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FRESHWATER ECOLOGY

Increasing concentration of polyunsaturated fatty acids in browning boreal lakes is driven by nuisance alga *Gonyostomum*

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Abstract. Elevated concentrations of dissolved organic carbon (DOC) promote browning of lakes, which alters the physicochemical properties of water and ecosystem functioning. However, browning-induced changes in basal production of polyunsaturated fatty acids from the n-3 and n-6 families (n-3 and n-6 PUFA) in lakes are not fully understood. The concentrations of PUFA, which are micronutrients required to maintain growth and reproduction of consumers, have been suggested to either rise or decline in seston as a response to lake browning. Elevated DOC concentrations may also promote bacterial biomass and production and thus potentially increase the concentration of bacterial fatty acids (BAFA) in seston. We analyzed phytoplankton, bacteria and heterotroph biomasses, the taxonomic composition of phytoplankton, and the concentrations and mass fractions of bioseston PUFA, BAFA, eicosapentaenoic, and docosahexaenoic acids in ten boreal lakes in eastern Finland, with DOC concentration ranging from 2.8 to 18.7 mg/L. Our results showed that the abundance of PUFA in seston depended on the responses of phytoplankton biomass and community composition to lake browning. Lake browning increased seston PUFA and BAFA concentrations (expressed as $\mu\text{g/L}$) but not the contents (expressed as $\mu\text{g/mg}$ bioseston C). Although low DOC lakes had a favorable phytoplankton community (in terms of PUFA content), the phytoplankton biomass in these oligotrophic lakes was so low that the concentration of PUFA remained low compared to high DOC lakes. The increasing concentration of PUFA in bioseston along the DOC gradient was mainly due to the increasing biomass of nuisance alga *Gonyostomum semen*. However, *Gonyostomum* may be too large for small-sized zooplankton to ingest, and thus, the trophic transfer of PUFA may be impaired. The trajectories for lake browning and the basal production of PUFA also may depend on the source of carbon and associated nutrient loading; DOC and nutrient loading from agricultural areas may promote cyanobacteria dominance and decrease PUFA availability in lakes, while DOC runoff from more acidic and nutrient-poor peatlands may promote *Gonyostomum* dominance and increase seston PUFA concentration.

Key words: dissolved organic carbon (DOC); docosahexaenoic acid (DHA); eicosapentaenoic acid (EPA); phytoplankton.

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INTRODUCTION

Increased concentrations of allochthonous dissolved organic carbon (DOC) are causing browning in a vast number of temperate and boreal lakes in Europe and North America. Lake browning has far-reaching ecological consequences because elevated DOC concentrations and the associated increase in watercolor alter physicochemical properties in lakes (e.g., light conditions and nutrient availability) and, consequently, ecosystem functioning (Karlsson et al. 2009, Weyhenmyer et al. 2014, Creed et al. 2018). The browning of lakes is caused by increased transport of terrestrial carbon from watersheds due to various processes, such as recovery of soil from acidification, as well as shortened soil frost periods and increased precipitation due to global climate change (e.g., Monteith et al. 2007, Lepistö et al. 2014). Browning is further accelerated by anthropogenic activities, particularly land use practices in peatland-dominated watersheds (Nieminen 2004, Nieminen et al. 2015). All direct and indirect effects of lake browning have pronounced impact on the biomass and production of basal producers, and phytoplankton community composition in lakes (Lepistö and Rosenström 1998, Bergström and Karlsson 2019). Deteriorated light conditions in brown lakes affect the photosynthetic capacity of algae and thus may decrease algal production (Kelly et al. 2018, Bergström and Karlsson 2019). However, certain phytoplankton taxa may compensate for decreased photosynthetic capacity with behavioral or physiological adaptations. Lake browning is expected to favor algal taxa that are able to adjust their position in the water column (Lepistö and Rosenström 1998, Carey et al. 2012), are adapted to low light conditions (Oliver and Ganf 2000), are efficient in scavenging essential nutrients bound to dissolved organic matter (Trick and Kerry 1992, Sorichetti et al. 2014), or are mixotrophic and thus capable of utilizing organic carbon sources (Jones 1992, Deiningner et al. 2017). Taxa that are commonly associated with lake browning include mixotrophic chrysophytes, cryptophytes and dinoflagellates, as well as raphidophytes (Lepistö and Rosenström 1998). In particular, raphidophyte *Gonyostomum semen* is abundant in brown lakes with high DOC concentrations (Lepistö et al. 1994, Lebrecht

et al. 2018). Moderate browning of oligotrophic temperate lakes favors cyanobacteria, thus deteriorating water quality and usability to humans (Senar et al. 2019).

Changes in basal resources, bacterial and algal biomasses, and phytoplankton community composition may have cascading effects on the food chains (Taipale et al. 2018). Basal producers not only fuel energy production but also that of essential micronutrients, which may limit secondary production (Müller-Navarra et al. 2000, Brett et al. 2009, Peltomaa et al. 2017). Polyunsaturated fatty acids, specifically eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3), are among the most important micronutrients that may limit secondary production (Müller-Navarra et al. 2000, Brett et al. 2009). In aquatic ecosystems, algae are the main producers of EPA and DHA, and other physiologically important polyunsaturated fatty acids (PUFA), such as arachidonic (ARA; 20:4n-6), α -linolenic (ALA, 18:3n-3), and linoleic acid (LIN; 18:2n-6). However, the production of PUFA is highly taxon-specific (Taipale et al. 2013, Galloway and Winder 2015) and phytoplankton community composition significantly affects the abundance of PUFA in seston (Strandberg et al. 2015b, Senar et al. 2019). Many browning-induced algal taxa are rich sources of n-3 and n-6 PUFA, while cyanobacteria are known to be poor in these PUFAs (Taipale et al. 2013, Galloway and Winder 2015, Strandberg et al. 2015b). Thus, the impact of browning on seston PUFA content and concentration will be determined by the responses of phytoplankton biomass and community structure to browning.

