

Browning reduces the availability—but not the transfer—of essential fatty acids in temperate lakes

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Abstract

1. Atmospheric changes are leading to the browning of northern lakes (i.e. increases in catchment-derived dissolved organic matter [DOM]), consequently altering phytoplankton biomass and community composition.
2. We hypothesised that lake browning and the concurrent increase in nutrients drive a shift towards greater cyanobacteria biomass. We further hypothesised that, as a consequence of this shift in phytoplankton, the content of ω -3 (n-3) essential fatty acids (EFA) in seston would decline, affecting the subsequent transfer of EFA to consumers across the plant–animal interface in pelagic regions of lakes.
3. We tested these hypotheses in the epilimnion of 30 temperate lakes in Ontario (Canada), representing a gradient of lake browning, with dissolved organic carbon (DOC) ranging from 2 to 10 mg/L and total phosphorus ranging from 6.0 to 48.5 μ g/L. In each of these lakes, the concentration and composition of DOM, the biomass of phytoplankton and cyanobacteria, and the EFA content of seston, cladocerans, and copepods were measured.
4. An increase in aromatic DOM was associated with increased phytoplankton and cyanobacteria biomass. Due to the lower content of the EFA eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) in cyanobacteria, this increase in phytoplankton biomass was associated with a decline in EPA and DHA content in lake seston. However, there was no significant change in EFA content of cladocerans and copepods. This homeostatic (diet-independent) EFA composition in zooplankton suggested that, as the phytoplankton community shifted towards more cyanobacteria with lower EFA content, the cladocerans and copepods may have met their nutritional requirements by relying on alternative food sources (e.g. heterotrophic ciliates and flagellates) capable of either trophically upgrading phytoplankton-produced EPA and DHA, or synthesising EPA and DHA de novo.
5. Results from this study indicate that increasing DOC from low (2 mg DOC/L) to moderate levels (15 mg DOC/L) may increase the importance of the microbial pathway in the trophic transfer of EPA and DHA from basal resources to zooplankton. However, this supplementary transfer of EFA through the microbial

food web may not sustain high EPA and DHA levels in zooplankton when lake browning starts to limit primary production (>15 mg DOC/L).

KEYWORDS

climate change, food webs, nutrient cycling, phytoplankton, zooplankton

1 | INTRODUCTION

Climate-change-driven browning in northern lakes (Creed et al., 2018; Kritzberg & Ekström, 2012; Monteith et al., 2007), caused by increases in allochthonous dissolved organic matter (DOM) loads, is altering the species composition, biomass, and nutritional quality of primary producers (Urrutia-Cordero, Ekvall, & Hansson, 2016) available to primary consumers (Karlsson et al., 2009; Kissman, Williamson, Rose, & Saros, 2017; Sterner, Elser, & Hessen, 1992). Allochthonous DOM is composed of darker, more refractory compounds (i.e. aromatic and with a high molecular weight) than autochthonous DOM and thereby alters the physico-chemical environments of freshwater lakes (McKnight, Hood, & Klapper, 2003). For example, allochthonous DOM reduces light penetration (Karlsson et al., 2009), resulting in increased heat retention and a shallower, warmer, and more stable epilimnion (Houser, 2006; Porcal, Koprivnjak, Molot, & Dillon, 2009). In addition, allochthonous DOM can function as an energy (C) and nutrient (nitrogen [N], phosphorus [P], iron [Fe]) source for primary producers (Ged & Boyer, 2013); however, the bioavailability of the increased nutrient pool declines with shifts to more refractory DOM (Findlay, 2003; Soares et al., 2017; Sorichetti, Creed, & Trick, 2016). Finally, increases in allochthonous DOM supplies in clear oligotrophic lakes can enhance primary production by supplying additional energy and nutrients to phytoplankton (Seekell et al., 2015; Tanentzap et al., 2014), but high contents of allochthonous DOM (e.g. >15 mg dissolved organic C [DOC]/L) in oligotrophic lakes have been associated with low primary production due to light limitation (Ask et al., 2009).

Lake browning induces changes in phytoplankton communities (Finstad, Helland, Ugedal, Hesthagen, & Hessen, 2014; Solomon et al., 2015). Specific phytoplankton groups, including cyanobacteria, have developed adaptations to browning-driven changes in light and nutrient conditions that allow them to outcompete eukaryotic algae (Jones, 1998; Urrutia-Cordero et al., 2016). For example, cyanobacteria have phycobiliproteins, accessory pigments that allow them to photosynthesise under lower-light conditions (Oliver & Ganf, 2000). Some cyanobacteria (e.g. *Aphanizomenon* sp.) can regulate their buoyancy and thus their position in the water column to take advantage of nutrient accumulation in deeper waters (e.g. at or below the metalimnion) (Carey, Ibelings, Hoffmann, Hamilton, & Brookes, 2012). Some cyanobacteria (e.g. *Dolichospermum* sp.) are able to synthesise organic ligands (i.e. siderophores) that scavenge Fe from DOM and transport it into the cell (Sorichetti, Creed, & Trick, 2014; Trick & Kerry, 1992). Finally, some cyanobacteria (e.g. *Cyanothece* sp.) resort to mixotrophy to access C and other nutrients (including P and Fe) from DOM (Deininger, Faithfull, & Bergström, 2017; Jones, 1998)

when light and nutrients are limiting. In addition to cyanobacteria, mixotrophic flagellates, including cryptophytes, dinoflagellates, and raphidophytes, are typically found in highly-coloured lakes, as they can also adjust their position in the water column, thereby benefiting from spatially discrete organic nutrient pools (Arvola, 1984).

The cascading effects of lake browning translated up the food web have been debated (Brett et al., 2017). The debate has focused on the essential fatty acid (EFA) composition of basal resources (Galloway et al., 2014; Kelly, Solomon, Weidel, & Jones, 2014). Essential fatty acids are polyunsaturated fatty acids (PUFA; i.e. fatty acids with more than two double bonds in their C chain) that are involved in maintaining cell structure, metabolism, growth and reproduction. Essential fatty acids generally cannot be synthesised *de novo* by consumers in sufficient quantities to meet their needs (Brett & Müller-Navarra, 1997; Vance & Vance, 2008); however, recent studies have observed the presence of specific desaturases that support the synthesis of ω -3 (n-3) unsaturated fatty acids from saturated ones in several lineages of invertebrates (Kabeya et al., 2018). Allochthonous DOM is enriched in long-chain saturated fatty acids (LC-SFA), fatty acids without double bonds and with 20 or more C atoms in their chains, but its PUFA content is restricted to mostly ω -6 (n-6) PUFA (Hixson, Sharma, Kainz, Wacker, & Arts, 2015; Taipale et al., 2014). In contrast, phytoplankton synthesise higher amounts of both n-3 and n-6 PUFA, although there are exceptions among taxa (Strandberg, Taipale, et al., 2015). Having an obligate requirement for both n-3 and n-6 PUFA, consumers must rely on autochthonous resources to meet their n-3 requirements (Hixson et al., 2015). Among n-3 PUFA, eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) are primarily synthesised by phytoplankton and are essential for the growth, neural development and reproductive success of consumers (Brett, Müller-Navarra, Ballantyne, Ravet, & Goldman, 2006; Ravet, Brett, & Müller-Navarra, 2003).

