistent with patterns observed for primary production (Section 2.4), although these effects were not statistically significant.

Table 1. Results of linear mixed-effects models relating DOC concentration to response variables, with warming treatment and sampling period included as fixed effects and pond identity as a random intercept. Models are: DOC + Temperature base models (i.e., lmer(logY ~ log(DOC) + TempTreat + Sampling + (1|pond)) and interaction models (i.e., lmer(logY ~ log(DOC) x TempTreat + Sampling + (1|pond). Significant ($p<0.05$) results are in bold. For Sampling, *p*-values refer to the overall Type III test of the categorical factor rather than a single slope estimate. ΔAIC$_{Int–Base}$ was calculated as AIC$_{interaction\ model}$ − AIC$_{base\ model}$; negative values indicate improved fit of the interaction model. R^2m is marginal R^2 (i.e., variance explained by fixed effects: DOC, warming, sampling) for Base model and Interactive model.

Model/ Response	**Predictor**	**Estimate**	**SE**	**df**	**t value**	***p* value**	**R^2m**	**ΔAIC$_{Int – Base}$**	**Interpretation**
log(DOC:TN)	log(DOC)	0.72	0.06	93	11.73	**< 0.001**	0.72		Browning is associated with increases in DOC:TN (C enrichment relative to TN)
	Warming (vs ambient)	0.04	0.11	93	0.34	0.71			No effect
	Sampling (time)	—	—	—	—	**< 0.001**			Strong seasonal control
	Int Model: log(DOC) × Warming	0.09	0.07	94	1.41	0.16	0.75	0.03	No statistically supported interaction for DOC effects on DOC:TN
log(DOC:TP)	log(DOC)	0.72	0.09	93	8.0	**< 0.001**	0.48		Browning is associated with increases in DOC:TP (C enrichment relative to TP)
	Warming (vs ambient)	0.12	0.17	93	0.72	0.48			No effect
	Sampling (time)	—	—	—	—	**< 0.001**			Strong seasonal control
	Int Model: log(DOC) × Warming	−0.07	0.09	93	−0.76	0.45	0.51	1.45	No interaction: DOC effects on DOC:TP do not depend on temperature
log(TN:TP)	log(DOC)	-0.12	0.06	93	-1.92	0.06	0.51		Marginal effect
	Warming (vs ambient)	0.03	0.03	93	1.34	0.21			No effect
	Sampling (time)	—	—	—	—	**< 0.001**			Strong seasonal control of TOC:TP
	Int Model: log(DOC) × Warming	−0.11	0.08	103	−1.44	0.15	0.53	0.34	No clear evidence for DOC × temperature interaction on TN:TP
log(DIC:DIN)	log(DOC)	−0.34	0.16	38	−2.13	**0.04**	0.24		Browning significantly decreases DIC:DIN
	Warming (vs ambient)	-0.07	0.06	38	−1.18	0.26			No effect
	Sampling (time)	—	—	—	—	**0.008**			Seasonal variation detected
	Int Model: log(DOC) × Warming	-0.09	0.31	25	−0.32	0.75	0.27	1.9	No interaction
log(DIC:P PO$_4^{3-}$)	log(DOC)	-2.32	0.44	38	-5.37	**<0.001**	0.42		Browning significantly decreases DIC: PO$_4^{3-}$

