

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~~ microbial 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-rich 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 did not 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 cDOM-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.

1. Introduction

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

Browning interacts strongly with warming. High cDOM and associated 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 (Houser, 2006; Pilla et al., 2018). Shallow lakes can experience strong and prolonged thermal stratification when high concentrations of dissolved organic matter increase light attenuation, causing heat to be absorbed near the surface, and disproportionally increasing surface water temperatures (Puts, Ask, Deininger, et al., 2023). This creates a stable density gradient that suppresses wind-driven mixing despite the lakes' shallow depth (Fee et al., 1996). 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 cDOM and nutrient concentrations in surface waters (Bergström & 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 cDOM (approx. DOC < 11 mg/L) can stimulate algal production through nutrient supplementation, whereas high cDOM (DOC > 11 mg / L) suppresses algal growth via light limitation (Hanson et al., 2003; S. E. Jones & 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 cDOM gradient, as well as with higher temperatures (Berggren, Laudon, Haei, et al., 2010; Berggren, Laudon, Jonsson, et al., 2010; Del Giorgio & Cole, 1998), resulting in a trend toward greater heterotrophy at higher levels of browning and warming. Growing evidence therefore points to interactive effects of cDOM and warming that together may shift lake ecosystems from algal-dominated to bacterial-dominated production regimes, with implications for CO₂ dynamics

and lake metabolism (Ask et al., 2012; Bogard & del Giorgio, 2016; Duarte & Prairie, 2005; Jonsson et al., 2003; Lapierre et al., 2013).

Despite these advances, the mechanistic links connecting browning and warming to 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 (Hampton et al., 2017). 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 cDOM (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 resource limitation in these 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 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 versus heterotrophic production) in boreal freshwaters.

2. Material & Methodology

2.1. Experimental approach and setup

We experimentally established a 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 BP, PP, 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 & 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 eDOM gradient was created by continuously mixing eDOM rich water from the nearby Hörneån river with low eDOM municipal tap water. The seasonal average DOC concentrations ranged from 1.4 to 21.3 mg L⁻¹. DOC served as an effective proxy for eDOM, and the warming treatment was successfully established with an average temperature difference of 1.8 °C between warmed and ambient ponds (Puts, Ask, Myrstener, et al., 2023). 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 + NH₄⁺), and PO₄³⁻), DOC, and pH every three weeks starting 7 May during seven samplings, and dissolved inorganic carbon (DIC) in three samplings (concoerted with PP). Water for DOC, DIN, and PO₄³⁻ was filtered (0.45 µm; Sarstedt Filtropur) and DOC samples were acidified to 12 mM HCl and refrigerated, and TN, TP, DIN, and PO₄³⁻ frozen. DOC was 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 PP measurements) were prepared by injecting 4 mL air-free water into N₂ 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 N₂ 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 (³H, 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 (1992). We measured PP during 3 sampling events, using a ¹⁴C 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* (chl_a) 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 NH₄NO₃ and KH₂PO₄ 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 chl_a as endpoint, by filtering 100 mL on GF/F Whatman filters and storing them in the freezer before further analyses. We extracted chl_a 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 (“interaction model”) 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:PO₄³⁻, DIN:PO₄³⁻, 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 (¹⁰log) 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 versus interaction model ($\Delta\text{AIC}_{\text{int-Base}}$). 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). DOC and DIC represent different carbon pools that affect different ecological processes. When ecosystem metabolism is considered, DIC is particularly relevant for PP because it is directly assimilated by photoautotrophs during photosynthesis. In contrast, DOC is generally unavailable to primary producers and is mostly utilized in heterotrophic metabolism, hence more closely linked to BP. The same goes for the dissolved inorganic nutrient pools versus total pools: the dissolved inorganic pool is directly available to phytoplankton (which could be autotrophic or heterotrophic), whereas the total nutrient pools include organic fractions, which would need (enzymatic) preprocessing, and which can have varying bioavailability (Berggren et al., 2025; Rulli et al., 2022, 2026; Soares et al., 2017). Both pools are therefore informative, but different. This is confirmed in our brown ponds; expressing the dissolved inorganic nutrient pools relative to DOC rather than DIC yields an average Redfield normalized carbon value of $95 \pm 11\%$, compared with $80 \pm 16\%$ (average $\pm$ SD) when normalized to DIC (Appendix Fig. 4). 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* (M. R. Smith et al., 2025).