Essential fatty acid content and composition in phytoplankton varies. Some phytoplankton (e.g. chrysophytes, cryptophytes, diatoms, dinoflagellates, and raphidophytes) are considered high quality resources due to their ability to synthesise significant amounts of EPA, DHA, and other n-3 PUFA. Other phytoplankton (e.g. chlorophytes and cyanobacteria) are considered low quality resources due to the low content or absence of EPA and DHA (Brett, Müller-Navarra, & Park, 2000; Brett & Müller-Navarra, 1997; Strandberg, Taipale, et al., 2015); however, chlorophytes and also cyanobacteria can contain appreciable quantities of shorter chain n-3 PUFA (Napolitano, 1999), α -linolenic acid (ALA; 18:3n-3), and stearidonic acid (18:4n-3) that are precursors of EPA and DHA. While differences in PUFA content among phytoplankton taxa are greater than

differences within each taxon (Galloway & Winder, 2015; Taipale et al., 2016), environmental conditions (e.g. light or nutrient availability) can influence PUFA content in phytoplankton (Fuschino et al., 2011; Guschina & Harwood, 2009). In fact, shifts in phytoplankton community composition driven by major changes in environmental conditions (e.g. browning and eutrophication) may lead to changes in the production and subsequent transfer of PUFA in lake pelagic food webs (Strandberg, Hiltunen, et al., 2015; Taipale, Kahilainen, Holtgrieve, & Peltomaa, 2018; Taipale et al., 2016).

Both autochthonous and allochthonous C is transferred from the base of the food web to higher trophic levels by zooplankton that graze phytoplankton and particulate organic matter (Cole et al., 2011) and by bacteria that assimilate decomposing phytoplankton and extracellular substances from living phytoplankton (i.e. the microbial loop; Tranvik, 1992). Heterotrophic bacteria are typically poor-quality resources that contain little or no PUFA; rather, they contain odd-chain-length and branched-chain SFA and mono-unsaturated fatty acids (referred from this point forward as bacterial fatty acids, BFA; Ratledge & Wilkinson, 1988; Hiltunen, Strandberg, Taipale, & Kankaala, 2015). However, heterotrophic flagellates can trophically upgrade fatty acids with each trophic transfer through the microbial loop where they are then preferentially retained by consumers (Bec, Martin-Creuzburg, & von Elert, 2006; Desvillettes

& Bec, 2009; Hiltunen, Honkanen, Taipale, Strandberg, & Kankaala, 2017). Consequently, the microbial loop can improve the quality of the C transfer pathway despite the loss of energy due to the upgrade of PUFA. The transfer of PUFA to higher trophic levels is further dependent on the feeding modes, life histories, reproduction cycles and PUFA requirements of primary consumers (Perhar, Arhonditsis, & Brett, 2013; Persson & Vrede, 2006). For example, cladocerans are generalist filter-feeders (although they exhibit some selectivity, particularly regarding particle size; DeMott, 1982), with larger EPA contents (Hiltunen, Strandberg, Keinänen, Taipale, & Kankaala, 2014; Hiltunen, Taipale, Strandberg, Kahilainen, & Kankaala, 2016; Persson & Vrede, 2006) and faster growth and reproduction rates than copepods. Copepods are more selective, with greater DHA content than cladocerans (Hiltunen et al., 2014, 2016; Persson & Vrede, 2006). However, certain zooplankton species can actively retro-convert dietary DHA back to EPA (in the case of cladocerans, see Fink & Windisch, 2018) and EPA to DHA (in the case of copepods, see Sargent, Bell, Bell, Henderson, & Tocher, 1995), although this process is considered to be energetically expensive.

Browning-driven changes in phytoplankton production and community composition could reduce the production and transfer of PUFA in lakes (Hessen, Andersen, & Lyehe, 1990). Browning may influence the PUFA pool size and transfer by: (1) supplying additional

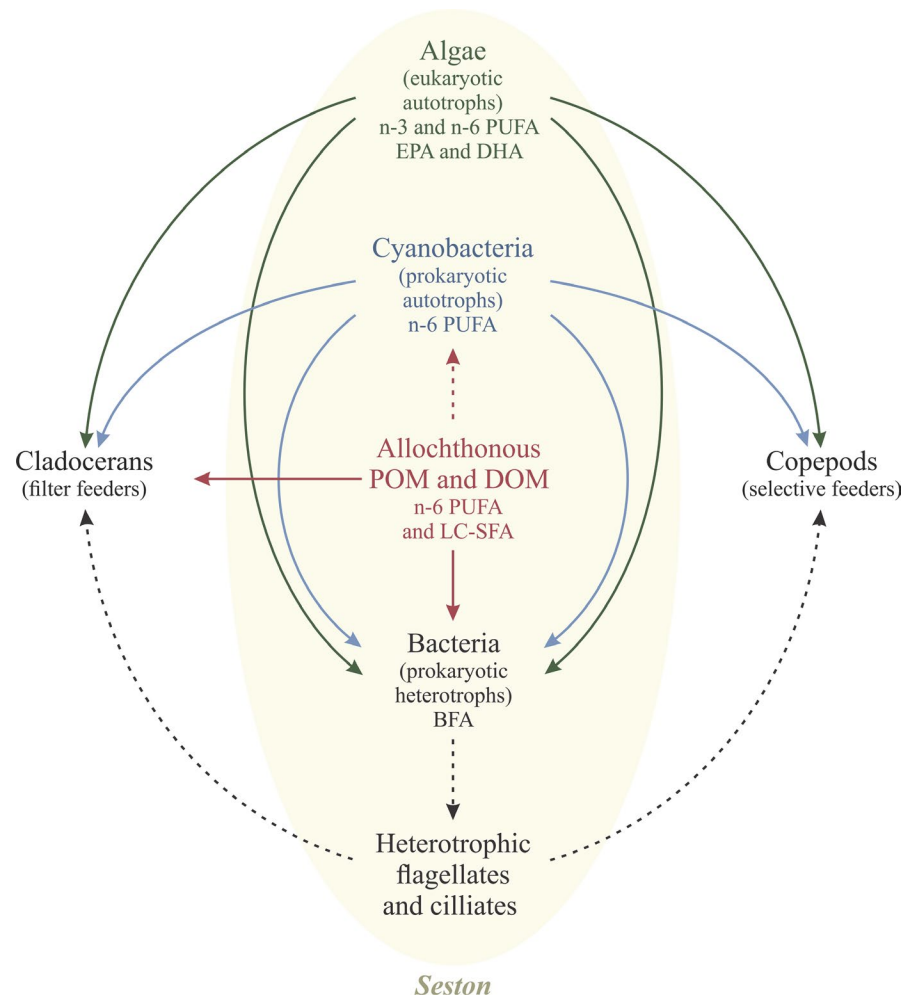


FIGURE 1 Diagram of a pelagic food web that identifies possible diet sources and fatty acid pathways to primary consumers; dashed arrows represent fatty acid transfer through the microbial loop. BFA, bacterial fatty acids; DHA, docosahexaenoic acid; DOM, dissolved organic matter; EPA, eicosapentaenoic acid; LC-SFA, long-chain saturated fatty acids; POM, particulate organic matter; PUFA, polyunsaturated fatty acids