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Model/ Response	Predictor	Estimate	SE	df	t value	*p* value	R²m	$\Delta AIC_{Int-Base}$	Interpretation
	Warming (vs ambient)	0.07	0.17	38	0.43	0.66			No effect
	Sampling (time)	—	—	—	—	**0.04**			No evidence of seasonal variation in DIC: PO_4^{3-}
	Int Model: log(DOC) × Warming	-0.001	0.85	45	-0.001	0.99	0.44	1.86	Warming does not alter DOC effect
log(DIN: PO_4^{3-})	log(DOC)	-1.09	0.30	104	-3.81	**0.0002**	0.32		Browning significantly decreases DIN: PO_4^{3-}
	Warming (vs ambient)	0.22	0.10	104	2.10	**0.038**			Significant warming effect
	Sampling (time)	—	—	—	—	**0.041**			Strong seasonal control
	Int Model: log(DOC) × Warming	-0.31	0.42	104	-0.86	0.39	0.33	1.3	No interaction
log(PP)	log(DOC)	0.36	0.23	44	1.71	0.09	0.47		No evidence that browning affects PP
	Warming (vs ambient)	0.10	0.16	44	0.71	0.52			Warming does not significantly alter PP
	Sampling (time)	—	—	—	—	**< 0.01**			Strong seasonal control of PP
	Int Model: log(DOC) × Warming	−0.49	0.33	44	−1.52	0.14	0.53	0.24	No interaction
log(BP)	log(DOC)	0.43	0.11	79	3.88	**0.0003**	0.47		Browning increases BP
	Warming (vs ambient)	−0.04	0.09	79	−0.48	0.7			Warming has no effect on BP after accounting for DOC
	Sampling (time)	—	—	—	—	**< 0.001**			Strong seasonal variation in BP
	Int Model: log(DOC) × Warming	−0.16	0.15	75	−1.05	0.29	0.52	0.95	No evidence that warming modifies the DOC effect on BP
log(PP:BP)	log(DOC)	-0.15	0.25	44	-0.38	0.69	0.36		Browning does not significantly alter PP:BP
	Warming (vs ambient)	0.11	0.18	44	1.37	0.18			No effect
	Sampling (time)	—	—	—	—	**0.022**			Strong seasonal control of PP:BP
	Int Model: log(DOC) × Warming	−0.73	0.39	44	−2.04	**0.047**	0.37	−1.99	Warming alters the DOC–PP:BP relationship, with PP:BP declining more strongly with DOC under warming

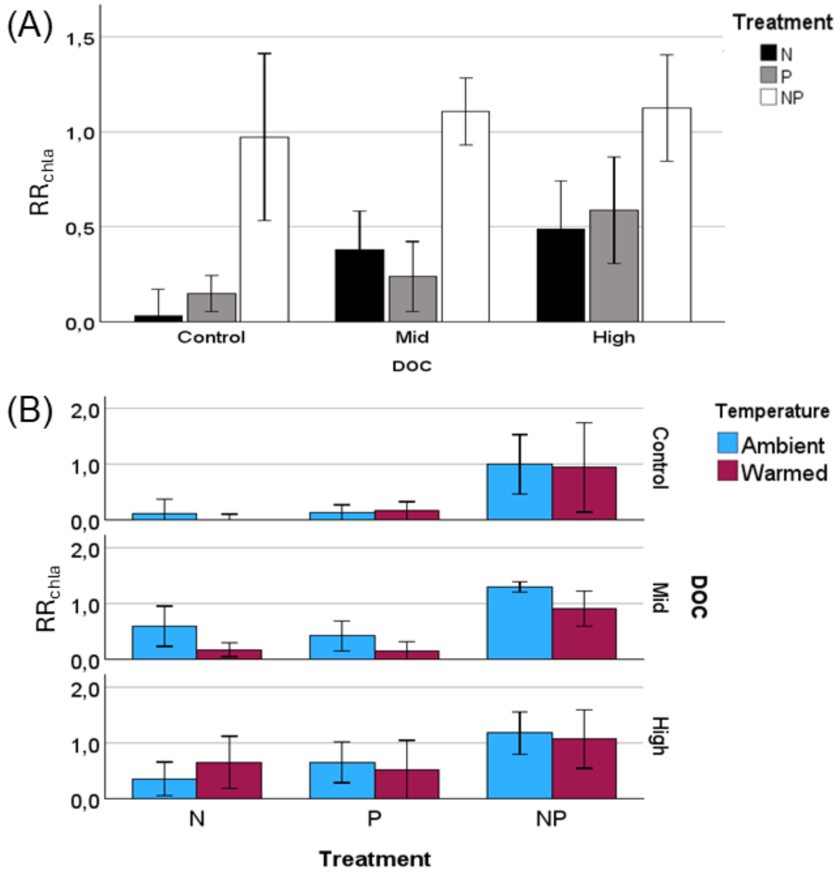


Figure 3. (A) Mean seasonal chlorophyll-a response ratios (RR_{chla}) to nutrient-addition assays (N, P, or NP) per DOC treatment, pooled across temperature treatments. (B) Mean seasonal RR_{chla} per added nutrient, and DOC treatment (panel), per temperature treatment.

