

Dissolved organic matter mediates the effects of warming and inorganic nutrients on a lake planktonic food web

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Abstract

Lakes are undergoing striking physicochemical changes globally, including co-occurring increases in dissolved organic carbon and nutrient concentrations, water color, and surface temperature. Although several experimental studies of lake browning and warming have been conducted over the last decade, knowledge remains limited as to the structural and functional responses of multitrophic plankton communities, especially under environmentally relevant physicochemical conditions. Using reverse osmosis to manipulate naturally occurring dissolved organic matter (DOM), we performed an enclosure experiment to evaluate the response of a planktonic food web (zooplankton–phytoplankton–bacterioplankton) to individual and combined increases in DOM and temperature, while accounting for changes in inorganic nutrients associated with DOM enrichment. We found that concomitant increases in DOM and temperature or inorganic nutrients elicited substantially greater biotic effects, but infrequently led to interactive effects. Overall, major plankton groups responded differently to manipulated factors, with most effects observed in standing stocks, community composition, and trophic structure, while metabolic (primary production and respiration) rates appeared to be generally less responsive. DOM enrichment had a clear stimulatory effect on phytoplankton, but weakly affected zooplankton. More specifically, DOM enrichment, alone or combined with inorganic nutrient amendments, decreased zooplankton : chlorophyll *a* ratios, implying a reduced trophic transfer efficiency and altered trophic structure. Warming generally increased bacterial abundance and cyanobacterial dominance, especially under DOM-enriched conditions. Collectively, these results demonstrate that increasing local DOM, even by only ~ 2 mgC L⁻¹, can enhance plankton responses to rising temperature or inorganic nutrients in the near-surface layer of a clearwater lake, with potential implications for ecosystems facing co-occurring environmental changes.

Dissolved organic matter (DOM) concentrations—operationally measured as dissolved organic carbon (DOC)—have increased substantially in many freshwater ecosystems across

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Additional Supporting Information may be found in the online version of this article.

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[Correction added on 27 July 2022, after first online publication: Figure 3, as originally published did not show the gray areas in panels 3B–3F. This has been corrected.]

Author contribution statement: MPH performed the research, statistically analyzed the data, and wrote and revised the manuscript. CS performed the Bayesian inferences for the metabolic estimates. MPH, CS, GFF, and BEB all contributed to materials or developed new methods, discussed the study, and reviewed and approved the manuscript.

Special Issue: Cascading, interactive, and indirect effects of climate change on aquatic communities, habitats, and ecosystems

Northern Europe and northeastern North America (Evans et al. 2005; Monteith et al. 2007), often accompanied with increases in water color (Roulet and Moore 2006). Commonly referred to as *browning*, this phenomenon is likely the result of several environmental drivers, including recovery from acidification, changes in land use or cover (Finstad et al. 2016; Kritzberg et al. 2020), and changes in climate such as increased precipitation and temperature (Weyhenmeyer and Karlsson 2009; Zhang et al. 2010; de Wit et al. 2016). Recent work suggests that climatic factors may have become increasingly important in explaining DOM trends (de Wit et al. 2021), even in regions where acid deposition has decreased. Irrespective of the causal mechanisms, there is a consensus that anthropogenic forcing plays a major role, stressing the co-occurrence of DOM and other climate-driven environmental changes in lakes, with corresponding ecological consequences. In light of the co-occurring increasing trends in DOC concentrations, water color, and temperatures

(Larsen et al. 2011; O'Reilly et al. 2015), an appreciable number of experimental studies of browning and warming have emerged over the last decade, addressing effects on communities and metabolic processes (e.g., Rodriguez et al. 2016; Vasconcelos et al. 2019). Yet, knowledge remains generally limited as to the structural and functional responses of food webs, especially based on realistic experimental manipulations of water physicochemistry.

The ways in which DOC regulates pelagic food web productivity and structure represents a long-standing question in limnology (Jones 1992; Williamson et al. 1999, 2015). Central hypotheses rely on the dual effects on primary producers, with increasing DOM inputs enhancing basal resources such as nutrients (fueling primary production) and reducing light penetration in the near-surface layer (inhibiting primary production), potentially resulting in cascading effects on upper trophic levels (Karlsson et al. 2009). Recent advances to predict the relationship between the delivery of DOM and primary production include the consideration of DOM chromophoricity, nutrient : carbon stoichiometry, and lake size (Kelly et al. 2018; Olson et al. 2020). Rising DOC loads may also promote bacterial growth and respiration, strengthening competition with phytoplankton for DOM-related nutrients (del Giorgio and Peters 1994; Jansson et al. 2000). Greater bacterial abundances can in turn stimulate secondary production via microplankton predation or passive filter-feeding (Berggren et al. 2010). From a community structure perspective, DOM-driven decreases in light penetration and increases in bacterial availability may favor mixotrophs (e.g., phytoflagellates; Creed et al. 2018), with possible indirect effects on zooplankton (Vad et al. 2021). Although the prevailing assumptions regarding DOM effects on higher trophic levels are often resource-based, planktonic consumers can be directly affected by oxidative stress due to DOC photoactivation (Wolf et al. 2017), or habitat changes from attenuated light, offering protection from visual predators and ultraviolet radiation (Williamson et al. 2020). Thus, investigating food web responses to changing DOM is complicated by many indirect effects, which may become harder to predict with concomitant environmental shifts.

A vast body of evidence supports profound effects of pervasive and rapid climate warming on lake food webs (O'Connor et al. 2009; O'Gorman et al. 2012). Based on the temperature dependency of biological rates, previous studies suggested that warming may increase primary production, but also top-down effects of consumers (via enhanced growth and/or feeding; Rall et al. 2010). How warming may shift the balance between these influences remains uncertain, however, with evidence for shifts toward both bottom- and top-heavy food webs (Petchey et al. 1999 vs. Shurin et al. 2012). Similarly, metabolic processes may show differential temperature constraints, with respiration thought to be more sensitive to warming than primary production, implying declines in net production (Yvon-Durocher et al. 2010). Warming can also alter

community structure by selecting taxa expressing wider thermal windows or higher optima, such as certain cyanobacterial species (Kosten et al. 2012); which are also expected to be favored under DOM-enriched conditions (Creed et al. 2018). Notably, experimental work showed that warming and browning can enhance bacterial and mixotrophic algal abundances (Wilken et al. 2018), as well as cyanobacterial dominance (Hansson et al. 2013), suggesting that cumulative effects could result when such environmental changes occur together.

Relative to the extensive experimental research on warming effects, manipulative studies of DOM effects under realistic physicochemical conditions are limited, in part owing to the more challenging methodological constraints. Common approaches to manipulate DOC concentrations and water color include the addition of (1) DOC-rich water collected from adjacent ecosystems or (2) commercial agents such as soil-based leonardite products manufactured for agricultural practices (oxidized lignite). Although useful to establish fundamental links between DOC concentrations and organisms, such techniques have limitations that restrict the transposability of conclusions to natural environments. Indeed, the first approach implies the introduction of a natural but exogenous DOM inoculum that may also include other chemicals and non-native microbial communities; while the second may harm organisms, in addition to also representing an input of exogenous DOM. The use of leonardite products, such as SuperHume and HuminFeed, can notably result in highly different DOM chemical properties, rapid loss of water color, greater particle formation (flocculation), altered bacterial communities, and zooplankton mortality (Lennon et al. 2013; Saebelfeld et al. 2017; Scharnweber et al. 2021). Recently, Scharnweber et al. (2021) compared methods through field and laboratory trials and concluded that the use of natural concentrates of local lake water via reverse osmosis (RO) may be most promising to preserve ecological realism in DOM experiments. Moreover, regardless of the method employed, another common issue (confounding variable) is the parallel increase in both DOM and inorganic nutrients when adding a DOM-enriched inoculum, further hampering the identification of DOM effects. Recent advances to partition inorganic nutrient and DOM effects include in situ nutrient limitation assays (Isles et al. 2020) or whole-lake nutrient enrichment (Bergström and Karlsson 2019) along naturally occurring DOC gradients. However, within-lake experimental studies exposing controlled communities to local DOM additions while accounting for changes in inorganic nutrients remain quite rare, let alone the consideration of other climatic factors.