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 chl a concentration in each replicate per treatment ($\text{chl}_{a, \text{replicate, treatment}}$) by the average chl a concentration of the control of each treatment ($\text{chl}_{a, \text{control average, treatment}}$), so that $\ln \text{RR}_x = \ln (\text{chl}_{a, \text{replicate, treatment}} / \text{chl}_{a, \text{control average, treatment}})$.

3.2. Results & discussion

3.1.2.1. Warming versus 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. While

increased cDOM and DOC concentrations increase the absolute amount of nutrients (open data), DOC strongly increased DOC:TN (Est. = 0.72, $p < 0.001$) and DOC:TP (Est. = 0.72, $p < 0.001$), indicating progressive carbon 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 (Est. = -0.12, $p=0.06$; 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:PO₄³⁻ (Est. = -2.32, $p < 0.001$) and DIN:PO₄³⁻ (Est. = -1.09, $p=0.0002$), indicating decreasing inorganic carbon enrichment relative to dissolved nutrients along the DOC gradient (Fig 1D-F). The increasing importance of carbon in the total nutrient pools (DOC:TN and DOC:TP), but decreasing importance of carbon in the inorganic pools (DIC:DIN and DIC: PO₄³⁻), reflects the increasing importance of organic nutrients along the DOC gradient (Andersen et al., 2025; Corman et al., 2018; Stetler et al., 2021). The ternary plots with Redfield normalized C:N:P ratios, confirm that ponds are consistently carbon-rich, and therefore $\overline{NP}$ and $\overline{PN}$ -poor relative to Redfield proportions in both dissolved (DIC–DIN– PO₄³⁻) and total (DOC–TN–TP) nutrient pools throughout the sampling season (Fig. 2B-C; Fig. S1). Most observations fell within the P-depleted and co N&P-depleted, and occasionally N-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. Most importantly, browning increased the C:N and C:P ratios in the dissolved carbon and total nutrient pools, indicating lower TN and TP relative to DOC, while decreasing these ratios in the dissolved fractions, indicating lower DIC relative to DIN and PO₄³⁻ (Fig. 1A-B, D-E). This contrasting pattern suggests that browning enhances the availability of carbon substrates for heterotrophs while reducing the relative carbon resources available to photoautotrophs.

As the ponds are consistently enriched in carbon relative to N and P (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 log₁₀-transformed value of 1.2 (¹⁰log (16:1)) for our molar concentrations (Figure 1). Ratios around this value indicate likely nitrogen–phosphorus co-limitation, whereas higher values suggest increasing P limitation and lower values indicate N limitation (Redfield, 1958). In our experiment, N:P ratios spanned this critical range, from just below 1.0 to >1.5, thereby capturing conditions from N limitation to P 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 a DOC concentration of 2.5 mg L⁻¹ 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; S. E. Jones & Lennon, 2015). Accordingly, the magnitude of the algal production peaks near this transition (~11 mg L⁻¹ DOC) is additionally driven by N:P nutrient stoichiometry (Kelly et al., 2018; Puts, Ask, Deininger, et al., 2023). 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 PP-(Section 2.3), as light limitation in our system is unlikely (Section 2.3.4).

3.2.2.2. 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:PO₄³⁻, which was significantly affected by warming (Est. = 0.22, 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 (month-to-month) biogeochemical cycling (Kolzau et al., 2014). The ternary plots (Fig. 2) indicate P limitation early in the season, with ~~nitrogen-N~~ 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 N 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 did not modify these effects except for DIN:PO₄³⁻. However, seasonal effects, where temperature is compounded, are observed. The observed effect of warming on DIN:PO₄³⁻ 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 & Follstad Shah, 2012) or influenced by variation in microbial and phytoplankton community composition that can dampen the direct effects of temperature on nutrient cycling.