3.4. Microbial production under browning and warming

Microbial production responded differently to browning and warming. Both BP and PP ($p < 0.001$ and $p < 0.01$, respectively), as well as PP:BP ($p = 0.022$) varied significantly across sampling times, indicating strong seasonal influence on microbial responses. Bacterial production (BP) increased strongly with DOC (Est. = 0.45, *p* = 0.0003; Fig. 1H) and was unaffected by warming or DOC–warming interactions (Fig 1H; Table 1). In contrast to primary producers, bacterial (heterotrophic) producers are well known to assimilate organic carbon efficiently, have a more flexible stoichiometry (Cotner et al. 2010) and are a substantial part of carbon cycling and the microbial foodweb via the "microbial loop" (Fenchel 2008). Indeed, primary production (PP) showed no significant response to DOC or warming although it showed a trend of higher values at low DOC that declined more sharply with DOC under warming, resulting in lower PP at high DOC in warmed treatments (Table 1; Figure 1G). Accordingly, warming amplified DOC-related declines in the PP:BP ratio (interaction: Est. = −0.73, p = 0.047) indicating that the ponds shifted toward stronger heterotrophic dominance, although net heterotrophy (PP:BP < 1) was not reached. Net heterotrophy was likely not reached because measurements were taken at a single, well-lit depth (0.6 m) rather than integrated over the full water column (Klaus et al. 2022), and because shallow lakes and ponds (≤4 m) typically exhibit high rates of both primary and heterotrophic production and large variability in net ecosystem production (Rabaey et al. 2024).

This pattern, indicating interactive effects of DOC and warming on metabolic balance (PP:BP), can only be disentangled in an experimental setting, as in natural lakes temperature variations are confounded by other factors that govern microbial production, such as nutrients, light, variability in DOC quality and quantity. Our finding of inhibited primary production due to warming contrasts with observations from Swedish Arctic lakes, where primary production is typically temperature-limited (Faithfull et al. 2011; Bergström et al. 2013; Puts et al. 2023a). Instead, our results controlled the confounded factors, and show that browning promotes net heterotrophy, but that this shift is not driven solely by increased bacterial production, it can also arise from warming-induced declines in primary production, so called "thermal compensation" (Liu et al. 2022). Indeed, temperatures during this experiment were unusually high (see section 2.1), meaning the additional +2 °C warming may have pushed the system beyond the thermal optimum of primary producers, whose growth declines sharply at temperatures higher than their thermal optimum (Kontopoulos et al. 2020). Moreover, we showed (section 2.1) that along the DOC gradient inorganic nutrient availability declines, which would impact production negatively. In less warm years, where primary producers are not passing optimal growth temperatures, the effect of temperature may not be negative, still, our results show that with browning PP will decrease. Our results indicate that bacterial production is thriving under 2°C warming and browning, whereas primary producers struggle, likely due to 1) thermal compensation and 2) Reduced inorganic nutrients, and 3) suboptimal stoichiometry.

With the increasing occurrence of heatwaves (Perkins-Kirkpatrick and Lewis 2020) and the concentration of browning and warming in surface waters (Pilla et al. 2018; Puts et al. 2023a), the conditions observed in our ponds are likely to become more common. Altogether, our results indicate that in ponds and natural lakes, net ecosystem respiration, or heterotrophy would be likely under browning and warming, particularly as warming intensifies epilimnetic temperatures (Duarte and Prairie 2005; Sobek et al. 2007; Bogard and del Giorgio 2016).