allochthonous resources (rich in LC-SFA); (2) favouring heterotrophic bacteria growth (resulting in enriched BFA); (3) enhancing the microbial loop and subsequently the fatty acid transfer through this pathway; and (4) provoking shifts phytoplankton composition, from higher-quality autotrophic eukaryotes to lower-quality cyanobacteria (Figure 1). Here, we hypothesised that browning of lakes enhances primary production but selects for cyanobacteria, and as primary production and associated fatty acid pools increase, cyanobacteria will shift the fatty acid pool to lower n-3 EFA in phytoplankton and thereby lower PUFA in consumers. We tested these hypotheses in a comparative space-for-time substitution study (Carpenter et al., 1991), representing the effects of browning on content and composition of DOM in 30 lakes in the temperate biome.

2 | METHODS

2.1 | Study lakes

Lakes were situated in the Ontario temperate biome, a region where the average annual temperature is 5.2°C and the average annual precipitation is 832 mm (weatheroffice.gc.ca). Lakes were situated on the Precambrian Shield that is composed of metasedimentary rocks and soils dominated by acidic podzols (Chapman & Putnam, 1973). Catchments that drained into lakes were generated in ArcMap 10.2 (ESRI, 2014) with lake polygons (Ontario Hydrographic Network; waterbodies) used as pour points on a 20-m digital elevation model (Ontario Digital Elevation Model, version

2.0.0) that was hydrologically conditioned using a depression filling algorithm (Tarboton, Bras, & Rodriguez-Iturbe, 1991). Catchments were covered by forests and wetlands. Forests were dominated by sugar maple (*Acer saccharum* Marsh), yellow birch (*Betula lutea* F. Michx), and beech (*Fagus grandifolia* Ehrh), but with some white pine (*Pinus strobus* L.), balsam fir (*Abies balsamea* L. Mill), and eastern hemlock (*Tsuga canadensis* L. Carr) (Perera, Euler, & Thompson, 2011). Wetlands ranged from 0.5 to 50% of the catchment area, based on the 2013 Ontario Wetland Inventory (Ontario Ministry of Natural Resources). As wetlands are an important source of organic C (Creed, Beall, Clair, Dillon, & Hesslein, 2008; Creed, Webster, Braun, Bourbonniere, & Beall, 2013), lake selection was based on the proportion of wetlands in their watershed (from <5% to c. 50%; Figure 2).

2.2 | Lake samples

Each lake was sampled at its deepest point in 2016 during the peak growing season for phytoplankton (August–September). Temperature measurements were taken at a 1-m interval down the water column using a YSI EXO-2 multiparameter sonde (YSI Incorporated, Yellow Springs, OH, USA). Thermocline depth was estimated as the depth of greatest change in temperature in the water column. Composite epilimnion water samples were collected by combining three water samples collected at equal intervals, from surface to thermocline depth, using a Van Dorn sampler. The three depths at which water was collected were equally spaced, and the

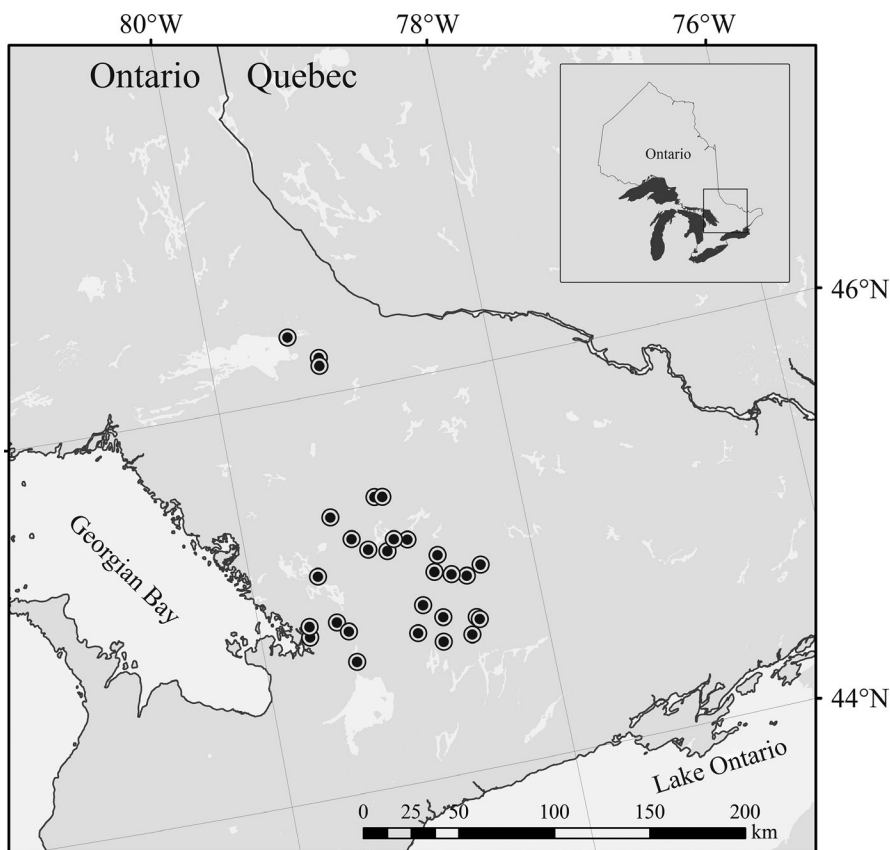


FIGURE 2 Location of study lakes located in the temperate biome in central Ontario

shallowest and deepest sample were at the same distance from the surface and the thermocline, respectively.

A 50-ml subsample from the composite epilimnion sample was collected in a borosilicate tube and used to measure P. All subsamples were kept on ice in coolers during transport and then at 4°C prior to analysis in the laboratory. P in the water sample was converted to orthophosphate in sulfuric acid-persulfate medium in an autoclave at 121°C for 30 min and then measured by colorimetry in a Skalar San++ Continuous Flow Analyzer (Skalar Analytical, Breda, The Netherlands). Lake trophic status was assigned based on total P (TP; µg/L). Oligotrophic lakes had a TP concentration <10 µg/L, mesotrophic lakes had a TP concentration between 10 and 35 µg/L, and eutrophic lakes had a TP > 35 µg/L (Dodds, Jones, & Welch, 1998; OECD, 1982).