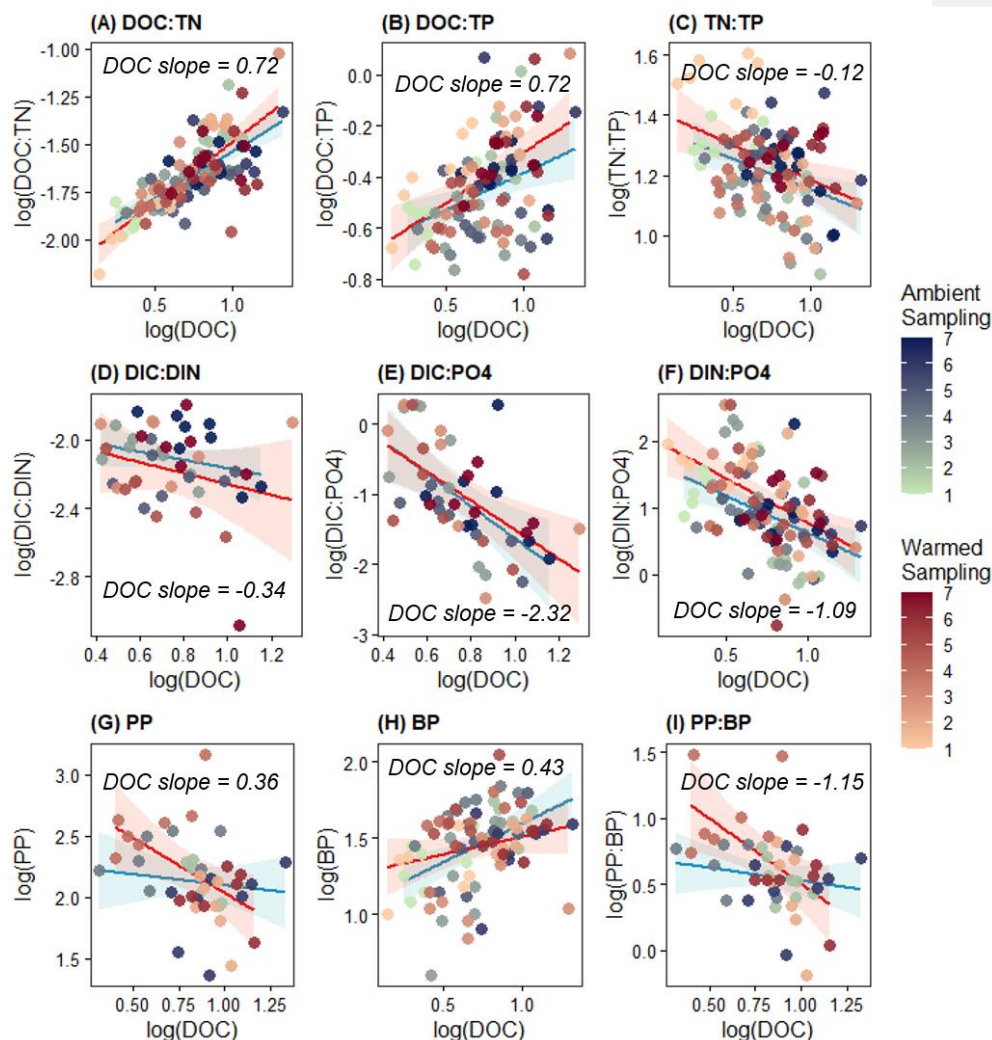
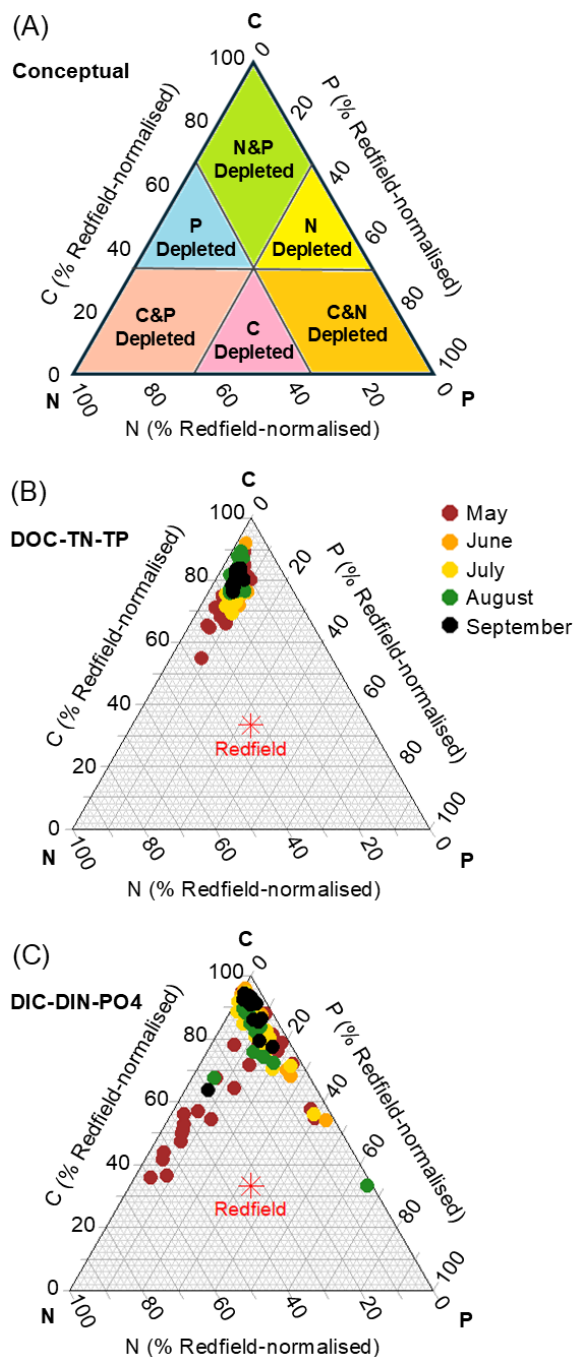