Using in-lake enclosures, we investigated planktonic food web responses to individual and combined increases in naturally occurring DOM (via RO) and temperature, paralleled with a nutrient treatment that matched inorganic nutrient concentrations increased via DOM enrichment. We tracked temporal variation across zooplankton, phytoplankton, and bacterioplankton, examining responses in terms of (a) community

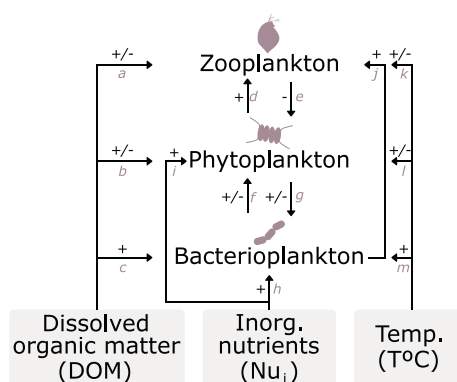


Fig. 1. Predicted effects of increased DOM, inorganic nutrients (Nu_i), and temperature ($T^\circ C$) on a lake planktonic food web (bacterioplankton, phytoplankton, and zooplankton). Direction of effects represented with signs (+/-). Note that this simplified scheme presents anticipated effects on plankton biomass/abundance or growth; not composition, trophic structure, nor metabolic flux, and excluding feedback effects of plankton responses on water chemistry. General predictions are identified with letters, with corresponding supporting references outlined in Supporting Information Table S1.

composition, (b) standing stocks, (c) mass ratios between plankton groups or relative to basal resources (reflecting trophic structure or resource use), and (d) metabolic flux (primary production and respiration). Our main objective is to compare effects of individual vs. dual physicochemical changes among plankton groups and response types, with a focus on interactive (between treatments) and cascading (across trophic levels) effects. We present high-level predictions for plankton groups (Fig. 1) exposed to elevated temperature ($\sim 3^\circ C$), DOM ($\sim 57\%$ DOC), or inorganic nutrients. Our study provides evidence for greater cumulative effects (both in terms of prevalence and magnitude) of DOM enrichment when co-occurring with higher temperatures or inorganic nutrient loads, pointing to the potential role of DOM in mediating food web responses to other environmental changes.

Methods

Experimental systems, design, and manipulations

We conducted a field-based experiment using a floating facility deployed in a protected lake (Lac Hertel) located at McGill University's Gault Nature Reserve (Québec, Canada; $45^\circ 32'N$, $73^\circ 08'W$). Lac Hertel is an oligo-mesotrophic clear-water lake, with mean annual concentrations of $\sim 15 \mu gP L^{-1}$ (total phosphorus [TP]), $\sim 0.3 mgN L^{-1}$ (total nitrogen [TN]), and $\sim 3 mgC L^{-1}$ (DOC). On 25 July 2018, we filled 24 1200-L enclosures (a.k.a. mesocosms; depth = 1.5 m) up to 90% of their capacity with lake water and planktonic organisms collected at 1 m depth. On 26 July, we topped up enclosures to full capacity and applied treatments. Our experiment ran for 61 d, from 27 July to 25 September.

We designed this study to reflect projected increases in lake DOC concentrations and temperature in northeastern North

America and developed methodologies to maintain more realistic environmental conditions. To manipulate DOC while preserving lake DOM constituents, biodegradability, and optical properties, we concentrated freshly collected lake water through an RO setup. This procedure also concentrated inorganic nutrients; a dual increase that may also occur when browning is driven by climate change and increased runoff. To disentangle DOM and inorganic nutrients effects, we incorporated an inorganic nutrient treatment, matching the increased supply in inorganic nutrients from our DOM-enriched treatment. We used a partial factorial design, with six treatments and four replicates ($N = 24$) to accommodate the electrical power (and thus warming) capacity of the facility. Prioritizing treatments addressing DOM effects, our design included: *control*, *+DOM*, *+DOM + nutrients*, *+nutrients*, *+temperature*, and *+DOM + temperature*; thus, excluding the two combinations centered on warming and inorganic nutrient interactions, which were not the focus of this study. As per future scenarios for the next decades (Couture et al. 2012; IPCC 2014), we targeted a 50% increase in DOC concentrations (~ 1.5 ppm increase in this system) and a $2^\circ C$ increase in mean daily temperatures. Target and attained physicochemical parameters across treatments are presented in Fig. 2 and Supporting Information Table S2 and Fig. S2.

The use of RO to manipulate naturally occurring DOM remains uncommon in browning experiments. Consequently, the temporal stability of DOC and water color, the chemical properties, and biodegradability of RO concentrates in outdoor experimental systems are not well documented. To tackle such uncertainties, we measured a suite of physicochemical and biological parameters before and after each round of concentration (Supporting Information Table S3), over a pilot trial, and through our main (61-d) experiment. We tracked concentrations in DOC, TP, TN, dissolved P and N (DP, DN), soluble reactive P (SRP), dissolved inorganic N (DIN; nitrites/nitrates and ammonium/ammonia; $N-NO_x$ and $N-NH_x$), and iron (Fe; which can influence water color; Kritzberg and Ekström 2012). We also measured the absorption coefficient at 320 nm (α_{320} ; as a proxy for colored DOM; cDOM; Williamson et al. 1999) and the specific UV absorbance at 254 nm normalized to DOC concentration ($SUVA_{254}$; proxy for relatively less degradable aromatic contents and, to some extent, terrestrial C and molecular weight; Weishaar et al. 2003; Hansen et al. 2016), bacterial abundance (BA), chlorophyll *a* (Chl *a*), and DOC biodegradability (through lability incubations held in parallel to the main experiment).

Over the 4 d preceding the experiment, we concentrated lake water for DOM enrichment with a closed-circuit filtration system, using customized RO membranes manufactured by H₂O Innovation. We prefiltered water at $1 \mu m$ using an inflow pipe directly connected to Lac Hertel, and concentrated each 1200-L batch into 10 \times -concentrated solutions (120 L) under semi-dark conditions; repeated 12 \times to obtain enough concentrates. All concentrates were pooled and stored in carboys. Equal volumes

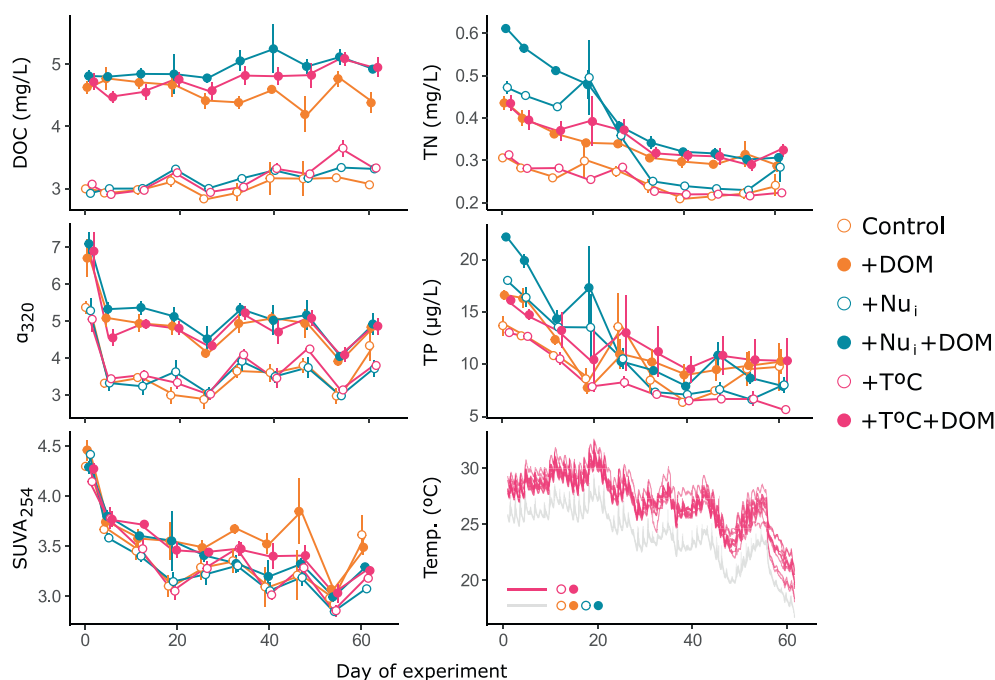


Fig. 2. Efficacy of experimental manipulations of water physicochemistry across 24 in-lake enclosures over 61 d. Manipulated factors are color-coded as shown in the legend; control = ambient lake water physicochemistry, + DOM = increased naturally occurring lake DOM via RO, +Nu_i = addition of manufactured Nu_i to match Nu_i concentrations increased via RO, + T°C = warming, and treatment combinations. Temporal dynamics are presented for DOC concentrations, colored DOM (cDOM) estimated as α_{320} (absorption coefficient at 320 nm; m⁻¹), specific UV absorbance normalized to DOC (SUVA₂₅₄; L mg⁻¹ m⁻¹), TN and TP concentrations, and temperature (°C). Error bars represent standard errors across replicated enclosures. An offset (jitter) on x-axis values was inserted to facilitate data visualization. Additional information on target and achieved levels of physicochemical parameters are presented in the Supporting Information.