Lake browning indicators included DOC (mg/L) as a proxy for DOM quantity; specific UV absorbance (SUVA; L mg⁻¹ m⁻¹) as a proxy for DOM quality (Weishaar et al., 2003); and water colour (true colour units, TCU). Subsamples for DOM quantity and quality were collected from the composite epilimnion sample. The composite epilimnion sample (1.5 L) for water chemistry analysis was filtered through a nylon filter (Nitex) of 80-µm porosity. A 500-ml subsample was collected in a polyethylene terephthalate bottle for analysis of DOC and water colour; and a second 500-ml subsample was collected in a dark Nalgene™ bottle for determination of SUVA. Dissolved organic carbon was measured from pre-ashed GF/F-filtered water samples by colorimetry using a Technicon AutoAnalyzer II (Seal Analytics). The absorbance measurement for the determination of SUVA—the ratio of absorbance at 254 nm to DOC concentration—was performed on a Cary 300 Bio UV-Visible spectrophotometer (Agilent Technologies) in a 1-cm path quartz cuvette. Water colour was determined by absorbance at 410 nm in a Shimadzu UVmini-1240 spectrophotometer (Shimadzu). Absorbance measurements were then converted into TCU using a calibration regression built with Hazen's Cobalt-Platinate Standards (1 mg Pt/L = 1 TCU).

Phytoplankton biomass and community composition were assessed as chlorophyll-*a* (chl-*a*, µg/L; a proxy for phytoplankton biomass; Boyer, Kelble, Ortner, & Rudnick, 2009) from a 100-ml subsample of the composite epilimnion sample. Chlorophyll-*a* was measured *in vivo* on the day of the sample collection in an AlgaeLabAnalyser (bbe Moldaenke GmbH, Schwentinental, Germany) in a 25-ml glass cuvette. The AlgaeLabAnalyser is a fluorometer that allows for a quick and reliable analysis of total chl-*a* measurements broken down into four fractions corresponding to the pigment concentration of cyanobacteria, green algae, brown algae (diatoms and dinoflagellates), and cryptophytes (Li, Parkefelt, Persson, & Pekar, 2017). For this study, we decided to combine the chl-*a* of diatom, dinoflagellates, and cryptophytes into a single *high-quality phytoplankton* measurement, as these groups represent high-quality food for aquatic consumers due to their generally high EPA and DHA content.

Fatty acid content was measured in seston and zooplankton. Seston and zooplankton samples were collected for fatty acid analysis by towing 60-µm (seston) and 156-µm (zooplankton) mesh-size plankton nets from the thermocline to the lake surface in each of the 30 lakes. Nets were towed until enough biomass was collected.

Seston samples were subsequently filtered, on-site, through an 80-µm mesh-size filter to remove zooplankton and other large suspended particles, and then filtered through 0.45-µm nitrocellulose filters (Sigma Aldrich), then backwashed with 2 ml of lake water. The filtered seston samples were stored in Falcon tubes® at -80°C. Zooplankton samples were collected in Whirl-Pak™ bags and kept frozen on dry-ice during transport at -80°C prior to laboratory analysis. Zooplankton samples were thawed and then sorted into cladocerans and copepods while ensuring that no phytoplankton remained on them. Seston, cladocerans, and copepod samples were freeze-dried prior to fatty acid extraction and quantification. Lipids were extracted from the freeze-dried samples with chloroform-methanol (2:1 by volume); methanolic sulfuric acid was added as a catalyst and samples were heated at 90°C for 90 min to transmethyrate the fatty acids. Fatty acids were then extracted to hexane, concentrated, and analysed through gas chromatography on a Shimadzu GC-210 with a flame ionisation detector using an SP-2560 column (Supelco Inc.). Helium was used as carrier gas (average flow of 20 cm/s) and a splitless injection technique was used. The temperature was maintained at 50°C for 1 min, then increased to 180°C at the rate of 15°C min⁻¹, then increased to 240°C at a rate of 2°C min⁻¹, where it was maintained for 23 min. The standard fatty acids methyl ester mix GLC68F (Nu-Chek Prep.) was used for peak identification and quantification. Additionally, gas chromatography with a mass selective detector (Agilent, USA) was used for peak identification. Seston and zooplankton C content was measured from freeze-dried samples through mass spectrometry by dry combustion using an EA (Costech Analytical Technologies), where percentage of C in the samples was converted to mg C per mg dry-weight based on the original weight measured for the analysis. Fatty acid concentrations were subsequently converted from dry weight to C ratios (µg FA/mg C).

2.3 | Statistical analyses

Significant differences in fatty acid composition between seston, cladocerans, and copepods (as µg FA/mg C) were assessed through one-way ANOVA in Sigma Plot 12.0 (Systat Software).

Direct and indirect effects on the availability and transfer of EFA in lakes caused by browning were studied through structural equation modelling (SEM) using the *lavaan* package (Rosseel, 2012) in R (R Development Core Team, 2008). Structural equation modelling is a statistical framework, often applied to ecological research, to understand links within systems and to test hypotheses about causal networks (Grace, Anderson, Olff, & Scheiner, 2010). Structural equation modelling tests indirect effects where specific variables can be simultaneously predictors and responses (like in this study, where seston EFA content is controlled by environmental conditions and in turn drives consumer EFA content; Dion, 2008). While it could be argued that a relationship between predictor and response variables does not necessarily imply causality, the use of SEM relies on a theoretical framework built prior to the analysis that identifies all potential causation pathways (Bollen & Pearl, 2013). The model was built using log-transformed data, and indicators of lake browning (DOC,

SUVA, water colour), and trophic status (TP) were used as predictors of phytoplankton biomass (using chl-*a* assigned to high-quality phytoplankton (diatoms, dinoflagellates, and cryptophytes), green algae and cyanobacteria as proxies). Indicators of lake browning, trophic status, and phytoplankton biomass were in turn used as predictors of EPA, DHA, and BFA content in seston. Finally, indicators of phytoplankton biomass and seston fatty acid content were used as predictors of EPA, DHA, and BFA content in cladocerans and copepods (LC-SFA content was excluded from this analysis as no significant correlations were identified). Relationships between EPA, DHA, and BFA within each group (seston, cladocerans, and copepods) were not tested to avoid multicollinearity (Tarka, 2018); instead, it was assumed that if EPA, DHA, and BFA presented significant covariance they were all driven by the same process. A number of iterations of

the SEM model were run, where non-significant ($p > 0.05$) pathways between predictors and responses were removed before each iteration. The best-fitting model was selected among all the iterations using Akaike's information criterion, using the *bbmle* package in R (Bolker & Bolker, 2017). Akaike's information criterion was used instead of the model's p -value as χ^2 is not a reliable metric of model fit when the sample size is low (Hox & Bechger, 1998).