Figure 1: Relationships between dissolved organic carbon (DOC) and (A-C) stoichiometry of total nutrients pools, (D-F) stoichiometry of dissolved nutrient pools and (G-I) production metrics, derived from linear mixed-effects models. Panels show responses of (A) DOC:TN, (B) DOC:TP, (C) TN:TP, (D) DIC:DIN, (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 sampling occasion is indicated with color intensity for the warmed (red) versus ambient (blue) ponds. The colored lines are fitted trends from the mixed model (not raw regressions through all points) for the ambient (blue) versus warmed (red) ponds. The slopes ("Est." in text) show average proportional response to browning, e.g., a 1% increase in DOC corresponds to a ~0.43% increase in BP after accounting for warming, sampling

282 date and pond identity (random effect). Note that DOC concentrations in the calculated ratios are given
283 on a molar basis, whereas the x-axis presents DOC in mg L^{-1} .



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

290 **3.3.2.3. Phytoplankton nutrient limitation assays**

291 The nutrient limitation assay shows consistent N–P co-limitation across all DOC treatments, with chl_a
292 responses strongest when N and P were added together (Fig. 3A). Responses to P in the highest DOC
293 treatment increased significantly compared to the control with low DOC (Fig. 3A). Responses to N and
294 P alone were low to moderate, with slight increases at higher DOC concentrations. Our results are in
295 line with large spatial studies across Sweden showing phytoplankton in southern lakes are co-limited by
296 N and P, while phytoplankton in carbon-poor northern lakes were mainly N-limited (Bergström, 2010;
297 Bergström et al., 2008). DOC enrichment (browning) therefore shifts the system toward stronger nutrient
298 stress for phytoplanktonic algal (chl_a) production, confirming that browning leads to less favorable
299 nutritional requirements for algal production (section 3.2.1).