were added to each DOM-enriched enclosures on 26 July. We then added the same volume of untreated lake water, filtered at 1 μ m, to other sites. This procedure enabled the standardization of water volumes and initial plankton densities.

We quantified readily bioavailable inorganic P and N in the forms of SRP, N-NO_x, and N-NH_x in DOM-enriched water (based on initial measurements of RO concentrates early in the week) and added similar amounts in the nutrient enrichment treatment on 26 July. We prepared nutrient solutions using KH₂PO₄, K₂HPO₄, and NH₄NO₃. To heat water, we built thermally insulated units using double-layered, air-containing aluminum shields (Resisto), inserted between two layers of polyethylene film in the submerged part of the enclosures. To control for light effects, opaque plastic layers were inserted in the non-heated enclosures. We secured 300-W heaters (Eheim) using a PVC structure and added identical structures in nonheated sites to standardize substrate surface area.

Sampling and measurements

We recorded highly resolved temperature data with Hobo pendant loggers (at 0.75 m), and dissolved oxygen (DO) data with MiniDOT loggers (Precision Measurement Engineering; at 0.5 m). We deployed one MiniDOT logger in each

treatment, which were rotated across replicates of a given treatment every 3–5 d.

We quantified DOC biodegradation rates in incubations run in parallel to the main experiment to compare C bioavailability between ambient and DOM-enriched water. On day 1, we inoculated incubations with water collected from control and DOM-enriched enclosures. For each site, we filtered 1.5 L of water through precombusted 2.7- μ m GF/D filters (Whatman) to preserve most bacteria, and divided filtrates into two replicates. We quantified DOC loss rates from each site based on averaged duplicates. All incubations were carried out in acid-washed glass bottles left in the dark over 47 d at 20°C.

We sampled on 10 sampling occasions: days 1, 5, 12, 19, 26, 33, 40, 47, 54, and 61. We collected water with an integrated tube sampler at multiple locations within enclosures and stored water samples in pre-assigned dark Nalgene bottles for DOC, TN, TP, Chl *a*, and optical measurements. For zooplankton, we sieved 3 L of water with a 53- μ m mesh. We anesthetized zooplankton with carbonated water and fixed samples with ethanol (final concentration ~ 75%). We measured a standard suite of physicochemical parameters (conductivity, pH, temperature, and DO) using a YSI multiparameter sonde. In addition, on three occasions (days 1, 33, and 61), we

measured a set of more refined parameters: BA and concentrations of DP, SRP, DN, NO_x , NH_x , and Fe.

From the bulk water samples, we directly poured 40 mL in glass tubes for TN and TP measurements and filtered water at $0.45\ \mu\text{m}$ for all dissolved fraction measurements. Fe samples were fixed with HNO_3 trace metal grade (final concentration 2%). For BA, 1 mL aliquots were fixed with glutaraldehyde (final concentration 1%) in cryotubes and stored at -80°C . We quantified Chl *a* based on in vivo fluorescence using a FluoroProbe (BBE Moldaenke) via the excitation of Chl *a* and accessory pigments. We determined the concentration of total Chl *a* and four groups: golden-brown algae (pigments associated with chrysophytes, diatoms, and dinoflagellates), green algae (pigments associated with chlorophytes and euglenophytes), blue-green algae (cyanobacteria), and cryptophytes.

We processed water samples in the Groupe de recherche interuniversitaire en limnologie (GRIL) laboratories based at Université du Québec à Montréal and Université de Montréal. We oxidized TP, DP, TN, and DN samples with a potassium persulfate digestion. We measured TP, DP, and SRP via colorimetric detection (molybdenum method) at 890 nm with a spectrophotometer (Ultrospec 2100 pro, Biochrom). For TN, DN, and N-NO_x , we used a continuous flow analyzer (OI-Analytical Flow Solution 3100) coupled with a cadmium reactor. We measured N-NH_x with the same flow analyzer, using a chloramine reaction with salicylate to form indophenol blue dye. We quantified DOC concentrations with an OI-1030 TIC-TOC Analyzer (OI Analytical Aurora) using persulfate oxidation, and then measured water absorbance at 254 and 320 nm with a spectrophotometer (Ultrospec 2100 pro, Biochrom) with a quartz cuvette. We measured Fe concentrations under helium flow with a triple quadrupole inductive coupled plasma mass spectrometer (8900 ICP-MS/MS, Agilent Technologies). Stability controls were performed running reference materials CALA and SLRS-6 with recuperation yields of 107.4% and 97.3%, respectively. We stained BA samples with SYTO-13 (ThermoFisher) and counted cell densities using a BD-Accuri-C6 flow cytometer (100–1000 events s^{-1}). We counted all crustacean individuals in the zooplankton samples, identifying organisms at genus or family levels using an Olympus dissecting microscope.

Data manipulations and statistical analyses

Data manipulations and statistical analyses were performed in R version 4.0.0 (R Development Core Team 2020). For optical measures, we determined the Napierian absorption coefficient at 254 and 320 nm using the following equation: $\alpha_\lambda = A_\lambda \times \ln(10)\ \text{L}^{-1}$, where A_λ is the absorbance at given wavelength and L is the optical path-length in m (2-cm cuvette). We converted zooplankton abundance to biomass using taxon-specific dry mass estimates and allometric coefficients provided in Hébert et al. (2016a,b).

We estimated gross primary production (GPP) and community respiration (CR) rates from time series of high-frequency

DO measurements. GPP and CR were estimated based on the inverse modeling technique with Bayesian inference (Appling et al. 2018), using the R package *StreamMetabolizer*. Although initially developed for streams, this package relies on equations transposable to other types of aquatic ecosystems (Soued and Prairie 2021), whereby the gas exchange rate coefficient (K_{600}) can be estimated with model fitted values (independent of ecosystem type or measurements); an advantage given the lack of direct K_{600} measurements in our study. *StreamMetabolizer* is also frequently updated. We assigned 1.5 m as the depth of the mixed layer (i.e., the depth of enclosures), given the homogeneous temperature profiles indicating weak to no stratification. We based the prior probability of GPP, CR, and K_{600} on normal distributions with means derived from the literature. For GPP and CR, we used a linear empirical relationship with TN (derived from a large lake assessment in Québec by Bogard et al. 2020); yielding 1.07 (GPP) and 1.13 (CR) $\text{gO}_2\ \text{m}^{-2}\ \text{d}^{-1}$. Standard deviations of prior distributions were set to $10\ \text{gO}_2\ \text{m}^{-2}\ \text{d}^{-1}$, conferring flexibility to the model. The prior probability of K_{600} was based on direct measurements made in similar experimental systems (Czerny et al. 2013), ranging from 0.43 to $0.6\ \text{m}\ \text{d}^{-1}$. The K_{600} prior distribution mean was defined as the average of this range ($0.52\ \text{m}\ \text{d}^{-1}$, equivalent to $0.34\ \text{d}^{-1}$ for 1.5-m-deep units), and the standard deviation was set to $1.12\ \text{d}^{-1}$ (amplitude range multiplied by 10). We retained daily GPP and CR estimates with a high goodness of fit based on modeled vs. measured DO (Pearson correlation coefficient ≥ 0.9). The retained modeled K_{600} values ranged from 0.25 to $2.74\ \text{d}^{-1}$ (0.38 to $4.11\ \text{m}\ \text{d}^{-1}$).