Current information on the impact of lake browning on seston PUFA content and concentration is contradictory. Lake browning has been suggested to both increase and decrease the content of PUFA in seston (Gutseit et al. 2007, Taipale et al. 2016, Senar et al. 2019). Decreased availability of PUFA to consumers has been suggested based on the observation that bacteria and degraded terrestrial matter do not contain PUFA or have a very low PUFA content (Brett et al. 2009, Taipale et al. 2013, Hiltunen et al. 2019). Increased lake browning due to allochthonous inputs of carbon is typically linked with increased bacterial biomass and heterotrophy (Hessen 1992, Jones 1992). However, the

abundance of PUFA in seston is strongly dependent on the phytoplankton community composition (Strandberg et al. 2015b), and thus, the impacts of lake browning on PUFA availability are likely more diverse. The variability among past studies is most likely due to different approaches: direct measurements vs. theoretical estimates, number of lakes included, seasonal averages vs. sampling within a season, browning intensity (color, concentration of iron and DOC in the lake), and other factors such as the location of the sampled lakes (temperate vs. boreal) and the degree of anthropogenic nutrient loading from the watershed (Gutseit et al. 2007, Taipale et al. 2016, Senar et al. 2019). For example, phytoplankton biomass and community composition are strongly affected by temperature, among other factors, and thus, increased DOC loading to temperate vs. boreal lakes may have contrasting effects on phytoplankton community composition and seston fatty acids.

In Nordic boreal streams, rivers, and lakes, the median annual increase in DOC concentrations has been +1.4% during years 1990–2013, and future increases in precipitation are expected to further increase DOC loading (de Wit et al. 2016). In this study, we investigated the link between lake DOC concentration and the quantity and quality of basal producers, and consequently the availability of polyunsaturated fatty acids in Nordic boreal lakes. We analyzed phytoplankton community composition and biomass as well as concentrations and contents of n-3 and n-6 PUFA (18:2n-6, 18:3n-3, 18:4n-3, 18:5n-3, 20:4n-6, 20:5n-3, and 22:6n-3) as well as bacterial fatty acids (BAFA: sum of branched and odd-chained fatty acids) in pelagic basal producers in 10 lakes with varying DOC concentrations.

MATERIAL AND METHODS

We sampled ten lakes along a DOC gradient of 2.8–18.7 mg C/L in eastern Finland. Details on the locations as well as lake morphology and water chemistry and watershed characteristics can be found in Strandberg et al. (2016). In each lake, seston samples were collected from three locations between 30 July and 13 September 2013. Seston samples were collected as composites (0–2 m from the surface) with a Limnos sampler (volume 2 L). Seston fatty acids were analyzed as

triplicates from each lake, while phytoplankton community structure and the biomass of phytoplankton, bacteria, and heterotrophic flagellates were analyzed from two randomly chosen locations within each lake. Additional water samples were collected for chemical analyses (total nitrogen, phosphorus, total organic carbon, dissolved organic carbon, chlorophyll-*a*). Details of the chemical analyses are presented in Strandberg et al. (2016). Total carbon to nitrogen (C/N) ratios of seston have been used as an indicator of the origin of the C: Higher C/N ratios are related to increased proportions of peatland in the watershed (Rantakari et al. 2004, Mattsson et al. 2009). Another variable that provides information on the origin of C and nutrients is pH: Lower pH is typically associated with increased loading DOC from the acidic peatlands rather than from agricultural land (Rantakari and Kortelainen 2008). We also analyzed the molecular size distribution of dissolved organic matter (DOM) in lakes and measured specific UV absorbance (SUVA₂₅₄, Weishaar et al. 2003). Spectral absorbance was measured at the wavelength range 200–700 using a Shimadzu UV-1700 spectrophotometer (Suzhou Instruments Manufacturing, Suzhou, China). High-performance size-exclusion chromatography (Agilent 1100; Agilent Technologies, Santa Clara, California, USA) was used to analyze the molecular size distribution of DOM, specifically the proportion of high molecular weight components (HMW%; details in Akkanen et al. 2012). Seston samples, used for analyses of phytoplankton community structure and fatty acid composition, were sieved through 50 µm mesh, to exclude zooplankton (including majority of rotifers). Consequently, large algal cells or algal colonies were excluded from samples. Algae larger than 50 µm are in most cases too large for zooplankton to ingest. For microscopic analyses of phytoplankton and bacteria enumeration, we fixed samples with Lugol's solution (0.5 mL/250 mL) and stored them in dark bottles at +4°C. Viable phytoplankton and heterotroph cells were counted by allowing a 10–50 mL seston subsample to settle overnight, after which cells were counted and measured from 25 fields with two magnifications (500× small cells, 313× large cells) with an inverted microscope (Leitz Labovert FS, Leica Microsystems; Utermöhl 1958). The biovolumes were converted to carbon biomass based

on equations from Menden-Deuer and Lessard (2000). The contribution of heterotrophs (protozoan flagellates and ciliates) to total viable cell counts was also estimated. Other groups, such as rotifers, were occasionally observed, but these were not counted in the bioeston estimate. Bacterial biomass was estimated from the same Lugol-preserved samples, decolorized with thiosulphate, stained with DAPI (4'-diamidino-2-phenylindole; Porter and Feig 1980), and filtered (1 mL) on black polycarbonate filters (Osmonics, pore size 0.22 μm). Bacterial cells were counted, and their size was measured from at least ten random fields with an epifluorescence microscope (1000 \times magnification; Olympus BX60, Olympus Optical, Tokyo, Japan) using analySIS 3.2. Soft Imaging System. The total bacterial cell volume was converted to carbon using a factor 0.36 pg C/ μm^3 (Tulonen 1993). Bioeston carbon biomass (bioeston C) was calculated as the sum of phytoplankton, bacteria, and small heterotrophs (ciliates and protozoan flagellates).

For fatty acid analyses, seston was collected on 5- μm PVDF membrane filters (Durapore). We acknowledge that because we used 5- μm filters, most likely part of the bacterial community was not captured on the filter, and we therefore likely underestimated the concentrations of BAFA in the seston samples. However, we note that the concentrations of BAFA strongly correlated with the bacterial biomass (see *Results* and *Discussion* for details). Most likely part of the bacteria attached or aggregated on particles (algae, heterotrophic flagellates, ciliates) and thus remained on the filter. We also note that the bacteria biomass was calculated from 0.22- μm filters.