300 Temperature had a minimal and insignificant effect (Fig. 3B). Nonetheless, the highest RR_{chl_a} values
301 were observed under Ambient temperature conditions, and warming generally reduced response,
302 particularly in Mid and High DOC treatments, although this response was not significant. Overall, higher
303 DOC enhanced nutrient-driven responses, particularly under NP additions, while warming tended to
304 dampen these effects, consistent with patterns observed for PP (Section 3.2.4), although these effects
305 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., $\text{lmer}(\log Y \sim \log(\text{DOC}) + \text{TempTreat} + \text{Sampling} + (1|\text{pond}))$ and interaction models (i.e., $\text{lmer}(\log Y \sim \log(\text{DOC}) \times \text{TempTreat} + \text{Sampling} + (1|\text{pond}))$. 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. $\Delta\text{AIC}_{\text{Int-Base}}$ was calculated as $\text{AIC}_{\text{interaction model}} - \text{AIC}_{\text{base model}}$; negative values indicate improved fit of the interaction model. R^2_{m} is marginal R^2 (i.e., variance explained by fixed effects: DOC, warming, sampling) for base model and interaction model.

Model/ Response	Predictor	Estimate	SE	df	t value	p value	R^2_{m}	$\Delta\text{AIC}_{\text{Int-Base}}$	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) $\times$ Warming	0.09	0.07	94	1.41	0.16	0.75	0.03	No interaction
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) $\times$ Warming	-0.07	0.09	93	-0.76	0.45	0.51	1.45	No interaction
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
	Int Model: log(DOC) $\times$ Warming	-0.11	0.08	103	-1.44	0.15	0.53	0.34	No interaction
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			Strong seasonal control
	Int Model: log(DOC) $\times$ Warming	-0.09	0.31	25	-0.32	0.75	0.27	1.9	No interaction
log(DIC:PO ₄ ³⁻)	log(DOC)	-2.32	0.44	38	-5.37	< 0.001	0.42		Browning significantly decreases DIC: PO ₄ ³⁻

Model/ Response	Predictor	Estimate	SE	df	t value	p value	R ² m	$\Delta\text{AIC}_{\text{Int}} - \text{Base}$	Interpretation
	Warming (vs ambient)	0.07	0.17	38	0.43	0.66			No effect
	Sampling (time)	—	—	—	—	0.04			Strong seasonal control
	Int Model: log(DOC) × Warming	-0.001	0.85	45	-0.001	0.99	0.44	1.86	No interaction
log(DIN: PO ₄ ³⁻)	log(DOC)	-1.09	0.30	104	-3.81	0.0002			Browning significantly decreases DIN: PO ₄ ³⁻
	Warming (vs ambient)	0.22	0.10	104	2.10	0.038	0.32		Warming significantly affects DIN: PO ₄ ³⁻
	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			No evidence that browning affects PP
	Warming (vs ambient)	0.10	0.16	44	0.71	0.52	0.47		No effect
	Sampling (time)	—	—	—	—	< 0.01			Strong seasonal control
	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			Browning increases BP
	Warming (vs ambient)	-0.04	0.09	79	-0.48	0.7	0.47		No effect
	Sampling (time)	—	—	—	—	< 0.001			Strong seasonal control
	Int Model: log(DOC) × Warming	-0.16	0.15	75	-1.05	0.29	0.52	0.95	No interaction
log(PP:BP)	log(DOC)	-0.15	0.25	44	-0.38	0.69			Browning does not significantly alter PP:BP
	Warming (vs ambient)	0.11	0.18	44	1.37	0.18	0.36		No effect
	Sampling (time)	—	—	—	—	0.022			Strong seasonal control
	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