3 | RESULTS

There was a range in environmental conditions (DOC, SUVA, water colour, TP) among the study lakes. Dissolved organic carbon ranged from 2.60 to 9.80 mg/L, SUVA ranged from 1.22 to 5.00 L mg C⁻¹ m⁻¹,

TABLE 1 Lake browning indicators predictors (dissolved organic carbon, DOC; specific UV absorbance, SUVA; water colour), trophic status (total phosphorous, TP) and phytoplankton community composition (cyanobacteria, green algae, high-quality phytoplankton) for the 30 study lakes

ID	DOC (mg/L)	SUVA (L mg C ⁻¹ m ⁻¹)	Colour (TCU)	TP (µg/L)	Cyanobacteria (µg chl- <i>a</i> /L)	Green algae (µg chl- <i>a</i> /L)	High-quality phytoplankton (µg chl- <i>a</i> /L)
BAS	5.80	2.76	20.70	10.70	0.84	0.68	1.69
BEL	2.60	1.98	8.44	6.50	0.26	0.00	1.26
BRA	9.50	5.00	135.00	48.50	4.17	1.19	1.02
COU	5.30	1.23	10.30	12.60	0.31	0.00	2.08
CRY	4.90	2.40	12.90	21.30	0.27	0.00	0.84
DAV	5.30	2.33	17.60	8.10	0.18	0.68	1.68
DEP	9.10	2.78	58.50	20.40	4.71	0.85	2.26
DEV	4.20	2.69	19.00	11.80	0.67	0.00	1.25
FOU	5.60	1.58	10.30	6.80	0.00	0.00	0.97
FOX	7.10	4.01	65.10	11.10	0.33	0.65	2.73
HEA	4.40	1.69	12.00	11.30	0.52	2.17	2.29
KAS	4.20	2.61	14.60	7.80	0.35	0.49	0.71
KOS	3.80	3.01	15.20	6.60	0.42	0.66	0.88
LOM	5.90	2.05	13.70	7.40	0.39	0.00	0.76
LON	5.20	2.78	18.40	9.30	0.69	0.25	1.42
MAC	7.40	2.32	32.00	18.70	1.18	2.62	1.60
MAP	3.70	2.91	16.40	7.50	0.27	0.20	0.99
MAR	5.10	3.39	35.60	12.40	0.47	1.12	1.07
MEN	7.10	3.93	58.70	11.40	0.25	1.83	0.99
MIN	9.80	2.85	53.10	18.40	0.52	1.86	3.63
MOR	5.50	2.91	29.30	9.00	0.64	0.66	0.79
AXB	4.00	2.75	17.00	7.20	0.58	0.58	0.38
PAI	3.90	2.52	20.00	10.40	0.48	0.85	1.60
RAV	3.50	2.59	16.20	6.00	0.52	0.74	1.48
RIL	4.10	4.83	28.40	10.90	0.26	0.97	0.97
SPA	4.90	2.32	16.80	13.40	0.49	0.59	1.51
TEA	6.30	2.82	27.70	7.50	0.42	0.51	1.40
TWE	3.20	2.39	14.40	8.10	0.13	0.00	2.95
WAS	7.90	3.34	54.60	31.80	0.23	2.08	2.07

Abbreviations: chl-*a*, chlorophyll-*a*; TCU, true colour units.

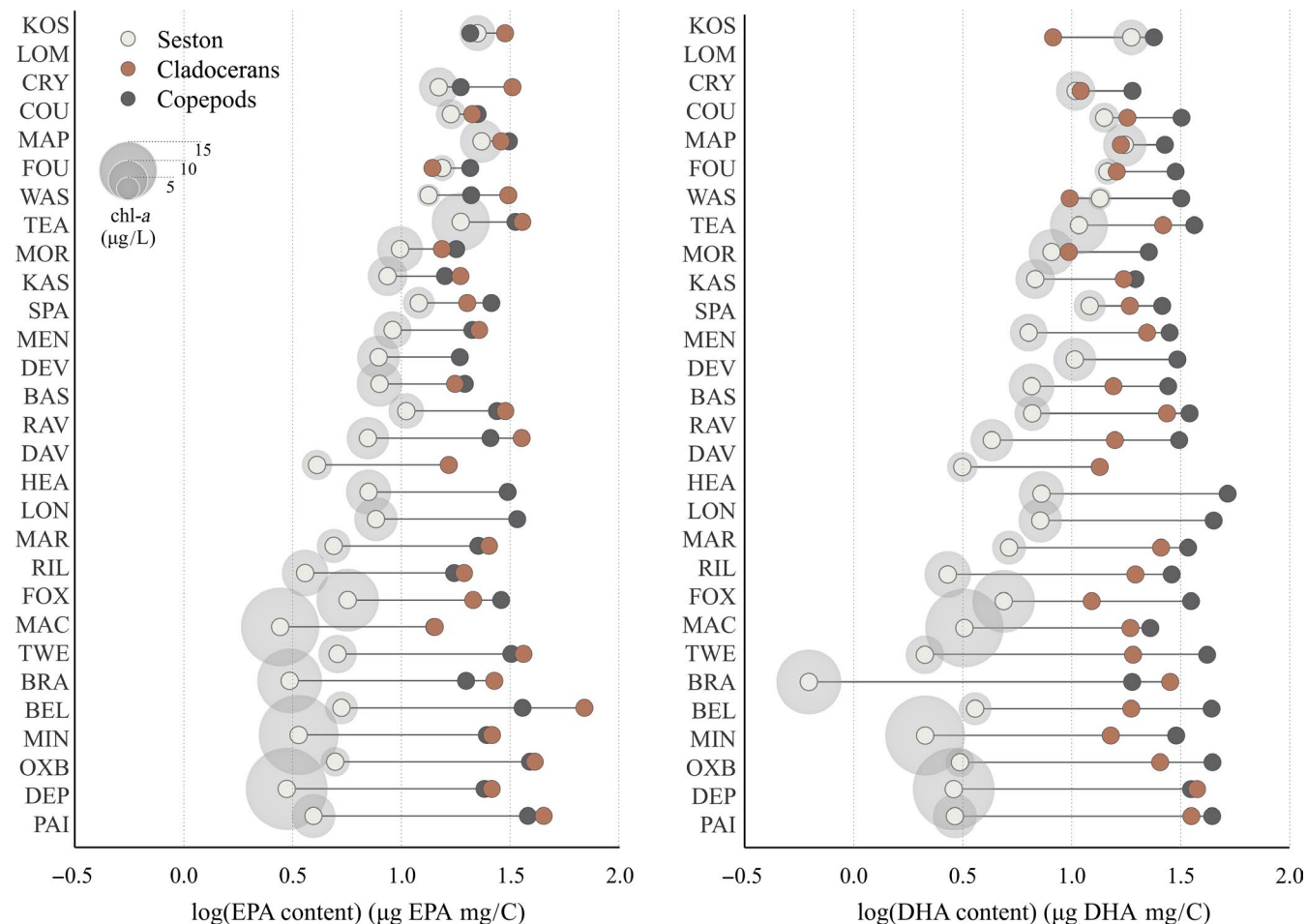
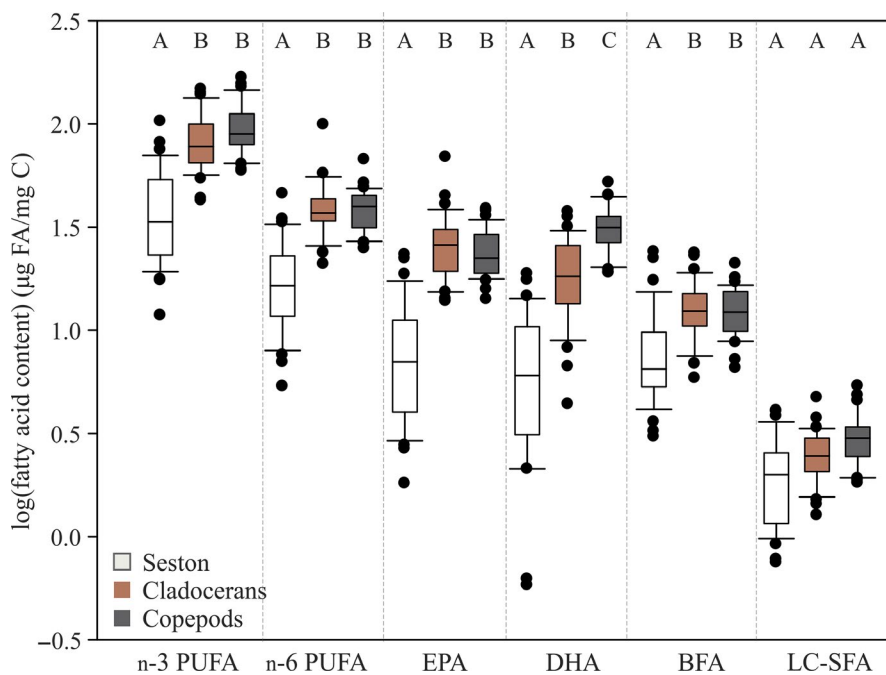