Filters were first placed in methanol, after which chloroform was added and lipids were extracted twice with chloroform-methanol (2:1 by volume). The extracts were pooled and evaporated to dryness under a nitrogen stream. Total fatty acids in seston were derivatized with an acid-catalyzed transmethylation reaction; immediately after evaporation, 1 mL of *n*-hexane and 2 mL of 1% sulfuric acid in methanol were added to the samples. The sample was flushed with nitrogen and heated at 90°C for 90 min, after which the reaction was stopped by adding water and the fatty acid methyl esters were extracted to *n*-hexane. In addition, we analyzed the phospholipid-derived fatty acids (PLFA) from a subset of

samples by using solid-phase extraction coupled with transmethylation as described above (details in Strandberg et al. 2015b). PLFA represent membrane-bound fatty acids, that is, fatty acids from living or recently deceased cells. Fatty acid methyl esters were analyzed at the University of Helsinki with Shimadzu Ultra GC-MS (Shimadzu) using an Agilent DB-23 (30 m \times 0.25 mm \times 0.25 μm) column. We used a splitless injection, and the inlet temperature was 250°C. Helium was used as a carrier gas with an average velocity of 36.3 cm/s. The oven temperature was as follows: initial temperature 50°C was maintained for 1 min, after which the temperature was increased 20°C/min to 140°C, and then 1.50°C/min to 190°C which was maintained for 3 min, and finally, the temperature was increased 5°C/min to 200°C and maintained for 10 min.

Statistical analyses

The effects of lake browning on the biomass of phytoplankton, bacteria, and small heterotrophs, as well as fatty acid concentrations and contents in bioeston, were evaluated with regression analysis using \log_{10} -transformed data. We also tested the Pearson correlation between lake DOC concentration and the proportion of phytoplankton, bacteria, and heterotrophs in total seston biocarbon content, using bootstrapping (1000 iterations). We also present the 95% confidence interval (bias corrected) of the bootstrapped correlations. The regressions and correlations were done with IBM SPSS statistics 25 and SigmaPlot 13.0.

We also used non-metric multidimensional scaling (NMDS) to illustrate the differences in lake phytoplankton composition, and cluster analysis to test how the lakes grouped without any a priori classification (group averages were used as the cluster mode). Prior to analyses, the phytoplankton community composition data were arcsine square root transformed and Euclidean distance was used as the resemblance matrix. The multivariate analyses were done with Primer 6.1.15 and Permanova 1.0.5.

RESULTS

Environmental variables

Several browning-related factors correlated strongly with lake DOC concentrations (see

details in Strandberg et al. 2016 and Appendix S1: Table S1). Briefly, a strong negative correlation ($r > -0.5$) was found between DOC concentrations and lake watersheds and morphometric variables: lake area as percentage of watershed, mean and maximum depth, relative depth percentage (relationship between lake area and maximum depth), and percentage of built environment and waterbodies (ponds, lakes, and rivers) in the watershed. A strong positive correlation ($r > 0.6$) was found between lake DOC concentration and the following parameters: lake nutrient and iron concentrations, the proportion of high molecular weight (HMW%) components, SUVA₂₅₄, C/N, and percentage of peatland in the watershed. In summary, clear lakes were large and deep, with low nutrient and iron concentrations, and had a higher proportion of human settlements, rivers, ponds, and lakes in their watershed (Table 1). Brown lakes were generally smaller and shallower, with higher nutrient and iron concentrations, and more peatland in their watershed (Table 1).

The quality of DOM varied between lakes: HMW% increased from about 28% to 84% with increasing DOC concentrations (Table 1). Similarly, SUVA₂₅₄ increased from 1.7 to 4.3 with increasing DOC concentrations. SUVA₂₅₄ and percent of HMW were strongly correlated ($r = 0.96$), indicating that both variables represent DOM quality equally well. SUVA₂₅₄, HMW%, and DOC concentrations were positively correlated with nutrient variables (totP and totN) as well as chlorophyll-*a* concentrations and C/N ratios. In summary, the increasing DOC

gradient of our study lakes was due to increased inputs of allochthonous DOC from peatland-dominated watersheds.

Bioseston biomass

Lake-specific mean bioseston biomass (sum of phytoplankton, heterotrophs, and bacteria biomasses) ranged from 27.9 to 216.7 $\mu\text{g C/L}$ (Fig. 1A). Bioseston biomass increased with lake DOC concentrations. Phytoplankton contributed the highest proportion of the bioseston, 67–87% of the total bioseston carbon, and increased with increasing DOC concentrations (Fig. 1B). The proportion of protozoan flagellates and ciliates in bioseston declined, and the proportion of bacteria did not change with increasing DOC concentrations (Fig. 1B).

Phytoplankton biomass increased from 18.6 to 188.1 $\mu\text{g C/L}$ with increasing DOC concentrations, and lake DOC concentrations explained 70% ($P = 0.003$) of the variation in phytoplankton biomass (Fig. 2A). Lake Ätäsö had lower phytoplankton biomass (53.1 $\mu\text{g C/L}$) than other lakes with high DOC concentrations. Bacterial biomass ranged from 2.6 to 10.8 $\mu\text{g C/L}$, and even though the proportion of bacteria from total bioseston carbon did not correlate with lake DOC concentration, the bacteria biomass significantly increased with lake DOC concentration ($r^2 = 0.75$, $P = 0.001$; Fig. 2A). The biomass of small heterotrophs was low in clear lakes and had a unimodal distribution along the DOC gradient, $r^2 = 0.67$, $P = 0.02$ (Fig. 2B). Note that this pattern differed from that between lake DOC concentrations and the proportional contribution

Table 1. Summary of water chemistry, basic lake morphometric, and DOC quality parameters in the study lakes.

Lake	DOC (mg/L)	TotP ($\mu\text{g/L}$)	TotN ($\mu\text{g/L}$)	Chl- <i>a</i> ($\mu\text{g/L}$)	pH	D_{max} (m)	D_{mean} (m)	C/N	C/P	SUVA ₂₅₄	HMW%
Kuorinka	2.8	1.6	169	1.3	7.1	32	10.5	17	1783	1.69	28
K. Pyhäjärvi	5.4	4.9	219	2.2	7.3	27	8	26	1160	2.40	55
Ylinen	6.9	3.6	315	2.3	7	35	12	22	1973	3.13	61
Kermajärvi	7.9	4.6	365	2.3	7.2	56	10.1	22	1754	2.88	58
Ätäsö	17.6	25.0	629	6.4	7.2	8	3.5	29	732	3.64	71
Koitere	11.9	11.1	286	6.5	6.3	46	6.7	44	1129	4.20	74
Harkkojärvi	16.6	18.3	458	8.2	6	10	3.5	37	917	3.87	74
Hattujärvi	16.2	23.3	377	7.4	6.4	9	3.3	44	707	4.26	75
Nuorajärvi	16.1	22.0	328	6.1	5.6	12	2.3	51	757	4.36	76
Mekrijärvi	18.7	28.8	556	9.5	6.2	3	1.8	34	657	4.10	75