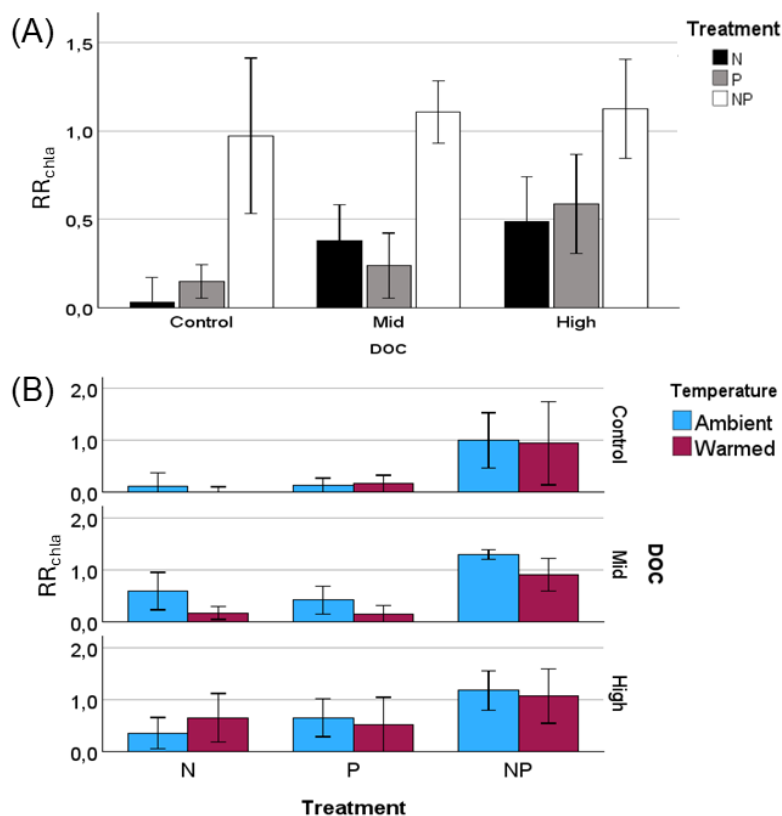


Figure 3. (A) Mean seasonal chlorophyll-a (chl-a) response ratios (RR_{chl-a}) to nutrient-addition assays (N, P, or NP) per DOC treatment, pooled across temperature treatments. (B) Mean seasonal RR_{chl-a} per added nutrient, and DOC treatment (panel), per temperature treatment.

3.4.2.4. 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. = 0.43, $p = 0.0003$; 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 food web via the “microbial loop” (Fenchel, 2008). Browning increased C:N and C:P ratios in the DOC versus total nutrient pools, but decreased them in the dissolved fractions (Fig. 1A-B, D-E), suggesting greater carbon availability for heterotrophs and reduced carbon availability for photoautotrophs. This pattern is consistent with

increasing BP and unaffected (though a trend of declining) PP and along the cDOM gradient (Fig. 1E,G; [Section 3.4](#)).

Indeed, while PP showed no significant response to DOC or warming (in the linear mixed-effects models), it showed a trend (linear regression) 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 PP due to warming contrasts with observations from Swedish Arctic lakes, where PP is typically temperature-limited (Bergström et al., 2013; Faithfull et al., 2011; Puts, Ask, Deininger, et al., 2023). Instead, our results controlled the confounded factors, and show that browning promotes net heterotrophy, but that this shift is not driven solely by increased BP, it can also arise from warming-induced declines in PP, 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 that along the DOC gradient inorganic nutrient availability declines (section [3.2.1](#)), 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 BP is thriving under 2 °C warming and browning, whereas primary producers struggle, likely due to 1) thermal compensation, 2) reduced inorganic nutrients, and 3) suboptimal stoichiometry.

With the increasing occurrence of heatwaves (Perkins-Kirkpatrick & Lewis, 2020)– and intensified browning and warming of surface waters (Pilla et al., 2018; Puts, Ask, Deininger, et al., 2023), 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 and heterotrophy would be likely under browning and warming, particularly as warming intensifies epilimnetic temperatures (Bogard & del Giorgio, 2016; Duarte & Prairie, 2005; Sobek et al., 2007).

3.5.2.5. Limitations of this study

As with the majority of ecosystem-scale experiments, our study simplifies natural shallow water systems. Although the EXEF ponds contain sediments, microbial communities, primary producers, consumers, and a natural shoreline, natural lakes have more variation in size and their catchments. Moreover, natural lakes have varying cDOM sources, with varying quality (Berggren et al., 2018). Thus, the experiment was designed to isolate the effects of browning and warming rather than replicate the full complexity of freshwater ecosystems. While DOC quality was constant across treatments (not seasons), natural lakes vary in cDOM composition, nutrient inputs, catchment influences, and mixing regimes, all of which can affect water physico-chemistry and productivity. Nevertheless, the observed patterns are consistent with large-scale studies of browning lakes. Our study does not ~~catch-capture~~ the full spectrum of DOC concentrations found in lakes: the control lake had relatively high DOC values already, and thus pristine mountain lakes are not captured in this gradient. Nonetheless, because our conclusions are still based on responses across a broader DOC gradient, we expect the direction of the responses to be robust, although their magnitude may vary among lake types and climatic conditions. The unusually warm summer during ~~the-our~~ study may also have amplified temperature effects. Accordingly, our findings should be viewed as evidence for the mechanisms underlying browning-warming interactions rather than as quantitative predictions for all boreal lakes.