FIGURE 3 Log-scaled sestion, cladoceran, and copepod eicosapentaenoic acid (EPA; left) and docosahexaenoic acid (DHA; right) content in the study lakes; seston circle sizes represent phytoplankton biomass (chlorophyll-*a*, chl-*a*)

FIGURE 4 Log-scaled fatty acid content in seston, copepods, and cladocerans. Letters represent significant differences between organism fatty acid content calculated from one-way ANOVA and post hoc Holm-Sidak test. BFA, bacterial fatty acids; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; LC-SFA, long-chain saturated fatty acids; PUFA, polyunsaturated fatty acids



and water colour ranged from 8 to 135 TCU. TP in the lakes ranged from 6.00 to 48.50 $\mu\text{g/L}$ (average TP = $12.85 \pm 8.92 \mu\text{g/L}$); 14 lakes were oligotrophic (TP between 0 and 10 $\mu\text{g/L}$), 15 lakes were mesotrophic (TP between 10 and 35 $\mu\text{g/L}$), and one lake (Brandy Lake) was eutrophic (48.50 $\mu\text{g/L}$) (Table 1). Seston EFA content was highly variable compared to consumer EFA content (Figure 3).

The fatty acid content was significantly smaller (but more variable) in seston than in cladocerans and copepods, except for LC-SFA which was low in all three groups (average log [LC-SFA] = 0.26 $\mu\text{g/mg C}$ in seston, 0.38 $\mu\text{g/mg C}$ in cladocerans and 0.47 $\mu\text{g/mg C}$ in copepods; Figure 4). Furthermore, DHA content was lower in cladocerans than copepods (average log [DHA] = 1.58 in cladocerans and 1.72 $\mu\text{g/mg C}$ in copepods), but there were no significant differences in the other fatty acids among zooplankton groups (Figure 4).

The SEM analysis identified significant relationships between lake environmental conditions (DOC, SUVA, water colour, TP), phytoplankton communities (cyanobacteria, green algae, high-quality phytoplankton) and fatty acids (EPA, DHA, BFA) in seston, cladocerans, and copepods (Table 2, Figure 5). The original model—with a total of 60 hypothesised relationships between predictor and response variables—was reduced after two iterations to include 18 significant ($p < 0.05$) relationships (Tables S1 and S2). In these relationships, standardised coefficients are often thought of as prediction coefficients (Grace et al., 2010) that indicate the direction (positive or negative) of the relationships, and r^2 values represent the

variance of response variables explained by the model. A significant positive relationship was identified between DOC and TP ($r^2 = 0.50$; $p < 0.01$), and increases in both DOC and SUVA resulted in greater (darker) water colour ($r^2 = 0.84$, $p < 0.01$). While water colour was the major predictor of cyanobacteria biomass ($r^2 = 0.51$, $p < 0.01$), DOC was positively related to green algae ($r^2 = 0.36$, $p < 0.01$), and none of the assessed environmental parameters related to high-quality phytoplankton. Cyanobacteria biomass was negatively related to seston EPA ($r^2 = 0.40$, $p < 0.01$) and DHA ($r^2 = 0.15$, $p = 0.04$). In turn, seston EPA was positively related to TP ($p = 0.03$) and green algae ($p = 0.03$), and seston BFA was positively related to DOC ($r^2 = 0.50$, $p < 0.01$), and negatively related to cyanobacteria ($p < 0.01$) and green algae ($p < 0.01$).

When analysing the major predictors of consumers' fatty acid content, seston EPA was positively related to EPA content in cladocerans ($r^2 = 0.10$, $p < 0.01$) and copepods ($r^2 = 0.23$, $p < 0.01$), but seston DHA was only related to copepod DHA ($r^2 = 0.08$, $p < 0.01$). Cyanobacteria was positively related to cladoceran DHA ($r^2 = 0.24$, $p < 0.01$), while green algae was positively related to BFA content in cladocerans ($r^2 = 0.33$, $p < 0.01$) and in copepods ($r^2 = 0.21$, $p < 0.01$). LC-SFA content in seston, cladocerans and copepods were not included in the SEM model as no significant relationships (with either browning indicators or phytoplankton biomass) were identified in preliminary analyses.

Results from the SEM analysis identified covariance (Figure 5) between cladoceran EPA and BFA ($p < 0.01$), copepod EPA and DHA ($p < 0.01$), and between copepod EPA and BFA ($p < 0.01$).