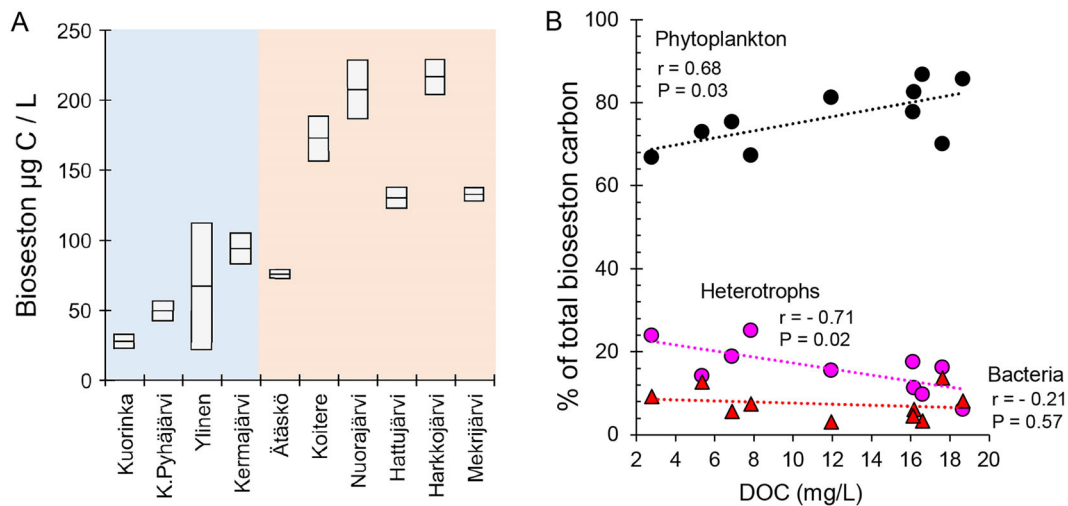


Fig. 1. (A) Mean and range of bioseston concentration (sum of phytoplankton, heterotrophs, and bacteria) in the study lakes. Lakes characterized as clear lakes are shaded with light blue and lakes characterized as brown lakes with pale orange. (B) Pearson correlation between lake DOC concentration and the mean proportion of phytoplankton, bacteria, and heterotrophs (ciliates and protozoan flagellates) in the total bioseston carbon. The 95% confidence intervals for the Pearson correlations were as follows: 0.55–0.98 for phytoplankton, –0.90 to –0.36 for heterotrophs, and –0.80 to 0.38 for bacteria.

of small heterotrophs to the bioseston pool (Fig. 1B); the proportion of heterotrophic flagellates and ciliates showed a linear decrease with increasing DOC concentration.

Phytoplankton community composition

Altogether 13 classes of algae were identified in the lakes. These algae accounted for 81.4–93.6% of all phytoplankton; thus, the proportion of unidentified algae ranged from 6.4% to 18.6% of total phytoplankton. The main algal classes were Raphidophyceae (range 0–55.1%), Dinophyceae (0–45.3%), Cryptophyceae (4.7–26.2%), Chlorophyceae (3.2–20.8%), Cyanophyceae (0.4–19.6%), Chrysophyceae (4.2–19.3%), Bacillariophyceae (1.9–14.4%), Synurophyceae (0–8.8%), and Euglenophyceae (0–3%; Fig. 3). Four classes, Charophyceae, Prasinophyceae, Tribophyceae, and Prymnesiophyceae, were present in low proportions (i.e., <2% of total phytoplankton in all the lakes) and were excluded from detailed evaluation.

The proportion of Raphidophytes increased from zero in clear lakes to 15–55% in high DOC lakes (Fig. 3). Raphidophytes were comprised almost exclusively of *Gonyostomum semen*

(hereinafter referred to as *Gonyostomum*), accounting for 100% of all Raphidophytes in all lakes except in Lake Harkkojärvi where *Gonyostomum* accounted for 95% of Raphidophytes. Cluster analysis based on phytoplankton community composition separated the lakes into two groups, with 22.7% similarity (cutoff level at 0.74; Fig. 4). Based on phytoplankton community composition, Lake Ätäskö clustered with the oligotrophic clear lakes instead of the other high DOC lakes, which were characterized by high proportion of Raphidophyceae. Lake Ätäskö was differentiated from the oligotrophic lakes because it had a higher proportion of cyanobacteria (Fig. 4). The dendrogram cutoff level for Ätäskö from the oligotrophic lakes was about 0.3 (data not shown). Phytoplankton community structure in clear lakes was more diverse, and no clear pattern was observed.

Fatty acids

In this study, we refer to the sum of 18:2n-6, 18:3n-3, 18:4n-3, 18:5n-3, 20:4n-6, 20:5n-3, and 22:6n-3 as PUFA (Appendix S1: Tables S2, S3). In addition, we separately present the concentration and content of EPA + DHA in bioseston. The

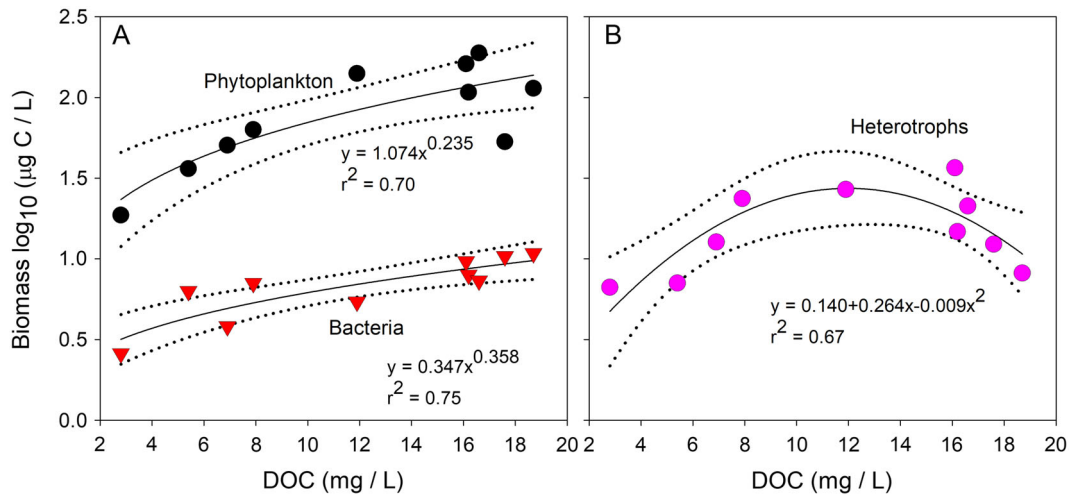


Fig. 2. (A) Non-linear regression, with 95% confidence intervals, between lake DOC concentration and the biomass of phytoplankton and bacteria in seston, and (B) between lake DOC concentration and the biomass of small heterotrophs (ciliates and protozoan flagellates). Note the logarithmic scale of response variables.