4.3. Conclusions

Our study demonstrates that browning (increasing DOC 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:PO₄³⁻, while combined with warming also 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-rich 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,

401 full net heterotrophy was not reached, likely due to shallow, well-lit conditions and high overall
402 productivity.

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

410 Appendix A: Material & Methodology

411 A1 Experimental approach and setup

412 We experimentally established a cDOM gradient under ambient and +2 °C warmed conditions. We
413 monitored effects on water chemistry (dissolved and total nutrients, and dissolved organic carbon
414 (DOC)) in seven samplings. We additionally monitored BP, PP, dissolved inorganic carbon (DIC), as
415 well as phytoplankton responses to nutrient amendments (nitrogen (N), phosphorus (P), N+P) in three
416 sampling events. The experimental treatments began in autumn 2017, and monitoring ran from 7 May
417 2018 to 3 September 2018, which was an exceptionally warm summer (Blunden & Arndt, 2019). The
418 experiment was conducted at the Experimental Ecosystem Facility (EXEF) in Umeå (Sweden) using 16
419 treatment ponds and 4 buffer ponds to separate temperature treatments. We aimed for a 4x2 factorial
420 design, with 4 DOC treatments in duplicate (DOC categories “Control”, “Low”, “Mid” and “High”) and
421 2 temperature treatments. We interpret actual DOC concentrations rather than categorical DOC
422 treatments.

423 The EXEF ponds (11.5 × 6.7 m and ~1.5 m deep) are naturally functioning ecosystems with soft
424 sediments, primary producers, and invertebrate consumers and a natural shoreline. The cDOM gradient
425 was created by continuously mixing cDOM-rich water from the nearby Hörneån river with low-cDOM
426 municipal tap water. The seasonal average DOC concentrations ranged from 1.4 to 21.3 mg L⁻¹. DOC
427 served as an effective proxy for cDOM, and the warming treatment was successfully established with
428 an average temperature difference of 1.8 °C between warmed and ambient ponds (Puts, Ask, Myrstener,
429 et al., 2023). Ponds were fish-free until 11 May 2018, when 43 sticklebacks were added to each pond,
430 and zooplankton biomass was low while periphytic biomass was relatively high compared to
431 phytoplankton production. One ambient control pond was excluded due to turbidity caused by a muskrat.
432 Specifics about the experimental water used, and management of the experimental treatments are
433 available in Capo et al. (2021); Koizumi et al. (2023); Puts et al. (2023b).

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A2 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 ($\text{NO}_3^- + \text{NH}_4^+$), and PO_4^{3-}), DOC, and pH every three weeks starting 7 May during seven samplings, and dissolved inorganic carbon (DIC) in three samplings (concerted with PP). 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 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 PP 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.

A3 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 (^3H , 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 (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* (chl*a*) 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 $\mu\text{g P L}^{-1}$ and 100 $\mu\text{g N L}^{-1}$, 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 at 0.6m depth. We did not filter water for zooplankton, but the biomass was very low due to high predation pressure (Koizumi et al., 2023). We measured chl*a* as endpoint, by filtering 100 mL on GF/F Whatman filters and storing them in the freezer before further analyses. We extracted chl*a* 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.