TABLE 2 Structural equation modelling results

Response	Predictor	SE	SE	p-value
TP	DOC	0.70	0.68	<0.01
Colour	DOC	0.60	0.12	<0.01
Colour	SUVA	0.53	0.27	<0.01
Green algae	DOC	0.60	0.06	<0.01
Cyanobacteria	Colour	0.71	0.06	<0.01
Seston EPA	TP	0.40	0.13	0.03
Seston EPA	Green algae	-0.38	1.71	0.03
Seston EPA	Cyanobacteria	-0.50	1.01	<0.01
Seston DHA	Cyanobacteria	-0.38	0.85	0.04
Seston BFA	DOC	0.68	0.35	<0.01
Seston BFA	Green algae	-0.60	1.20	<0.01
Seston BFA	Cyanobacteria	-0.63	0.54	<0.01
Cladoceran EPA	Seston EPA	0.32	0.10	<0.01
Cladoceran DHA	Cyanobacteria	0.49	0.51	<0.01
Cladoceran BFA	Green algae	0.58	0.66	<0.01
Copepod EPA	Seston EPA	0.48	0.09	<0.01
Copepod DHA	Seston DHA	0.28	0.11	<0.01
Copepod BFA	Green algae	0.46	0.77	<0.01

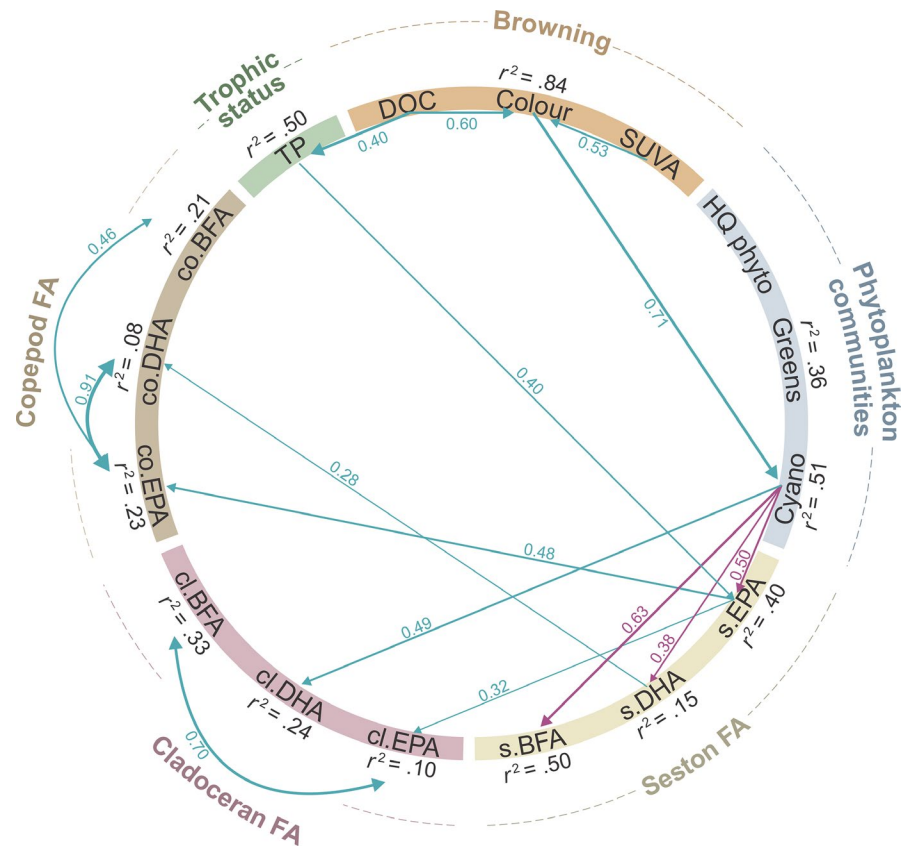
Abbreviations: DHA, docosahexaenoic acid; DOC, dissolved organic carbon; EPA, eicosapentaenoic acid; SUVA, specific UV absorbance; TP, total phosphorous.

4 | DISCUSSION

Lake browning alters the pathways and efficiency of energy and nutrient transfers in lake food webs (Finstad et al., 2014; Karlsson et al., 2009). This alteration may occur directly, by increasing the availability of low-quality allochthonous resources (Karlsson et al., 2009), changing the phytoplankton community composition (Jones, 1998), or promoting the microbial loop because of additional C resources (Tranvik, 1992). Alternatively, this alteration may occur indirectly, by altering the production of EFA by phytoplankton through changing lake environmental conditions (Guschina & Harwood, 2009; Solomon et al., 2015). We hypothesised that lake browning directly affects the phytoplankton community, increasing biomass and favouring cyanobacteria, consequently resulting in a reduction in the availability and transfer of EFA to primary consumers.

We found that lake browning was characterised by increased DOM quantity and shifts towards more refractory DOM (higher SUVA) and darker water colour (McKnight et al., 2003). We also found that browning was associated with increases in TP and shifts towards meso- and eutrophic status, suggesting that DOM acts as a nutrient vector (Ged & Boyer, 2013), or that the processes that drive increases in DOM (e.g. hydrologic intensification, Senar, Webster, & Creed, 2018) result in an increase in nutrient concentrations in the lake (Mengistu, Creed, Webster, Enanga, & Beall, 2014). The

FIGURE 5 Summary of the structural equation modelling analysis results related to the study hypothesis. Arrows within the circle represent (weighted) significant pathways between study variables (green = positive relationship, red = negative relationship); r^2 values represent variance of response variables explained by the model; and arrows outside the circle represent covariance between fatty acid content in cladocerans and copepods. Additional links identified by structural equation modelling are included in Table 2. BFA, bacterial fatty acids; DHA, docosahexaenoic acid; DOC, dissolved organic carbon; EPA, eicosapentaenoic acid; LC-SFA, long-chain saturated fatty acids; POM, particulate organic matter; PUFA, polyunsaturated fatty acids



additional refractory DOM inputs led to shifts in phytoplankton communities; while green algae responded positively to higher DOM quantity, cyanobacteria responded positively to shifts in water colour (i.e. changes in DOM quantity and quality, and thus light conditions and nutrient availability). Cyanobacteria have been found to have competitive advantages in lakes with moderate concentrations of refractory DOM (Sorichetti et al., 2014). Cyanobacteria have accessory pigments that allow them to photosynthesise under lower light conditions (Oliver & Ganf, 2000), some species can scavenge Fe from DOM-Fe complexes (Sorichetti et al., 2014, 2016), and some species can shift from autotrophy to mixotrophy to consume DOM (Poerschmann, Spijkerman, & Langer, 2004; Wilken et al., 2018) in nutrient-poor and dark lake waters. Irrespective of the strategy, the increase in the cyanobacteria could drive declines in the content of PUFA of seston (Müller-Navarra et al., 2004).