4. Conclusions

Our study demonstrates that browning (increasing dissolved organic carbon concentrations) is the dominant driver determining nutrient stoichiometry, phytoplankton responses, and ecosystem metabolism (ratio between primary production and bacterial production: PP:BP). Moderate warming (+2 °C) only affected DIN:$PO_4^{3-}$and PP:BP. Across all sampling periods, increasing DOC consistently altered C:N:P ratios both total and dissolved pools, leading to progressively stronger carbon enrichment in total nutrient pools, but decreasing dissolved inorganic nutrients. Although N:P ratios in both total and dissolved pools decreased with DOC indicating stronger N-limitation, both pools remained strongly carbon-saturated and relatively depleted in both nitrogen and phosphorus.

Phytoplankton nutrient limitation assays confirmed widespread N–P co-limitation across the DOC gradient, with the strongest responses occurring under combined (N+P) additions. Higher DOC amplified these nutrient-driven responses, indicating that browning intensifies stoichiometric constraints on algal production, although warming did not affect algal nutrient limitation. Browning promoted a shift toward greater heterotrophic dominance by strongly stimulating bacterial production (BP), while primary production (PP) showed no significant effect but tended to decline with increasing DOC, particularly under warming conditions. Although 2°C warming alone had no significant effects, we found interactive effects with DOC that significantly reduced the balance between autotrophy and heterotrophy (PP:BP), indicating a shift toward increased reliance on heterotrophic pathways. However, full net heterotrophy was not reached, likely due to shallow, well-lit conditions and high overall productivity.

Our results are novel in that they show that 2°C warming and browning alone, and combined, impact primary production differentially than bacterial production. When combined, it leads to a reduction of PP:BP. This identifies an additional mechanistic pathway contributing to the widespread occurrence of net ecosystem respiration in northern lakes. Given the widespread intensification of browning and warming events across northern lakes, our findings suggest that primary producers may become increasingly constrained, while heterotrophic bacterial production is maintained or enhanced, particularly under episodic heatwaves.

5. Data availability

The raw and processed data used for statistical analysis and the preparation of figures in the study are available at https://zenodo.org/records/20498522 (Paltsev and Puts 2026) (open access).

6. Disclaimer

7. Author contributions

AP and ICP contributed equally to the development of the study design, data interpretation, and manuscript preparation. ICP was responsible for conducting field sampling and laboratory analyses.

8. Competing interests

The authors declare that they have no conflict of interest.