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A4 Data analysis

We assessed whether the browning and temperature treatments independently (“base model”) or additively (“interaction model”) 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:PO₄³⁻, DIN:PO₄³⁻, 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 (¹⁰log) 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 versus interaction model ($\Delta AIC_{Int-Base}$). 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, PO₄³⁻, as well as DOC, TN, and TP, which we normalized relative to the Redfield ratio using the equations as in Andersen et al., (2025). DOC and DIC represent different carbon pools that affect different ecological processes. When ecosystem metabolism is considered, DIC is particularly relevant for PP because it is directly assimilated by photoautotrophs during photosynthesis. In contrast, DOC is generally unavailable to primary producers and is mostly utilized in heterotrophic metabolism, hence more closely linked to BP. The same goes for the dissolved inorganic nutrient pools versus total pools: the dissolved inorganic pool is directly available to phytoplankton (which could be autotrophic or heterotrophic), whereas the total nutrient pools include organic fractions, which would need (enzymatic) preprocessing, and which can have varying bioavailability (Berggren et al., 2025; Rulli et al., 2022, 2026; Soares et al., 2017). Both pools are

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therefore informative, but different. This is confirmed in our brown ponds; expressing the dissolved inorganic nutrient pools relative to DOC rather than DIC yields an average Redfield-normalized carbon value of $95 \pm 11\%$, compared with $80 \pm 16\%$ (average $\pm$ SD) when normalized to DIC (Appendix Fig. 1). 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* (M. R. Smith et al., 2025).

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 chl_a concentration in each replicate per treatment ($chl_{a,replicate, treatment}$) by the average chl_a concentration of the control of each treatment ($chl_{a,control average, treatment}$): so that $\ln RR_x = \ln (chl_{a,replicate, treatment} / chl_{a,control average, treatment})_x$.

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5.4. 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 & Puts, 2026) (open access).

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6.5. Disclaimer

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7.6. Author contributions

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

8.7. Competing interests

The authors declare that they have no conflict of interest.

9.8. Acknowledgements

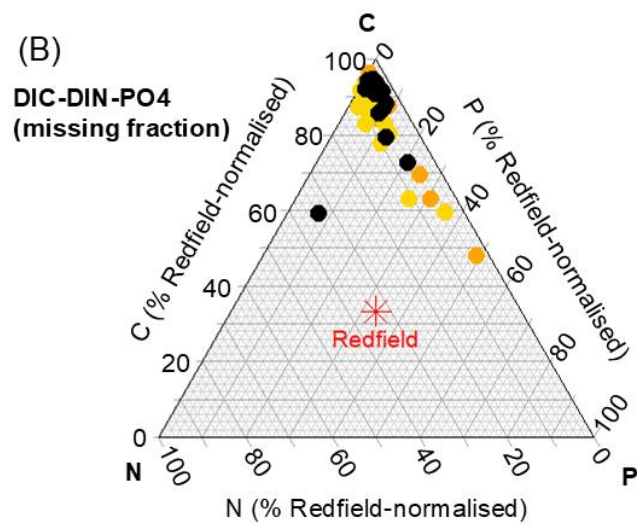
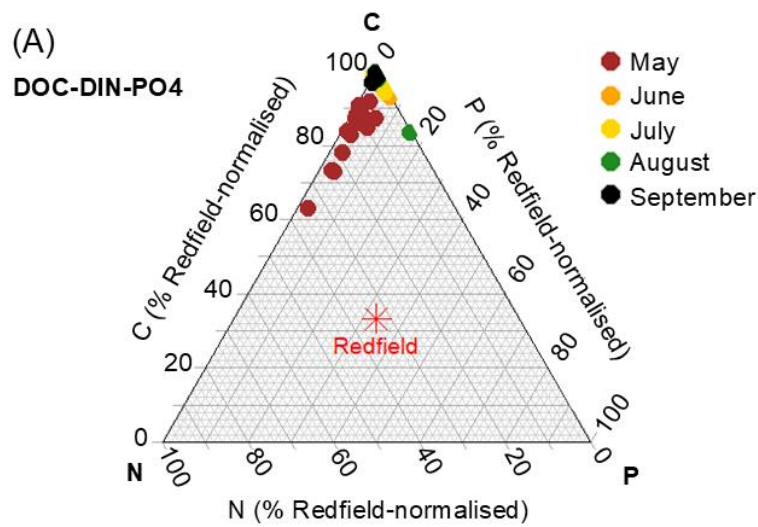
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536 chemistry. AI was used to optimize the modelling workflow, but no parts of the manuscript were
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541 **11.10. Appendix**



542

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.

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