Browning-driven changes did not influence directly the EFA content in seston (i.e. no significant relationships were identified between browning indicators—DOC, SUVA, water colour—and seston EFA content). However, lake browning indirectly affected seston EFA through changes in phytoplankton and cyanobacteria biomass. On one hand, increasing phytoplankton biomass could result in higher EFA availability. On the other hand, increasing cyanobacteria prevalence (outcompeting EFA-rich phytoplankton) could result in lower EFA content in seston (Müller-Navarra et al., 2004). In the study lakes, changes in phytoplankton community composition, particularly increases in cyanobacteria prevalence, were associated with declines in seston quality in terms of EPA, DHA, and BFA. The decline

in seston EFA content can be explained not only with increased cyanobacteria dominance but also by changes in environmental conditions driven by browning (Guschina & Harwood, 2009). For example, increases in temperature and CO_2 concentrations (Mayorga et al., 2005; Porcal et al., 2009) can decrease PUFA production in phytoplankton, as unsaturation of fatty acids is typically highest at low temperature (Fuschino et al., 2011) and CO_2 concentrations (Thompson, 1996). Furthermore, changes in nutrient conditions can decrease PUFA pools. Nutrient limitation can lead to slower growth and reduced cellular division resulting in greater cellular fatty acid stores (Guschina & Harwood, 2009; Thompson, 1996). Therefore, browning and associated increased nutrient loads (Jones, 1998) can decrease in PUFA pools by favouring faster growth and therefore reducing cellular fatty acid storage. Alternatively, decreases in the penetration of UV radiation (Williamson, Stemberger, Morris, Frost, & Paulsen, 1996) can increase PUFA pools by reducing PUFA oxidative damage (Harwood, 1998). However, results from the SEM analysis also identified a positive relationship between TP and seston EPA. While these results differ with previous studies that relate eutrophication with declines in EFA availability (e.g. Müller-Navarra et al., 2004), the lakes in this study are mostly oligo- and mesotrophic (with a single eutrophic lake), and therefore an increase in nutrients could be associated with an increase in phytoplankton production (rather than dominance of EFA-poor cyanobacteria). In addition to these links, SEM analysis identified that increases in DOC were related to increases in TP and green algae biomass, resulting in decreases in seston EPA content due to the relatively poor quality of green algae

compared to other phytoplankton groups (Hiltunen et al., 2015) and increases in seston BFA due to enhanced bacterial production.

Despite the observed difference in EFA content in seston (driven by the lower EFA levels in the cyanobacteria dominated lakes), we found no difference in EFA content in zooplankton. Compared to seston, zooplankton had a larger content of EFA (and BFA), reflecting the preferential retention of these fatty acids. Increases in EFA content from seston to herbivores, and from herbivores to carnivores, have been previously observed, as these compounds are preferentially retained to maintain somatic growth and reproduction (Persson & Vrede, 2006). Greater classification (to the species level) within zooplankton taxa could help unravel differences between feeding types (Guschina & Harwood, 2009; Hessen & Leu, 2006). However, differences in EPA and DHA were observed even at the class level, with cladocerans (filter-feeders) having a larger average EPA content and copepods (selective-feeders) having a larger DHA content. The DHA content in copepods makes them the major DHA transfer pathway to top predators (Strandberg, Hiltunen, et al., 2015). Importantly, there was no decrease in zooplankton PUFA content associated with lake browning. Instead, the PUFA content in zooplankton was relatively homogenous among lakes irrespective of DOM properties. The PUFA content of consumers is defined, in part, by phylogenetic origin and life history (Persson & Vrede, 2006), making them quasi-homeostatic (i.e. their nutritional characteristics are, to an extent, independent of their diet) to fatty acid variability in seston (Brett, Müller-Navarra, & Persson, 2009). Both, the enrichment and the consumers' quasi-homeostatic PUFA content suggest that zooplankton have strategies (e.g. greater consumption of PUFA-poor resources or reliance on the microbial loop) to compensate for the decline in seston quality.

We found an association between EFA and BFA in cladocerans and copepods (represented by the significant covariances between cladoceran EPA and BFA, and copepod EFA and BFA), indicating that BFA and EFA are controlled, to a certain extent, by the same process. These results suggest partial reliance on fatty acid transfer through the microbial loop (Hiltunen et al., 2015, 2017), given that this is the major pathway of BFA transfer to consumers. In the microbial loop, autochthonous and allochthonous C is assimilated by bacteria (although autochthonous C is more rapidly consumed and preferred by bacteria, as indicated by the negative relationship between SUVA and seston BFA; Kritzberg, Cole, Pace, Granéli, & Bade, 2004) and transferred to zooplankton via two additional trophic transfers (bacteria-ciliates-heterotrophic flagellates-zooplankton; Berglund, Muren, Båmstedt, & Andersson, 2007). Since each trophic transfer results in EFA enrichment, heterotrophic flagellates can have significantly higher PUFA content than the original resource; a phenomenon known as *trophic upgrading* (Bec, Martin-Creuzburg, & Von Elert, 2010; Bec et al., 2006). Under EFA-poor phytoplankton conditions, heterotrophic flagellates might be a greater quality resource for zooplankton (Desvillettes & Bec, 2009). Results from this study suggest that reliance on microbial loop transfer of EFA and trophic upgrading could be used as a strategy of consumers to uptake EFA during poor-quality seston conditions. Alternatively, and given that browning promotes

phytoplankton biomass, zooplankton—and especially cladoceran filterers—could increase their food intake, overcoming the loss in seston EFA quality (as seen in the positive relationship between cyanobacteria concentration and cladoceran DHA).

This study did a space-for-time substitution for lake browning. The degree of browning in our lakes was relatively low, as DOM concentrations ranged from 2 to 10 mg DOC/L, and changes in DOM composition from SUVA of 1 L mg C⁻¹ m⁻¹ (labile DOM) to 5 L mg C⁻¹ m⁻¹ (refractory DOM). Studies that reported greater degree of lake browning (i.e. DOM concentrations that are more typical in boreal lakes >15 mg/L; Taipale, Kainz, & Brett, 2015; Taipale et al., 2016) identified decreases in zooplankton EFA content with increasing DOC concentrations. Even though phytoplankton communities are typically dominated by EFA-rich raphidophytes in these darker systems (Taipale et al., 2016), primary production is low (Kelly et al., 2014), and raphidophytes are too large to be directly consumed by zooplankton, therefore limiting the transfer of EFA to consumers (i.e. consumers have a lower biomass) and eventually to top predators. In contrast, the degree of browning in our study lake indirectly resulted in declines in seston EFA (despite the increase in phytoplankton biomass), due to the increasing prevalence of EFA-poor cyanobacteria, but not in consumers EFA content. Furthermore, under EFA-poor seston conditions, consumers did not experience major changes in their EFA composition, nor did they present an increasing reliance on allochthonous fatty acids (LC-SFA). These results suggest that lake browning could increase the transfer of EFA through the microbial loop, allowing primary consumers to adapt to the lower quality of phytoplankton (i.e. consumers find alternative pathway for EFA).

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