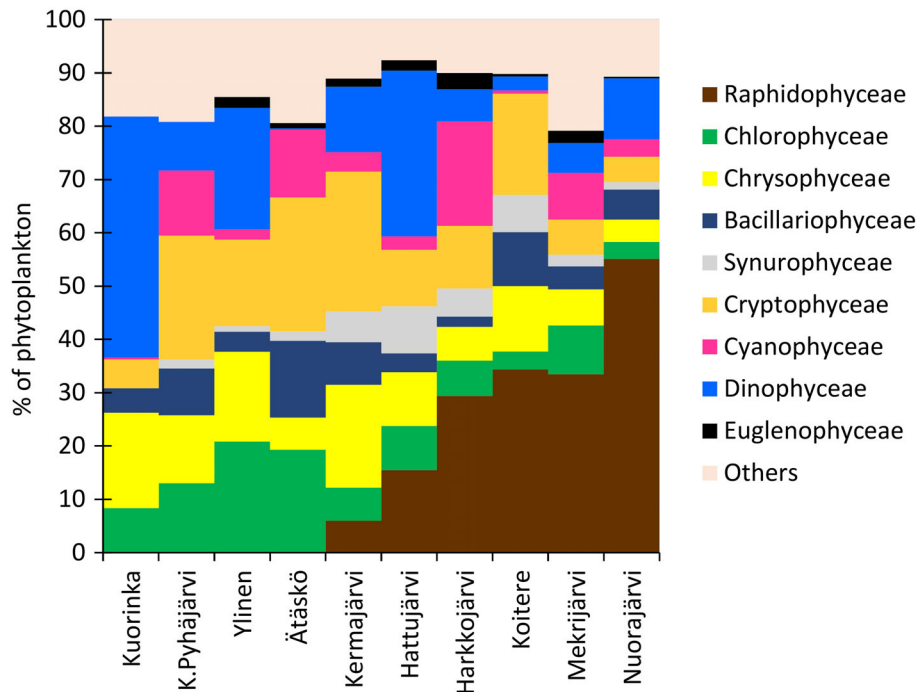


Fig. 3. Phytoplankton community composition in the 10 studied lakes from Eastern Finland. Lakes are presented according to increasing proportion of Raphidophytes in the phytoplankton community. Percentages are calculated from carbon biomass of each class/group. Taxa that account for at least 2% of the total phytoplankton community are represented. Category “others” is comprised of unidentified and rare taxa (<2% of total phytoplankton).

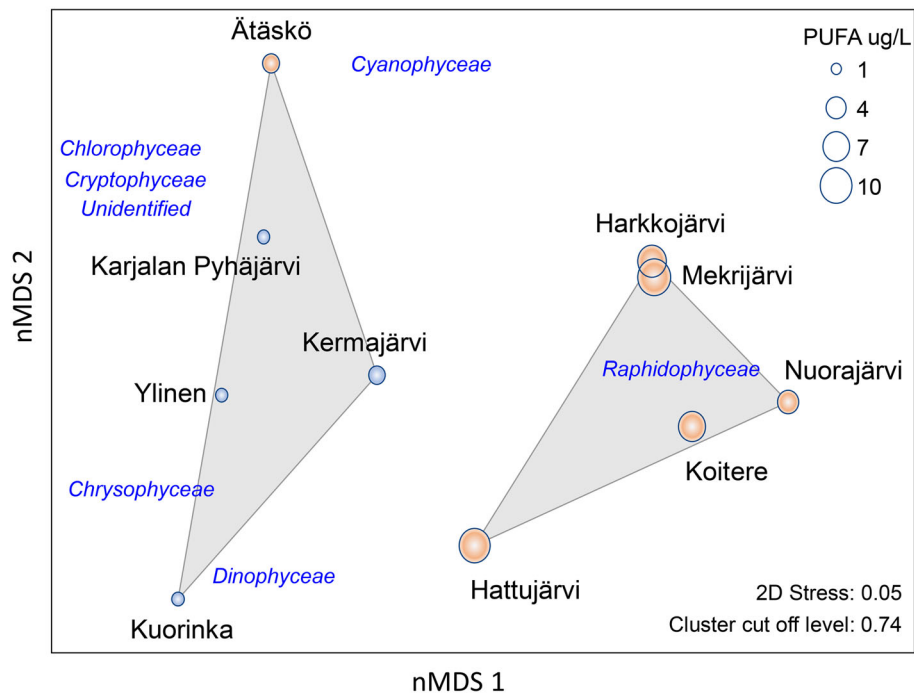


Fig. 4. Non-metric multidimensional scaling (nMDS) of phytoplankton composition in the study lakes. Blue dots mark lakes characterized as oligotrophic with low DOC concentration and orange dots lakes that are meso/eutrophic with high DOC concentration. The size of the dot indicates the concentration of n-3 and n-6 PUFA (sum of 18:2n-6, 18:3n-3, 18:4n-3, 18:5n-3, 20:4n-6, 20:5n-3, and 22:6n-3) in the seston (scale presented in the upper right corner). The gray area indicates clustering of samples based on phytoplankton community composition (without any a priori classification). The dendrogram was cut off at 0.74 and indicated a 22.7% similarity in phytoplankton community composition between the two lake clusters.