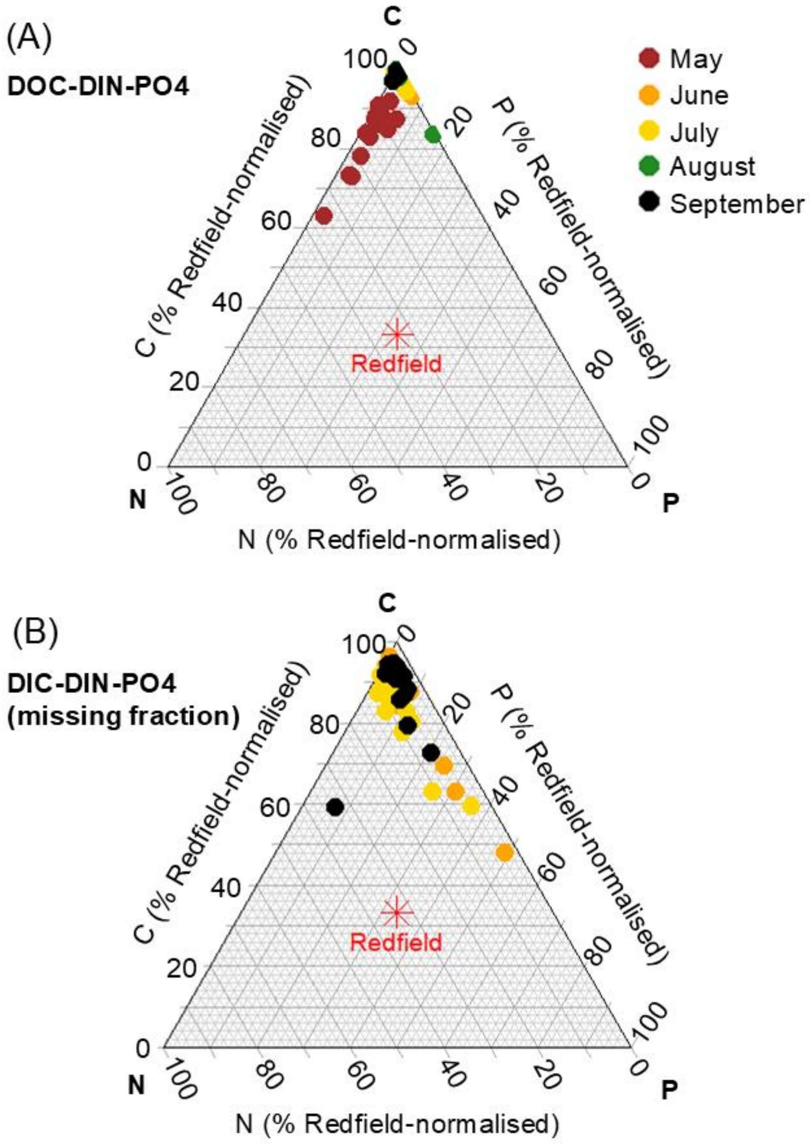
9. Acknowledgements

We thank Ann-Kristin Bergström with supporting with materials and extend our gratitude for her valuable career guidance.. We are grateful to Pelle Byström & Erik Geibrink for access and maintenance to the experimental ecosystems facility (EXEF), and Anders Jonsson & BAF for analyzing the water chemistry. AI was used to optimize the modelling workflow, but no parts of the manuscript were generated.

10. Financial support

This study was financed by Swedish Research Council FORMAS

11. Appendix



Appendix Figure 1. Redfield-normalized C:N:P ratios for dissolved nutrients and DOC (A) and dissolved nutrients and in situ DIC with data available for June, July and September (B), with points colored by sampling month.

12. References

Andersen, I. M., J. M. Taylor, D. Graeber, P. T. Kelly, A. K. Hoke, C. J. Robbins, and J. T. Scott. 2025. Redfield revisited: Insights into freshwater seston carbon : nitrogen : phosphorus stoichiometry. Limnol. Oceanogr. doi:10.1002/lno.70133

Ask, J., J. Karlsson, and M. Jansson. 2012. Net ecosystem production in clear-water and brown-water lakes. Global Biogeochem. Cycles 26. doi:10.1029/2010GB003951

Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. Fitting Linear Mixed-Effects Models Using lme4. J. Stat. Softw. 67. doi:10.18637/jss.v067.i01

Berggren, M., H. Laudon, M. Haei, L. Ström, and M. Jansson. 2010a. Efficient aquatic bacterial metabolism of dissolved low-molecular-weight compounds from terrestrial sources. ISME Journal 4: 408–416. doi:10.1038/ismej.2009.120

Berggren, M., H. Laudon, A. Jonsson, and M. Jansson. 2010b. Nutrient constraints on metabolism affect the temperature regulation of aquatic bacterial growth efficiency. Microb. Ecol. 60: 894–902. doi:10.1007/s00248-010-9751-1

Berggren, M., M. P. D. Rulli, A. K. Bergström, R. A. Sponseller, and G. Hensgens. 2025. Does Dissolved Organic Matter Composition Help Explain the Concentrations of Bioavailable Macronutrients in Organic Matter-Rich Freshwaters? Freshw. Biol. 70. doi:10.1111/fwb.70141

Berggren, M., R. A. Sponseller, A. R. Alves Soares, and A. K. Bergström. 2014. Toward an ecologically meaningful view of resource stoichiometry in DOM-dominated aquatic systems. J. Plankton Res. 37: 489–499. doi:10.1093/plankt/fbv018

Bergström, A. K. 2010. The use of TN:TP and DIN:TP ratios as indicators for phytoplankton nutrient limitation in oligotrophic lakes affected by N deposition. Aquat. Sci. 72: 277–281. doi:10.1007/s00027-010-0132-0

Bergström, A. K., C. Faithfull, D. Karlsson, and J. Karlsson. 2013. Nitrogen deposition and warming - effects on phytoplankton nutrient limitation in subarctic lakes. Glob. Chang. Biol. 19: 2557–2568. doi:10.1111/gcb.12234