To quantify effects on response variables while accounting for pseudoreplication (nonindependence) across temporally repeated measurements, we built linear mixed-effects models (LMMs) with the R package *lme4* and set “individual enclosure” as a random effect. We applied a $\log_{10}(1 + X)$ transformation on variables related to standing stocks and mass ratios. We used mass ratios between plankton groups as indicators of food web structure, and mass ratios between plankton at the base of the food chain and basal chemical resources as proxies for, not measures of, resource use efficiency (RUE; given that production was not assessed for all groups (Hodapp et al. 2019)). The LMM model structure was built to compare treatments relative to controls; when comparing effect sizes, we also scaled data. We graphically represented effect sizes with a heat map, scaling color saturation with model parameter estimates. We determined interactive effects of combined treatments in a set of LMMs allowing for statistical interactions. We only reported interactions for combined factors that had detectable effects when compared to controls; only three interaction effects were discarded. We tested temporal effects by converting “day of experiment” into a continuous variable; however, given the lack of strong time-dependent effects, we excluded this predictor from main models. To test whether DOC biodegradability varied over time between DOM-enriched and lake water, we

performed a separate LMM using time as a continuous variable (Supporting Information Fig. S1).

Finally, we explored pairwise relationships across physico-chemical (manipulated) and biotic (response) variables in a correlative analysis using the R packages *corrplot* and *psych*. Correlation strengths between plankton groups may uncover associations, but not causal cascading effects. Unlike LMMs, this correlative analysis relied on recorded continuous data (recorded temperature, DOM properties, and chemical concentrations), not treatment factor levels. We quantified Pearson correlation coefficients with nontransformed data and used temporal averages to preclude pseudoreplication, comparing three subsets (total duration and 1st and 2nd halves of the experiment). We corrected for multiple testing by adjusting significance values with a false discovery rate (FDR).

Results

Water physicochemistry

The addition of RO concentrates of lake water effectively increased DOC concentrations as of day 1, from $2.99 (\pm 0.03)$ to $4.72 (\pm 0.11)$ mg L⁻¹; a 57.4% increase, on average (Fig. 2).

Enhancing naturally occurring DOM also increased cDOM (α_{320}) by 28.4%, but only had weak to undetectable effects on DOM aromaticity (SUVA₂₅₄) and biodegradability (Fig. 2). The pool of DOC was thus similarly bioavailable across control and DOM-enriched sites (mean daily C loss of 0.68%; Supporting Information Fig. S1), despite increases in DOC and cDOM. Mean TP and TN also increased with DOM enrichment as of day 1, from $13.72 (\pm 1.05)$ to $16.64 (\pm 0.45)$ $\mu\text{gP L}^{-1}$ and from $0.306 (\pm 0.004)$ to $0.435 (\pm 0.015)$ mgN L⁻¹ (Fig. 2). DOC, TP, and TN concentrations remained higher in DOM-enriched sites, although nutrient levels progressively declined over time. Wherever DOM was enriched, greater TN and TP were generally maintained in the form of dissolved organic N (DON) and particulate P; DIN decreased rapidly over time, as did SRP, to some extent (Supporting Information Fig. S2). RO had no observable effect on Fe concentrations, indicating that increases in cDOM did not occur via changes in Fe. A more detailed overview of how RO modified water physicochemistry is presented in the Supporting Information (Tables S2-S3, Figs S1-S2).

As part of the inorganic nutrient enrichment (hereafter referred to as nutrient enrichment), increases in total and

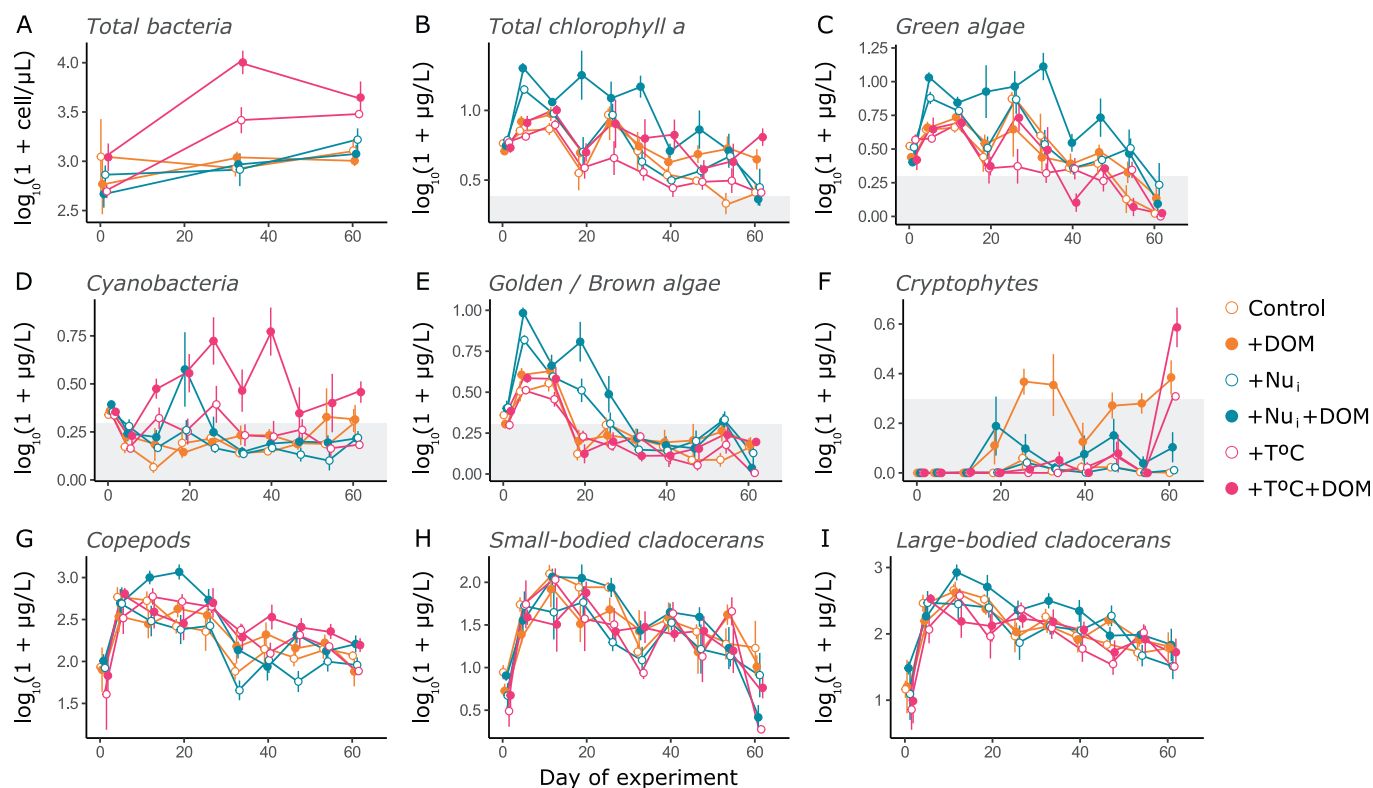


Fig. 3. Temporal dynamics (mean values across replicates \pm SE) across bacterioplankton, phytoplankton, and zooplankton. Bacterioplankton: (A) total bacterial cell abundance (BA). Phytoplankton: (B) total Chl *a* concentration and specific concentrations of (C) green algae, (D) cyanobacteria, (E) golden-brown algae, (F) cryptophytes; shaded areas denote algal concentrations $\leq 1 \mu\text{g L}^{-1}$. Zooplankton: (G) copepod biomass, (H) small-bodied cladoceran biomass (members of Bosminidae and Chydoridae), (I) large-bodied cladoceran biomass (members of Daphniidae and Sididae). An offset (jitter) on x-axis values was inserted to facilitate data visualization. [Correction added on 26 July 2022, after first online publication: Figure 3 is revised.]

dissolved N and P concentrations matched those associated with the DOM enrichment treatment relatively well (Fig. 2; Supporting Information Table S2). The initial proportions of DIN and DON were relatively greater in the nutrient and DOM enrichment treatments, respectively. Generally, DIN was more rapidly consumed than DON over time across DOM- and/or nutrient-enriched sites. Both types of chemical enrichment increased N : P molar ratios by approximately 16%; effects on nutrient stoichiometry rapidly vanished over time, however. Finally, the warming treatment exceeded the target daily increase of 2°C, reaching 3.01°C instead (range = 2.16–3.19°C; Fig. 2).