lake-specific content of n-3 and n-6 PUFA, in general, and EPA + DHA, in specific, in bioseston varied from 20.2 to 77.6 $\mu\text{g}/\text{mg}$ bioseston C and from 6.0 to 29.3 $\mu\text{g}/\text{mg}$ bioseston C, respectively (Table 2). No correlation was found between lake DOC concentrations and the mass fractions of n-3 and n-6 PUFA or EPA + DHA in bioseston ($\mu\text{g}/\text{mg}$ bioseston C; Fig. 5). However, PUFA and EPA + DHA concentrations as $\mu\text{g}/\text{L}$, which represent the quantity of PUFA and EPA + DHA in the lake, increased strongly with increasing DOC concentrations (Fig. 5). Lake-specific PUFA and EPA + DHA concentrations ranged from 1.4 to 10.3 $\mu\text{g}/\text{L}$ and from 0.4 to 3.8 $\mu\text{g}/\text{L}$, respectively (Table 2). We cannot separate the proportion of PUFA from algae or heterotrophs; bacteria are not known to contain PUFA. Bacterial fatty acids (BAFA) represent the sum of odd- and branched-chain fatty acids in the bioseston (iso-15:0, anteiso-15:0, iso-17:0, anteiso-17:0, 17:0). Lake-

specific mean concentrations of BAFA ranged from 0.1 to 2.2 $\mu\text{g}/\text{L}$, and the mass fraction from 1.5 to 16.9 $\mu\text{g}/\text{mg}$ bioseston C (Table 2). Concentrations of BAFA increased with increasing lake DOC concentrations, while no statistically significant linear trend was observed between BAFA mass fraction and lake DOC concentrations (Fig. 5). Concentrations of BAFA increased with increasing bacterial biomass (Fig. 6). The PLFA analyses verified that majority of fatty acids in the total fatty acid fraction were from living or recently deceased cells and not from degraded organic material (Appendix S1: Fig. S1). Data obtained from the total fatty acid analyses were used in all subsequent data analyses.

DISCUSSION

Overall, we found that browning of boreal lakes with peatland-dominated watersheds

Table 2. Mean and standard deviation of seston n-3 and n-6 PUFA (sum of 18:2n-6, 18:3n-3, 18:4n-3, 18:5n-3, 20:4n-6, 20:5n-3, and 22:6n-3), EPA + DHA (20:5n-3 and 22:6n-3), and BAFA (sum of odd- and branched-chain fatty acids) concentration and content ($\mu\text{g/L}$ and $\mu\text{g/mg}$ bioseston C, respectively) in ten boreal lakes from eastern Finland.

Lake	n-3 and n-6 PUFA				EPA + DHA				BAFA			
	$(\mu\text{g/L})$		$(\mu\text{g/mg}$ bioseston C)		$(\mu\text{g/L})$		$(\mu\text{g/mg}$ bioseston C)		$(\mu\text{g/L})$		$(\mu\text{g/mg}$ bioseston C)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Kuorinka	1.5	0.9	53.2	33.9	0.7	0.6	25.3	19.6	0.1	0.0	4.5	1.4
K. Pyhäjärvi	1.5	1.4	30.1	29.1	0.4	0.4	8.6	7.9	0.2	0.2	3.6	4.1
Ylinen	1.4	0.7	20.5	9.8	0.4	0.2	7.0	3.2	0.1	0.0	1.6	0.5
Kermajärvi	2.6	1.8	27.6	18.5	0.9	0.7	9.9	7.0	0.1	0.1	1.5	1.0
Ätäskö	2.8	1.3	36.7	17.4	0.9	0.3	12.8	4.0	0.5	0.4	6.2	5.0
Koitere	6.8	1.1	39.5	6.6	1.8	0.4	10.3	2.3	0.6	0.1	3.3	0.7
Harkkojärvi	7.9	2.3	36.3	10.7	1.9	0.5	8.6	2.4	0.8	0.3	3.8	1.5
Hattujärvi	9.3	4.7	71.3	36.2	3.8	2.3	29.3	17.1	1.9	1.8	14.7	14.0
Nuorajärvi	4.2	1.2	20.2	5.3	1.2	0.4	6.0	1.8	0.8	0.1	3.6	0.2
Mekrijärvi	10.3	1.6	77.6	12.0	2.8	0.4	21.0	2.8	2.2	0.7	16.9	5.2

Notes: Sampling depth was 0–2 m and $n = 3$. Bioseston C refers to the sum of carbon from phytoplankton, bacteria, heterotrophic flagellates, and ciliates.

increased the concentration (expressed as $\mu\text{g/L}$) of PUFA, EPA + DHA, and BAFA in seston, but did not have any significant effect on the content of PUFA, EPA + DHA, or BAFA in seston (expressed as $\mu\text{g/mg}$ C in bioseston). The finding that the concentrations, but not the content, of seston PUFA and EPA + DHA respond to lake browning demonstrates the need to consider the algal biomass when estimating the abundance and availability of these micronutrients to upper trophic level consumers. The main driver for the increasing concentration of PUFA along the DOC gradient was the increasing phytoplankton biomass, specifically that of the nuisance alga *Gonyostomum*. Our findings are supported by laboratory analyses, in which authors have reported high proportions of several PUFAs in *Gonyostomum* grown in autotrophic conditions, particularly EPA and C18 PUFAs, as well as a small proportion of DHA (Johansson et al. 2016, Taipale et al. 2016). The association that we found between PUFA and DOC concentrations is in accordance with a previous study on seston fatty acids in browning boreal lakes, but the values in the previous study were significantly higher (Gutseit et al. 2007). Both the concentration and content of many PUFA, including EPA and ALA, have been reported to increase

with lake browning (Gutseit et al. 2007). The values of seston PUFA content between our study and the previous study are not fully comparable because we only included carbon from phytoplankton, heterotrophs, and bacteria while the previous study presented their results relative to total particulate carbon, thus including also terrestrially derived carbon and other detritus (Gutseit et al. 2007). Additionally, prior to fatty acid analyses, water samples were sieved through 50 μm mesh in the current study and 100 μm mesh in the previous study (Gutseit et al. 2007). This suggests that some of the large-celled algae, including *Gonyostomum* (50–100 \times 20–24 μm), may have been excluded from the analyses of fatty acids and phytoplankton community composition in the current study. Note that both the fatty acid and phytoplankton community composition analyses were done from the same sieved fraction. *Gonyostomum* contributed a much higher proportion of the phytoplankton, and chlorophyll-*a* concentrations were at least three times higher in the study by Gutseit et al. (2007) compared to those in the current study. These differences may partly explain the differences in the amount of PUFA between the studies and further support the finding that *Gonyostomum* biomass is a key driver in determining