Bergström, A. K., and M. Jansson. 2000. Bacterioplankton production in humic Lake Ortrasket in relation to input of bacterial cells and input of allochthonous organic carbon. Microb. Ecol. 39: 101–115. doi:10.1007/s002480000007

Bergström, A. K., A. Jonsson, and M. Jansson. 2008. Phytoplankton responses to nitrogen and phosphorus enrichment in unproductive Swedish lakes along a gradient of atmospheric nitrogen deposition. Aquat. Biol. 4: 55–64. doi:10.3354/ab00099

Blunden, J., and D. S. Arndt. 2019. Takeaway points from the state of the climate supplement. Bull. Am. Meteorol. Soc. 100: 1625–1636. doi:10.1175/BAMS-D-19-0193.1

Bogard, M. J., and P. A. del Giorgio. 2016. The role of metabolism in modulating CO2 fluxes in boreal lakes. Global Biogeochem. Cycles 30: 1509–1525. doi:10.1002/2016GB005463

Capo, E., G. Spong, S. Koizumi, I. Puts, F. Olajos, H. Königsson, J. Karlsson, and P. Byström. 2021. Droplet digital PCR applied to environmental DNA, a promising method to estimate fish population abundance from humic-rich aquatic ecosystems. Environmental DNA 3: 343–352. doi:10.1002/edn3.115

Corman, J. R., B. L. Bertolet, N. J. Casson, S. D. Sebestyen, R. K. Kolka, and E. H. Stanley. 2018. Nitrogen and Phosphorus Loads to Temperate Seepage Lakes Associated With Allochthonous Dissolved Organic Carbon Loads. Geophys. Res. Lett. 45: 5481–5490. doi:10.1029/2018GL077219

Cotner, J. B., E. K. Hall, J. T. Scott, and M. Heldal. 2010. Freshwater Bacteria are Stoichiometrically Flexible with a Nutrient Composition Similar to Seston. Front. Microbiol. 1. doi:10.3389/fmicb.2010.00132

Creed, I. F. and others. 2018. Global change-driven effects on dissolved organic matter composition: Implications for food webs of northern lakes. Glob. Chang. Biol. 24: 3692–3714. doi:10.1111/gcb.14129

Duarte, C. M., and Y. T. Prairie. 2005. Prevalence of heterotrophy and atmospheric CO2 emissions from aquatic ecosystems. Ecosystems 8: 862–870. doi:10.1007/s10021-005-0177-4

Evans, C. D., D. T. Monteith, and D. M. Cooper. 2005. Long-term increases in surface water dissolved organic carbon: Observations, possible causes and environmental impacts. Environmental Pollution 137: 55–71. doi:10.1016/j.envpol.2004.12.031

Faithfull, C. L., A. K. Bergström, and T. Vrede. 2011. Effects of nutrients and physical lake characteristics on bacterial and phytoplankton production: A meta-analysis. Limnol. Oceanogr. 56: 1703–1713. doi:10.4319/lo.2011.56.5.1703

Fenchel, T. 2008. The microbial loop - 25 years later. J. Exp. Mar. Biol. Ecol. 366: 99–103. doi:10.1016/j.jembe.2008.07.013

Filiz, N. and others. 2020. Phytoplankton community response to nutrients, temperatures, and a heat wave in shallow lakes: An experimental approach. Water (Switzerland) 12. doi:10.3390/w12123394

Del Giorgio, P. A., and J. J. Cole. 1998. Bacterial Growth Efficiency in Natural Aquatic Systems.

Hanson, P. C., D. L. Bade, S. R. Carpenter, and T. K. Kratz. 2003. Lake metabolism: Relationships with dissolved organic carbon and phosphorus.

Harpole, W. S. and others. 2011. Nutrient co-limitation of primary producer communities. Ecol. Lett. 14: 852–862. doi:10.1111/j.1461-0248.2011.01651.x

Isles, P. D. F. 2020. The misuse of ratios in ecological stoichiometry.