Biotic responses

We examined different facets of food web structural and functional responses by tracking temporal dynamics in bacterioplankton, phytoplankton, zooplankton (Fig. 3), and metabolic rates (Fig. 4), with associated LMM results compiled in Supporting Information Table S4 and graphically summarized in Fig. 5. BA remained relatively stable in all nonheated sites, but increased substantially over time in warmed enclosures, with 3.6× greater mean BA by the end of the experiment (4950.7 ± 1552.5 vs. 1378.1 ± 168.7 cell μL^{-1} ; Fig. 3a). A LMM revealed that temperature enhanced BA, even more so when DOM was enriched (positive interaction effect; Fig. 5b). Total Chl *a* increased wherever DOM was enriched (Figs. 3b, 5b). Chl *a* levels were distinctly higher with DOM and nutrient co-enrichment (up to 22.1 ± 9.6 $\mu\text{g L}^{-1}$ on day 19), with trends driven by green (Fig. 3c) and gold-brown algae (Fig. 3e; first half of the experiment only). Cyanobacteria showed a clear response to increased DOM and temperature, with a positive interaction detected (Figs. 3d, 5b); mean levels remained

otherwise low elsewhere (generally ≤ 1 $\mu\text{g L}^{-1}$). DOM increased cryptophytes (up to 5 $\mu\text{g L}^{-1}$), although levels were generally ≤ 1 $\mu\text{g L}^{-1}$ (Fig. 3f). Dual increases in DOM and temperature had a negative effect on %green algae, and a positive effect on %cyanobacteria (relative to total Chl *a*; Fig. 5a), pointing to a shift mainly between these taxa.

For zooplankton, we observed an abrupt biomass increase between the two first sampling occasions, likely resulting from reduced predation and/or acclimation; an effect commonly observed in enclosures. Total biomass only showed minor increases to the co-enrichment of DOM and nutrients, with positive responses visible across all major groups, but only statistically significant in copepods, where a positive interaction occurred (Figs. 3g–i, 5b). Minor copepod biomass increases were also detected with elevated temperature and DOM (Fig. 5b). Copepods appeared to be slightly favored under DOM-enriched conditions relative to otherwise similar treatments (filled vs. empty dots in Fig. 3g). None of the treatments affected cladoceran biomass (Fig. 5b). However, the relative proportion of large-bodied cladocerans decreased in warmed enclosures (Fig. 5a), while that of small-bodied cladocerans declined with dual increases in DOM and nutrients or temperature—likely as copepods dominated. Concomitant decreases in %cladocerans and increases in %copepods suggest a shift in predominant crustaceans with warming (Supporting Information Fig. S4).

DOM and nutrient co-enrichment increased ratios between Chl *a* and nutrients or DOC (Fig. 5c). BA : DOC ratios were higher with elevated temperatures, but this effect was driven by increased BA (not DOC) with warming. In contrast, zooplankton : Chl *a* ratios were lower where DOM and nutrients were enriched, individually or jointly. Together, these

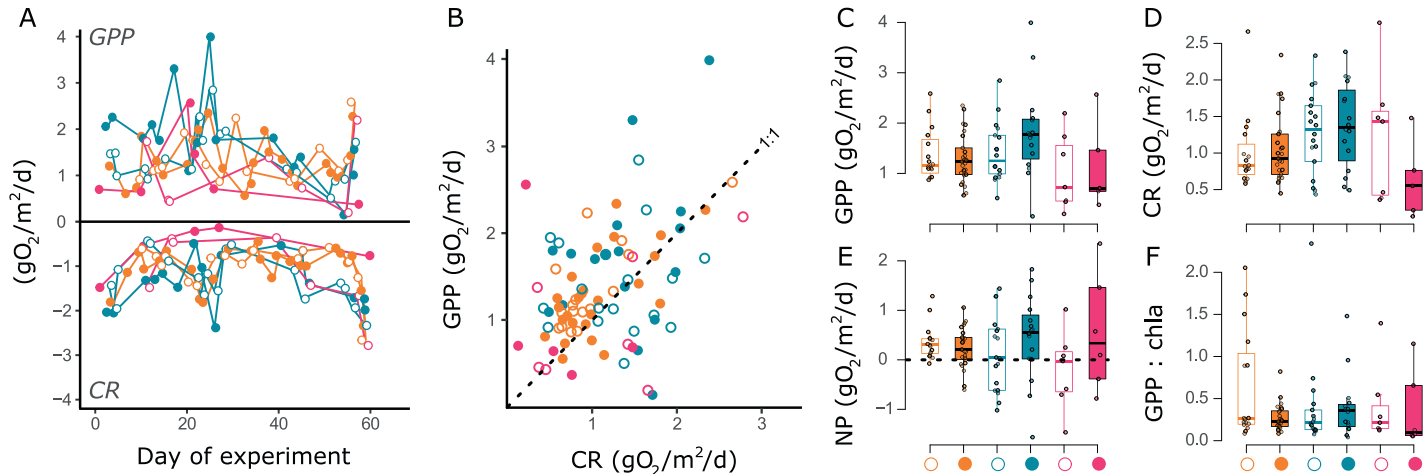


Fig. 4. Variation in daily metabolic estimates based on diel O_2 fluctuations. **(A)** Temporal dynamics in daily GPP and CR. **(B)** Relationship between GPP and CR, including the 1 : 1 line ($\text{GPP} - \text{gO}_2 \text{ m}^{-2} \text{ d}^{-1} : \text{CR} - \text{gO}_2 \text{ m}^{-2} \text{ d}^{-1}$). Overall differences in **(C)** GPP, **(D)** CR, **(E)** net production (NP), and **(F)** GPP : Chl *a* ratio among treatment types. Box plots median values (thick lines) and quartiles. Only metabolic estimates with a high goodness of fit were retained, resulting in an unequal number of estimates per treatment type. Color legend as in Figs. 2 and 3.

results indicate that effective energy transfer occurred from basal chemical resources to phytoplankton, but did not reach the zooplankton.

Overall, no clear pattern emerged in metabolic rates (Fig. 4), except for higher GPP with DOM and nutrient co-enrichment (Figs. 4c, 5d), especially during the first half of the experiment (peaking on day 26 at $3.99 \text{ gO}_2 \text{ m}^{-2} \text{ d}^{-1}$; Fig. 4a). CR was also visibly greater under co-enriched conditions over the same period (Fig. 4a,d), but effects were statistically undetectable. GPP : CR ratios remained generally > 1 across enclosures (Fig. 4b), with highest net production rates when DOM was jointly increased with nutrients or temperature (up to $2.35 \text{ gO}_2 \text{ m}^{-2} \text{ d}^{-1}$; Fig. 4e); although, neither GPP : CR ratios, nor net production rates showed statistically detectable responses. Examining GPP : Chl *a* ratios to assess changes in productivity flux per standing stocks, we found no clear pattern except for generally lower ratios with enriched DOM alone (Figs. 4f, 5d).

A high-level comparison of responses across the planktonic food web indicated that composition (a), standing stocks (b), and trophic structure/RUE (c) (horizontal comparison in Fig. 5a–c) did not strongly differ in their sensitivity to treatments. However, declines were only observed in metrics of composition or structure; not in plankton biomass (Fig. 5a,c vs. Fig. 5b). In contrast, metabolic rates appeared to be generally less responsive overall (Fig. 5d). When contrasting responses to individual vs dual treatments (first three rows vs. two last rows in Fig. 5), we found that combined increases in

DOM and temperature or nutrients had substantially greater effects—that is, co-occurring physicochemical changes increased the prevalence and magnitude of ecological responses. This occurred especially for standing stocks, composition, and trophic structure/RUE (Fig. 5a–c), with more than twice as many effects observed with combined (two last rows) than individual treatments (first three rows).