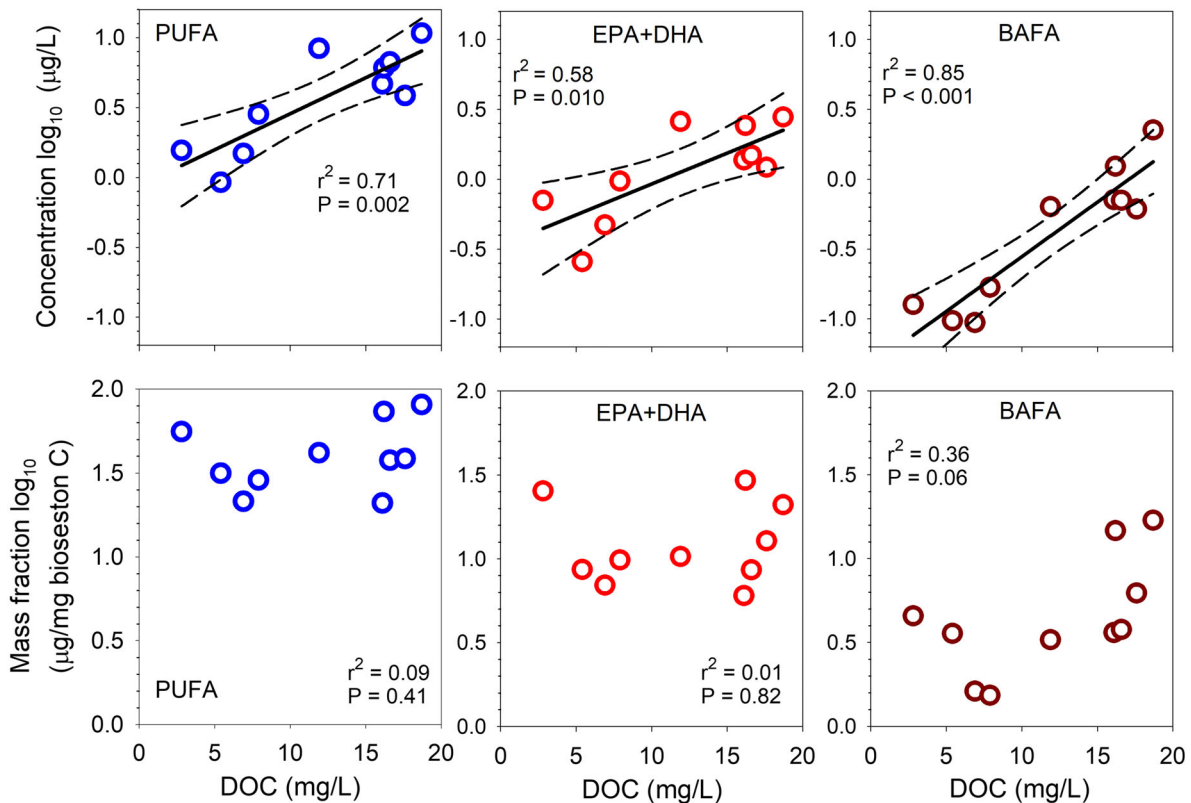


Fig. 5. Regression (with 95% confidence intervals) between lake DOC concentration (mg/L) and the concentration ($\mu\text{g/L}$) and carbon mass fraction ($\mu\text{g/mg}$ bioseston C) of n-3 and n-6 PUFA (sum of 18:2n-6, 18:3n-3, 18:4n-3, 18:5n-3, 20:4n-6, 20:5n-3, and 22:6n-3), EPA + DHA (20:5n-3 and 22:6n-3), and BAFA (sum of odd- and branched-chain fatty acids). Bioseston C refers to the total carbon biomass of phytoplankton, heterotrophs, and bacteria, thus excluding, for example, particulate detritus, terrestrial plant material. Data were \log_{10} transformed.

seston PUFA concentration in browning boreal lakes.

Gonyostomum has been invading lakes in northern Europe in the past few decades, causing fouling of fishing gear, boats, and docks, and may trigger skin irritation in bathers. The reasons behind the rapid invasion of *Gonyostomum* remain unclear. *Gonyostomum* seems to favor lakes that are small, have brown water color, low pH, and high DOC and phosphorus concentrations (Findlay et al. 2005, Peltomaa et al. 2013, Trigo et al. 2013), but the current expansion has brought *Gonyostomum* to lakes that are more eutrophic, larger, and have a higher pH (Hagman et al. 2015). *Gonyostomum* can form dense blooms in lakes in late summer, during which up to 95% of the phytoplankton biomass can consist of *Gonyostomum* (Peltomaa et al. 2013).

Phytoplankton biomass in the brown lakes we studied was higher than expected considering light limitation in these lakes. A previous study in Swedish lakes reported a unimodal relationship between phytoplankton biomass and lake DOC concentrations; phytoplankton biomass started to decline at 11 mg/L DOC concentrations because of light limitation (Bergström and Karlsson 2019). *Gonyostomum* is capable of osmotrophy (i.e., uptake of dissolved organic compounds by osmosis), which may compensate for light limitation and thus explain the higher than expected phytoplankton biomass in the current study lakes (Findlay et al. 2005, Rengefors et al. 2008, Hagman et al. 2019). *Gonyostomum* is also highly pigmented and thus efficient in absorbing solar energy in low light conditions (Eloranta and Råike 1995).

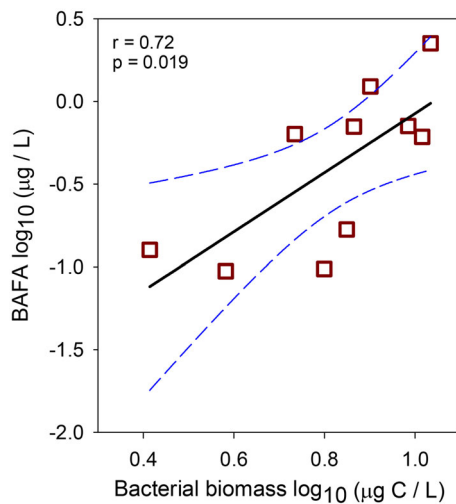


Fig. 6. Pearson correlation (with 95% confidence intervals) between the concentration of BAFA (sum of odd- and branched-chain fatty acids) in seston and bacterial biomass. Data were \log_{10} transformed.