Isles, P. D. F., I. F. Creed, and A. K. Bergström. 2018. Recent Synchronous Declines in DIN:TP in Swedish Lakes. Global Biogeochem. Cycles 32: 208–225. doi:10.1002/2017GB005722

Isles, P. D. F., A. Jonsson, I. F. Creed, and A. K. Bergström. 2020. Does browning affect the identity of limiting nutrients in lakes? Aquat. Sci. 82. doi:10.1007/s00027-020-00718-y

Jones, R. I. 1992. Dissolved Organic Matter in Lacustrine Ecosystems: Energy Source and System Regulator.

Jones, S. E., and J. T. Lennon. 2015. A test of the subsidy-stability hypothesis: the effects of terrestrial carbon in aquatic ecosystems.

Jonsson, A., J. Karlsson, and M. Jansson. 2003. Sources of carbon dioxide supersaturation in clearwater and humic lakes in northern Sweden. Ecosystems 6: 224–235. doi:10.1007/s10021-002-0200-y

Kelly, P. T., C. T. Solomon, J. A. Zwart, and S. E. Jones. 2018. A Framework for Understanding Variation in Pelagic Gross Primary Production of Lake Ecosystems. Ecosystems 21: 1364–1376. doi:10.1007/s10021-018-0226-4

Klaus, M., H. A. Verheijen, J. Karlsson, and D. A. Seekell. 2022. Depth and basin shape constrain ecosystem metabolism in lakes dominated by benthic primary producers. 67: 2763–2778. doi:10.2307/27362734

Koizumi, S., M. Hamdan, I. C. Puts, A. K. Bergström, J. Karlsson, and P. Byström. 2023. Experimental warming and browning influence autumnal pelagic and benthic invertebrate biomass and community structure. Freshw. Biol. 68: 1224–1237. doi:10.1111/fwb.14099

Kolzau, S., C. Wiedner, J. Rücker, J. Köhler, A. Köhler, and A. M. Dolman. 2014. Seasonal Patterns of Nitrogen and Phosphorus Limitation in Four German Lakes and the Predictability of Limitation Status from Ambient Nutrient Concentrations. PLoS One 9: e96065. doi:10.1371/journal.pone.0096065

Kontopoulos, D. G., E. van Sebille, M. Lange, G. Yvon-Durocher, T. G. Barraclough, and S. Pawar. 2020. Phytoplankton thermal responses adapt in the absence of hard thermodynamic constraints. Evolution (N. Y). 74: 775–790. doi:10.1111/evo.13946

Kritzberg, E. S., and S. M. Ekström. 2012. Increasing iron concentrations in surface waters - A factor behind brownification? Biogeosciences 9: 1465–1478. doi:10.5194/bg-9-1465-2012

Kuznetsova, A., P. B. Brockhoff, and R. H. B. Christensen. 2017. lmerTest Package: Tests in Linear Mixed Effects Models. J. Stat. Softw. 82. doi:10.18637/jss.v082.i13

Lapierre, J. F., F. Guillemette, M. Berggren, and P. A. Del Giorgio. 2013. Increases in terrestrially derived carbon stimulate organic carbon processing and CO 2 emissions in boreal aquatic ecosystems. Nat. Commun. 4. doi:10.1038/ncomms3972

Liu, K., B. Chen, and H. Liu. 2022. Evidence of partial thermal compensation in natural phytoplankton assemblages. Limnol. Oceanogr. Lett. 7: 122–130. doi:10.1002/lol2.10227

Paltsev, A. and others. 2024. Phytoplankton biomass in northern lakes reveals a complex response to global change. Science of the Total Environment 940. doi:10.1016/j.scitotenv.2024.173570

Paltsev, A., and I. C. Puts. 2026. Warming vs. browning: a dual mechanism behind net ecosystem production of shallow brown aquatic systems. Dataset. .doi:10.5281/zenodo.20498522

Perkins-Kirkpatrick, S. E., and S. C. Lewis. 2020. Increasing trends in regional heatwaves. Nat. Commun. 11: 3357. doi:10.1038/s41467-020-16970-7