Interaction effects of DOM \times temperature or DOM \times nutrients (asterisks in Fig. 5) did not occur frequently. Only 7 of the 23 effects ($\sim 30\%$) of combined factors were interactive, with most in standing stocks measures (5/10; Fig. 5b). Interactions between DOM and nutrients were observed in zooplankton and zooplankton : Chl *a* ratios, while those between DOM and temperature were in phytoplankton and bacterioplankton. Overall, combined increases in DOM and temperature or nutrients primarily elicited additive effects.

Relationships with environmental drivers and within the planktonic food web

We examined correlation strengths between physicochemical (manipulated) and biotic (response) variables, as well as between planktonic consumers and resources (Fig. 6; Supporting Information Figs. S5, S6). Recorded temperatures co-varied with BA and cyanobacteria and were related to compositional shifts in algae (increasing %cyanobacteria and declining %green algae) and zooplankton (increasing %copepods and declining %large-bodied cladocerans); in agreement

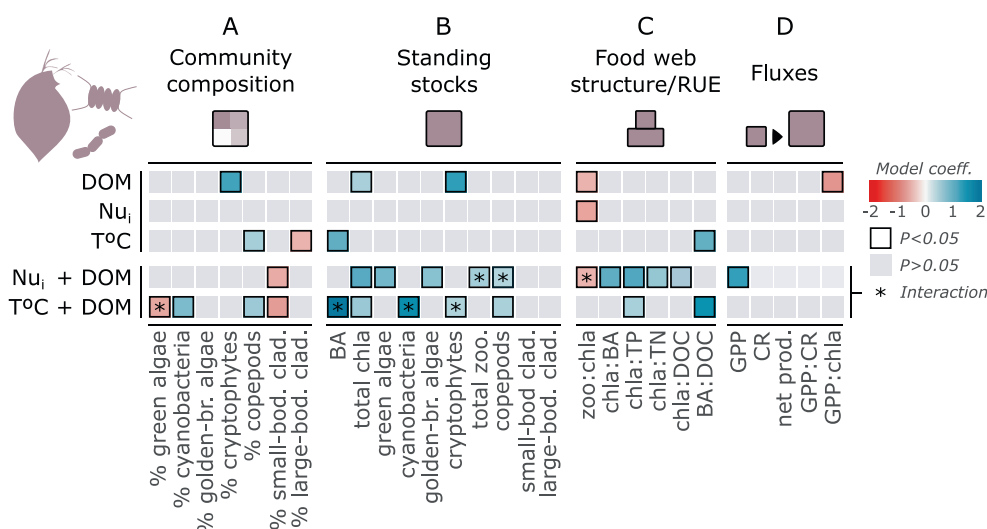


Fig. 5. Multifaced responses of planktonic food webs to individual vs. joint presence of DOM, inorganic nutrients (Nu_i), and temperature ($T^\circ\text{C}$) over the course of the experiment. Response variables are grouped based on whether they reflect changes in (A) community composition (% relative to either total Chl *a* or total zooplankton), (B) biomass stocks (units as in Fig. 3), (C) food web structure or RUE (mass ratios), and (D) metabolic parameters indicative of fluxes. Color type and saturation indicate overall direction and effect size of treatments, respectively, over the course of the experiment. Effect sizes (model coeff.) are expressed as scaled parameter estimates of LMMs; LMMs were built to quantify effects relative to controls and performed individually for each response variable. 95% Confidence intervals were used to assess significance; framed and gray slots are deemed significant and nonsignificant, respectively. All model parameters are listed in Supporting Information Table S4. For effects of combined factors (two lower rows), asterisks denote the presence of interaction effects (“DOM \times Nu_i ” or “DOM \times $T^\circ\text{C}$ ”); determined in a separate set of LMMs (see Methods).

with our LMM models. Recorded temperatures were, however, not associated with phytoplankton and zooplankton biomass in any of the temporal subsets (Supporting Information Fig. S5). Although DOC and nutrient concentrations often co-varied, we found different affinities for DOC vs. nutrient forms in phytoplankton (Supporting Information Fig. S6). Chl *a* was strongly linked with measured DOC and nutrient concentrations (all but SRP); while green and golden-brown algae were related to most nutrient fractions, but not DOC. Across algal taxa, DOC was only positively linked with cryptophytes. GPP was related to DON concentrations throughout the experiment. BA did not significantly correlate with any of the chemical resources. Likely owing to indirect effects, zooplankton biomass often correlated with nutrient concentrations; only copepods were positively associated with DOC and α_{320} .

Although limited in our ability to test for cascading effects, we inferred potential trophic linkages from strong planktonic consumers–resources associations. We found apparent relationships between Chl *a* concentrations and total zooplankton, copepod, and large-bodied cladocerans (Figs. 6); most discernable during the first half of the experiment (Supporting Information Fig. S5b). Correlations were stronger between Chl *a* and large-bodied cladocerans than with copepods, and none was found with small-bodied cladocerans. Positive relationships were identified between green algae and copepods or large-bodied cladocerans; although, for copepods, this correlation became slightly negative over time (Supporting Information Fig. S5c). Golden-brown algae were positively linked with large-bodied cladoceran densities. None of the other algal groups, nor BA, showed relationships with zooplankton. Furthermore, we found no links between BA and any algal groups, other than a correlation with cyanobacteria (which were likely captured in BA measurements). Specifically, BA was not related to any potential mixotrophic groups (e.g., cryptophytes or golden-brown algae) in our experiment.

Discussion

We used in-lake enclosures to evaluate the structural and functional responses of a planktonic food web to individual and combined increases in naturally occurring (local) DOM and temperature, while accounting for DOM-related inorganic nutrients. Despite the complexity of the effects observed, we found a marked contrast between responses to individual vs. combined changes in water physicochemistry, with dual increases in DOM and temperature or inorganic nutrients eliciting substantially greater ecological effects. Co-occurring environmental changes led to more prevalent and generally more pronounced responses, yet infrequently resulted in interactive effects (30% of effects detected; the remainder considered additive). Plankton groups responded differently to manipulated factors, with effects primarily observed in standing stocks, composition, and trophic structure. Warming mainly benefitted bacteria, cyanobacteria and, to a lesser

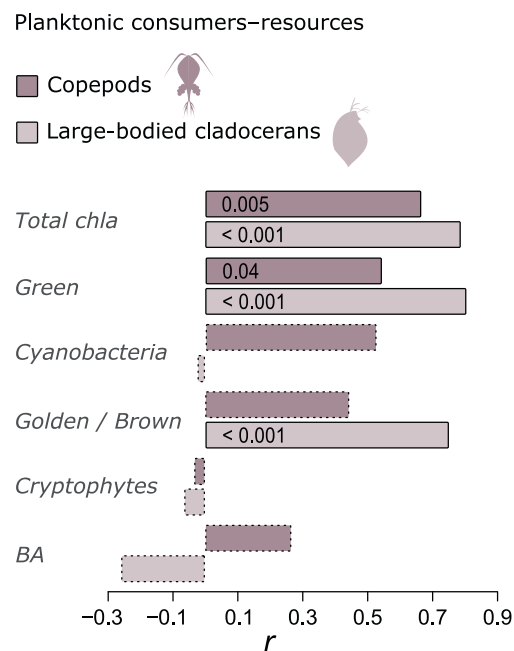


Fig. 6. Pearson correlations between planktonic consumers (zooplankton; copepods and large-bodied cladocerans) and resource availability (phytoplankton and bacterioplankton; total Chl *a*, green algae, cyanobacteria, golden-brown algae, cryptophytes, and BA) over the course of the experiment. Correlations were quantified on averages to preclude pseudoreplication; strength and significance of correlations are as in Supporting Information Fig. S5A. Solid and dashed frames represent significant and non-significant correlations, respectively. A FDR correction was applied on significance values to account for multiple testing; significant FDR-adjusted *p* values are shown. Note that correlations with small-bodied cladocerans were quantified (Supporting Information Fig. S5) but not reported because none were significant.

extent, copepods, whereas DOM and nutrient co-enrichment affected phytoplankton (total Chl *a*, green and golden-brown algae, and GPP), zooplankton (minor increases in copepods), and mass ratios (greater Chl *a* : chemical and lower zooplankton : Chl *a* ratios). Such ratio responses indicate an effective uptake of basal resources by phytoplankton, but a low transfer efficiency from phytoplankton to zooplankton, implying a reduced energy flow to higher trophic levels with DOM and/or nutrient enrichment. Although limited in our ability to identify indirect or cascading effects in this experiment, we found clear associations between some phytoplankton and zooplankton, but none with bacterioplankton. Our study demonstrates that increasing naturally occurring DOM via RO, even by only $\sim 2 \text{ mgC L}^{-1}$, can enhance plankton responses to other environmental changes in the near-surface layer of a clearwater, low-DOC lake.

DOM and inorganic nutrient enrichment

Enhancing DOM or nutrients individually weakly affected the planktonic food web. The most plausible explanation is that absolute increases in dissolved C, N, and P remained

minor when DOM or nutrients were enriched alone. Furthermore, the use of RO to concentrate DOM enhanced N to a greater extent than P (~ 16% increase in N : P ratio), resulting in a net DP increase of only $< 2 \mu\text{g L}^{-1}$ when either chemical treatment was applied individually. As a result, the limited effect on phytoplankton was predictable, especially in a system likely to be P-limited (all N : P molar ratios > 30). Nevertheless, our models showed that increasing local DOM alone, from ~ 3 to 4.7 mgC L^{-1} , was sufficient to enhance Chl *a* concentrations, despite 28% reductions in light availability. This result agrees with previous lake surveys reporting a positive DOM–Chl *a* relationship at low DOC concentrations; generally, $< \sim 11 \text{ mg L}^{-1}$ (Bergström and Karlsson 2019; Myrstener et al. 2022). Of the four algal groups, only cryptophytes responded to DOM increases. More prevalence of potentially mixotrophic cryptophytes would align with the predictions that mixotrophs are favored under elevated DOC and reduced light (Creed et al. 2018). Yet, absolute levels of cryptophytes remained very low, and their lack of relationship with BA suggests that mixotrophy was unlikely to be a central energy pathway in this experiment. Despite leading to greater Chl *a* concentrations, increasing DOM alone had no effect on zooplankton and was thus insufficient to affect higher trophic levels in our planktonic food webs.

Combined increases in DOM and inorganic nutrients led to markedly greater ecological responses compared to individual additions. The co-enrichment had a clear stimulatory effect on primary producers, enhancing total Chl *a*, green and golden-brown algae, and GPP. Both the prevalence and magnitude of algal responses were augmented with co-enrichment, likely because nutrient inputs were doubled (Fig. 2). Ratios between Chl *a* and TP, TN, and DOC were also all enhanced, reflecting effective chemical uptake and high RUE in phytoplankton. However, it should be noted that Chl *a* estimated via *in vivo* fluorescence can be influenced by altered algal physiological state, and that increased Chl *a* may also reflect greater cellular pigment concentration (subject to increase with attenuated light). That said, Chl *a* and (O_2 -based) GPP increased concomitantly and in similar magnitudes (effect sizes in Fig. 5) under co-enriched conditions, indicating that increases in Chl *a* resulted, at least partly, from algal biomass proliferation. Overall, primary production appeared to be limited by nutrient (not light) availability in our study. Contrary to conventional assumptions (Hessen 1992; Jansson et al. 1996), enhancing DOC and nutrients had no observable effect on BA or CR. Presumably, phytoplankton had stronger nutrient affinities than did bacterioplankton, due to greater nutrient limitation or competitive ability. Collectively, the bottom-up effects of rising local DOM by $\sim 2 \text{ mgC L}^{-1}$, alone or combined with inorganic nutrients, constitute evidence for the fuelling of pelagic primary producers, further supporting the resource hypothesis whereby small increases in DOC may enhance both GPP rates and standing stocks in clearwater lakes (Seekell et al. 2015; Bergström and Karlsson 2019).

Relative to primary producers, consumers only showed weak responses to dual increases in DOM and inorganic nutrients. Generally, most zooplankton groups showed highest biomass under co-enriched conditions; but only copepods (and consequently, total zooplankton) showed detectable minor increases. Copepods co-varied with DOC and water color across sites. Together, our data suggest that enhanced DOM had a positive (albeit minor) influence on copepods. While DOM shading effects can directly benefit zooplankton via UV protection (Williamson et al. 2020), the emergence of relationships between DOC and most nutrient forms, Chl *a*, and copepods, along with apparent phytoplankton–copepod associations, suggests that DOM enrichment favored copepods via increased algal availability (indirect effect). An alternative pathway through which DOM could also enhance resources is through the microbial–detrital food chain (“brown pathway”). Cyclopoids, the only type of copepods in our experimental systems, have been shown to assimilate DOM via the ingestion of bacterivorous microzooplankton such as rotifers or ciliates (Persaud et al. 2009; Berggren et al. 2014). Although the microbial–detrital food chain was not investigated, the absence of DOM–BA and BA–copepod relationships suggests that the brown pathway was unlikely to be the primary route to channel DOM to consumers. Thus, our zooplankton data also support the prevailing resource hypothesis whereby elevated DOM may benefit primary consumers (here, copepods) through the “green pathway,” as previously reported in within-lake experiments (Kissman et al. 2013; Kelly et al. 2016).

Despite clear phytoplankton–zooplankton associations, both individual and combined increases in DOM and inorganic nutrients decreased zooplankton : Chl *a* ratios, reflecting a more bottom-heavy planktonic food web and reduced trophic transfer efficiencies. Recently, Bergström et al. (2021) found a similar pattern in whole-lake fertilization experiments along a DOC gradient, attributing low transfer efficiencies between phytoplankton and zooplankton to declines in algal dietary quality and edibility with increasing DOC. Changes in algal community composition, nutritional quality, or edibility are thought to be important drivers through which changing DOC and nutrients may affect higher trophic levels in lakes (Creed et al. 2018; Lau et al. 2021). Although this explanation could also apply in the context of our study, we did not identify any shifts in phytoplankton taxa under such conditions, and our data remain too taxonomically coarse to infer changes in algal edibility. Irrespective of the underlying mechanisms, weaker trophic linkages in lakes undergoing chemical enrichment can have ecological repercussions, even on the short term, with implications for algal proliferation and food web processes.

Warming alone and combined with DOM enrichment

Regardless of DOM conditions, increasing water temperature by $\sim 3^\circ\text{C}$ enhanced BA but none of the metabolic rates.

The substantial increases in BA suggest that warming promoted bacterial proliferation in our experiment, which would be congruent with the literature on the temperature dependency of microbial abundance and activity (White et al. 1991; Gurung et al. 2001). The greater and interactive effect of warming under DOM-enriched conditions further suggests that higher BA was likely the result of greater bacterial production, and not from reduced predation. In stark contrast, the lack of metabolic response to warming, alone or with elevated DOM, was surprising given the well-established temperature sensitivity of CR, GPP and, to some extent, net production rates (López-Urrutia et al. 2006; Yvon-Durocher et al. 2010), especially with changing DOM (Rodriguez et al. 2016; Bogard et al. 2020). A possible explanation for the lack of warming effect is data limitation. Indeed, we only retained conservative GPP and CR estimates, with the lowest numbers of retained estimates being for both warming treatments ($N < 10$). As a result, it is possible that warming affected metabolic fluxes, but we were unable to statistically detect effects. That said, previous studies also reported a lack of metabolic response to warming, even when crossed with DOM enrichment (Feuchtmayr et al. 2019). Although we found no evidence of altered metabolic rates, nor cascading effects of greater BA on higher trophic levels, the magnitude of the long-lasting effect on BA ($> 3\times$ increases over ~ 2 months) has potential implications for microbial processes in the near-surface lake layer.