Pilla, R. M., C. E. Williamson, J. Zhang, R. L. Smyth, J. D. Lenters, J. A. Brentrup, L. B. Knoll, and T. J. Fisher. 2018. Browning-Related Decreases in Water Transparency Lead to Long-Term Increases in Surface Water Temperature and Thermal Stratification in Two Small Lakes. J. Geophys. Res. Biogeosci. 123: 1651–1665. doi:10.1029/2017JG004321

Ptacnik, R., T. Andersen, and T. Tamminen. 2010. Performance of the Redfield Ratio and a Family of Nutrient Limitation Indicators as Thresholds for Phytoplankton N vs. P Limitation. Ecosystems 13: 1201–1214. doi:10.1007/s10021-010-9380-z

Puts, I. C. and others. 2025. Impacts of hypoxia on boreal lake biogeochemistry and productivity: a 4-year whole-ecosystem BACI experiment. Biogeochemistry 168. doi:10.1007/s10533-025-01262-3

https://doi.org/10.5194/egusphere-2026-2970
Preprint. Discussion started: 24 June 2026

Puts, I. C., J. Ask, A. Deininger, A. Jonsson, J. Karlsson, and A. K. Bergström. 2023a. Browning affects pelagic productivity in northern lakes by surface water warming and carbon fertilization. Glob. Chang. Biol. 29: 375–390. doi:10.1111/gcb.16469

Puts, I. C., J. Ask, M. Myrstener, and A. K. Bergström. 2023b. Contrasting impacts of warming and browning on periphyton. Limnol. Oceanogr. Lett. 8: 628–638. doi:10.1002/lol2.10317

Rabaey, J. S. and others. 2024. Freshwater Biogeochemical Hotspots: High Primary Production and Ecosystem Respiration in Shallow Waterbodies. Geophys. Res. Lett. 51. doi:10.1029/2023GL106689

Rulli, M. P. D., A. K. Bergström, R. A. Sponseller, and M. Berggren. 2022. Seasonal patterns in nutrient bioavailability in boreal headwater streams. Limnol. Oceanogr. 67: 1169–1183. doi:10.1002/lno.12064

Schindler, D. W., R. V. Schmidt, and R. A. Reid. 1972. Acidification and Bubbling as an Alternative to Filtration in Determining Phytoplankton Production by the ^{14} C Method. Journal of the Fisheries Research Board of Canada 29: 1627–1631. doi:10.1139/f72-250

Sinsabaugh, R. L., and J. J. Follstad Shah. 2012. Ecoenzymatic Stoichiometry and Ecological Theory. Annu. Rev. Ecol. Evol. Syst. 43: 313–343. doi:10.1146/annurev-ecolsys-071112-124414

Soares, A. R. A., A. K. Bergstrom, R. A. Sponseller, J. M. Moberg, R. Giesler, E. S. Kritzberg, M. Jansson, and M. Berggren. 2017. New insights on resource stoichiometry: Assessing availability of carbon, nitrogen, and phosphorus to bacterioplankton. Biogeosciences 14: 1527–1539. doi:10.5194/bg-14-1527-2017

Sobek, S., L. J. Tranvik, Y. T. Prairie, P. Kortelainen, and J. J. Cole. 2007. Patterns and regulation of dissolved organic carbon: An analysis of 7,500 widely distributed lakes. Limnol. Oceanogr. 52: 1208–1219. doi:10.4319/lo.2007.52.3.1208

Solomon, C. T. and others. 2015. Ecosystem Consequences of Changing Inputs of Terrestrial Dissolved Organic Matter to Lakes: Current Knowledge and Future Challenges. Ecosystems 18: 376–389. doi:10.1007/s10021-015-9848-y

Stetler, J. T., L. B. Knoll, C. T. Driscoll, and K. C. Rose. 2021. Lake browning generates a spatiotemporal mismatch between dissolved organic carbon and limiting nutrients. Limnol. Oceanogr. Lett. 6: 182–191. doi:10.1002/lol2.10194

Weyhenmeyer, G. A., Y. T. Prairie, and L. J. Tranvik. 2014. Browning of boreal freshwaters coupled to carbon-iron interactions along the aquatic continuum. PLoS One 9. doi:10.1371/journal.pone.0088104