Warmer temperatures were generally associated with increasing proportions of copepods and decreasing proportions of cladocerans. In theory, this shift could be driven by several processes: (i) a selection in favor of warming-tolerant (copepod) taxa, due to variation in thermal niches; (ii) compensatory responses between crustacean groups, whereby warming-induced changes in one group could indirectly reduce or strengthen competition with the other group; or (iii) a bottom-up effect resulting from changes in food resources with warming (Gerten and Adrian 2002; O'Connor et al. 2009; Thompson and Shurin 2012). Our ability to identify zooplankton response mechanisms to combined warming and browning is limited given the lack of effect of individual treatment on absolute (not relative) biomass. Nonetheless, the effect of dual increases in temperature and DOM on the biomass of copepods, but not on cladocerans, suggests that compositional changes were not attributable to more sensitive cladocerans. Greater temperatures and DOM resources may have both benefitted copepods, as the former may facilitate population development and the latter may stimulate growth (Holste and Peck 2006). Lefebvre et al. (2013) also found that copepod biomass increased only under combined increases in temperature and DOM. The greater Chl *a* concentrations under similar conditions, along with associations between DOC and both Chl *a* and copepods across enclosures, suggest that positive copepod responses to co-occurring warming and browning were primarily modulated by DOM bottom-up effects.

Unlike warming alone, the combination of elevated temperature and DOM positively affected phytoplankton. Greater Chl *a* concentrations were likely achieved via DOM resource effects, given the positive Chl *a* response to DOM enrichment alone but not to warming alone. Graham and Vinebrooke (2009) also identified a positive influence of dual increases in temperature and DOM on phytoplankton biomass; but in contrast to their results, we did not find that warming amplified DOM effects. Increasing trends in Chl *a* were mainly driven by cyanobacteria and, to a lesser extent, cryptophytes; although, the overall low cryptophyte levels diminish the importance of their role. Warming and browning increased cyanobacteria (positive interactive effect), but distinctly strong effects were only found in two of the four enclosures (large effect size in LMMs, but high SEs in time series). More compelling was the greater % of cyanobacteria observed across all replicates (lower effect size in LMMs, but smaller SEs in time series), suggesting a growing dominance of cyanobacteria under warmer and browner conditions, possibly at the expense of green algae. Positive effects of temperature and DOM on cyanobacteria have been reported previously, both under individual or joint exposure (Kosten et al. 2012; Ekvall et al. 2013; Hansson et al. 2013). Greater cyanobacterial dominance would also be consistent with recent work suggesting their proliferation with climate change, thereby reducing food quality for consumers; notably in terms of fatty acids, stoichiometry, or toxicity (Creed et al. 2018; Lau et al. 2021; Wu et al. 2021). It is possible that increases in less edible phytoplankton such as cyanobacteria could have had inhibited consumer grazing in our experiment, explaining the observed increases in Chl *a* in warmer and browner enclosures; similarly, reduced dietary preferences for cyanobacteria could also be a plausible explanation for the declines in %cladocerans. Nevertheless, the absence of effects in zooplankton : Chl *a* ratios suggests that trophic transfers from phytoplankton to zooplankton remained overall unaffected by concurrent warming and browning.

Co-occurring environmental changes and ecological relevance

In many lakes worldwide, DOM concentrations, water color, and temperatures are increasing, representing striking changes in water physicochemistry with corresponding ecological consequences. With climate change, rising DOM loads due to increased runoff may be accompanied by greater inorganic nutrient supply (e.g., as observed in Isles et al. 2020), contributing to chemical enrichment. In this study, we used RO to manipulate the local pool of DOM and showed that increasing DOC concentrations by only $\sim 2 \text{ mgC L}^{-1}$ is sufficient to enhance planktonic responses to warming or inorganic nutrient inputs in a low-DOC system. Collectively, our results indicate that DOM can mediate planktonic responses to other environmental changes via resource effects, supporting the prevailing hypothesis whereby DOM may fuel

food webs in clearwater lakes. While extrapolation of experimental observations to natural ecosystems must be made with caution, our study is in line with previous space-for-time lake assessments, with potential implications for algal proliferation, trophic structure, and energy flow in pelagic food webs.

This study contributes to developing the implementation of RO in experimental limnology to preserve lake DOM constituents, properties, and (as a result) C bioavailability when manipulating DOC. While improving ecological realism on this front, our study includes common limitations of short-term enclosure experiments. For example, dual increases in DOM and inorganic nutrients had clear stimulatory effects on primary producers, but some of those effects progressively weakened over time, suggesting that chemical enrichment may differentially affect phytoplankton over longer time-scales. Similarly, 61 d may not have been sufficient to capture some ecological and adaptive processes that may only occur on the long term (Schaum et al. 2017), especially in organisms with longer generation time such as zooplankton. Long-term studies of DOM and water color effects on food webs, either under natural or experimental settings, remain limited, however (Williamson et al. 2020). Furthermore, given the methodological constraints associated with the use of RO, our experiment only included two DOC concentration levels, precluding the determination of a DOC threshold after which DOM-resource effects may be offset by DOM shading; that is, the unimodal hypothesis, as evidenced by empirical and modeling approaches (Bergström and Karlsson 2019; Olson et al. 2020). Future experimental research should establish concentration gradients of local DOC to test for the unimodal hypothesis with within-lake communities, as most empirical studies to date either include cross-lake comparisons or the use of exogenous DOM in within-lake experiments. Finally, another important aspect to consider when evaluating ecological responses to increasing DOM and temperature is lake size (area and depth), as changes in water color can mediate differently warming effects in the near-surface vs. deeper lake layers (Rose et al. 2016; Pilla et al. 2018). Irrespective of temperature, DOM concentrations or water color, lake size and depth are also key determinants of light limitation and the mixed layer depth in the water column, affecting the relationship between DOM and ecosystem-level primary production (Kelly et al. 2018). As such, our experimental study reports effects on pelagic organisms and processes in the near-surface layer only, constraining extrapolations to whole lake ecosystems and food webs.

A frontier topic in global change ecology is whether co-occurring environmental changes will lead to interactive effects in freshwater ecosystems (Birk et al. 2020), with mounting interest in browning and other stressors (Creed et al. 2018; Lau et al. 2021). In our experiment, treatment combinations led to greater cumulative effects, but effects primarily remained additive (as in Vasconcelos et al. 2019). The few interactive effects of DOM and nutrients inorganic were

detected in zooplankton and trophic ratios, while those of DOM and temperature were in phytoplankton and bacterioplankton. The emergence of interactive effects in different taxa or structural metrics may indicate that increasing DOC and nutrients or temperature may operate differently on lake food web components.

Investigating the ways in which global change may influence lake structure and function has been central to limnology over the last few decades. Experimental advances furthered our understanding of interactive and cascading effects of browning and warming, enabling the identification of causal chains in food web responses (e.g., Vasconcelos et al. 2018). Our study suggests that rising DOM may elicit greater ecological responses to other physicochemical changes. Given the complexity of interacting components, further experimental research under local, more realistic physicochemical conditions may serve as a key asset in disentangling intricate effects of co-occurring environmental changes in lakes.

Data availability statement

The data that support the findings of this study are openly available in Figshare at <https://doi.org/10.6084/m9.figshare.20069327.v2>. Additional data (physicochemical and other measurements during field/lab trials) are available from the corresponding author upon request.

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Acknowledgments

We are grateful to the staff of the Gault Nature Reserve, especially C. Normandin for their assistance with electrical circuits in the field. We thank K. Kagzi and M. Langley for their sustained assistance with field work, and acknowledge all occasional helpers, including C. Bazerghi, A. Shakra, R. Rolland, J. Benbahtane, E. Katkov, S. Massé, and I. Levade. We are thankful to D. Bélanger for insightful discussions regarding the development of experimental methodologies; as well as to C. Lechartre, O. Durrenberger, K. Velghe, and M. Robidoux for

their assistance with laboratory work. Finally, we wish to thank three anonymous reviewers for their supportive and insightful comments, and for making the revision process enjoyable. MPH acknowledges funding from the Natural Sciences and Engineering Research Council (NSERC), the Fonds de Recherche du Québec Nature et Technologies (FRQNT), the NSERC-CREATE Écolac program through the GRIL, and the PEO Sisterhood. CS was supported by a MITACS-Accelerate grant. This work was funded by NSERC through Discovery grants to GFF and BEB.

Conflict of Interest

None declared.

Submitted 15 February 2022

Revised 13 May 2022

Accepted 09 June 2022

Associate editor: Hans-Peter Grossart