The effects of browning on phytoplankton community structure and seston PUFA content may differ depending on watershed characteristics, the composition and concentration of DOC, and availability of macro- and micronutrients (Creed 2018). Moderate browning of temperate lakes (2–10 mg C/L) has resulted in cyanobacteria dominance and corresponding declines in EPA and DHA in seston (Senar et al. 2019). Cyanobacteria do not contain any EPA or DHA but may contain C18 PUFA (Los and Mironov 2015), thus maintaining some level of PUFA production (Strandberg et al. 2015b). The dominance of cyanobacteria in browning temperate lakes may be related to longer and warmer growing season and higher nutrient levels or less acidic conditions compared to the naturally brown boreal lakes in the current study (Palviainen et al. 2016, Senar et al. 2019). This is supported by the results from Lake Ätäskö in the current study, which was the only lake with any relevant anthropogenic input of carbon and nutrients (Palviainen et al. 2016). Lake Ätäskö was distinct from the other high DOC lakes due to its absence of *Gonyostomum* and correspondingly lower phytoplankton biomass and PUFA concentration (although not as low as in the oligotrophic lakes), which is in accordance with previous studies

(Bergström and Karlsson 2019, Senar et al. 2019). This suggests that trajectories of lake browning and the basal production of PUFA may differ depending on large-scale climatic factors and vegetation zones, as well as local factors, such as anthropogenic nutrient and carbon loading. Lake browning and its effects on seston PUFA concentration may differ between boreal and temperate lakes and are most likely strongly affected by landscape and the level of anthropogenic activity in the watershed. Moderate browning of temperate lakes together with elevated nutrient loading may promote cyanobacteria growth and decrease seston PUFA content (Senar et al. 2019). In boreal lakes, browning is often due to increased loading of DOC from nutrient-poor and more acidic peatlands, which may lead to the dominance of another nuisance alga, *Gonyostomum*, and increased seston PUFA concentration.

A previous theoretical estimate of PUFA levels based solely on the phytoplankton community composition indicated that both lake browning and eutrophication should decrease seston PUFA content (Taipale et al. 2016). However, this theoretical estimate did not include the contribution of PUFA from small heterotrophs and ciliates or account for the variation in algal PUFA content caused by other environmental factors. Phylogeny is the most important predictor of phytoplankton fatty acid composition (Galloway and Winder 2015), but other factors, such as temperature and nutrient concentrations, are known to significantly alter the content of PUFA in algae (Reitan et al. 1994, Juneja et al. 2013). Furthermore, heterotrophic flagellates may produce PUFA, and thus, seston PUFA concentrations may be higher than those estimated based upon phytoplankton community composition (Cronberg 2005, Hiltunen et al. 2017).

Seston is a highly diverse and complex mixture of particulate organic matter, which is comprised of phytoplankton, ciliates, protozoan flagellates, and bacteria, as well as degrading autochthonous and allochthonous organic material. Phytoplankton are the main source of PUFA, because bacteria do not contain PUFA and degrading terrestrial matter is poor in PUFA (Taipale et al. 2013, Galloway and Winder 2015, Hiltunen et al. 2019). However, small heterotrophs may be an alternative source of PUFA for zooplankton, because many algae are grazer-resistant and

certain heterotrophic flagellates may produce PUFA (Cronberg 2005, Hiltunen et al. 2017). In the current study, bioeston EPA content in clear lakes exceeded the suggested threshold levels for somatic growth and egg production in *Daphnia pulex* of 0.04–0.14 µg/mg C and 0.17–0.25 µg/mg C, respectively (Ravet et al. 2012). However, the concentration of EPA in the clear lakes was below the suggested threshold level 0.8 µg/L for somatic growth of another cladoceran zooplankton (*Daphnia galeata*; Müller-Navarra 1995). The results indicate that although oligotrophic clear lakes have high-quality seston, the quantity of seston (in terms of EPA concentration) is low. Thus, in these lakes the production of cladocerans, which are important prey items for zooplanktivorous fish, may be limited by this micronutrient. In brown lakes, bioeston PUFA and EPA contents were as high as those in clear lakes, and the concentration of EPA exceeded the suggested threshold values (Müller-Navarra 1995), suggesting that seston contained a sufficient amount of EPA provided that zooplankton feed on *Gonyostomum* (Johansson et al. 2013). In addition to EPA limitation, the reproductive success of copepods may be limited by DHA (Koski et al. 2006, Perhar et al. 2013). However, the specific nutritional requirements of freshwater copepods are far less studied compared to those of cladocerans.

Trophic transfer of PUFA from basal producers to consumers in the brown lakes depends on the grazing resistance and avoidance of *Gonyostomum* (Johansson et al. 2013a, b, 2016). *Gonyostomum* is large-celled compared to many other phytoplankton, has two unequally long flagella at the anterior end of the cell that are used in swimming, and consequently can perform diel vertical migrations (Cowles and Brambel 1936, Salonen and Rosenberg 2000, Cronberg 2005). In addition, *Gonyostomum* has trichocysts (long slimy threads) that can be ejected from inside the cell upon contact, and potentially act as a defense mechanism (Cronberg 2005). Lakes with a high biomass of *Gonyostomum* have a lower biomass of smaller, edible algae including chrysophytes, cryptophytes, and chlorophytes (Trigal et al. 2011, Johansson et al. 2013a). Regardless of the lower biomass of nutritionally valuable algae, zooplankton biomass has been noted to be similar between bloom and non-bloom lakes,

suggesting an alternative energy transfer pathway (Trigal et al. 2011, Johansson et al. 2013a).

In the current study, the bacterial biomass and correspondingly the concentration of BAFA in bioeston increased with lake browning. The strong correlation between BAFA concentrations and bacterial biomass indicates that the loss of bacteria due to our methodological approach (i.e., filter pore size) was similar across lakes. Thus, although we most likely underestimated the BAFA concentrations in our study lakes, the degree of underestimation is similar in all lakes, and the upward trend in BAFA concentrations along lake DOC gradient is not a methodological artifact. Both bacterial and phytoplankton biomasses increased with increasing DOC concentrations, but the bacterial biomass increased less than the phytoplankton biomass. Consequently, the proportion of bacteria in bioeston did not change along the DOC gradient even if bacteria biomass increased. Bacteria biomass may have experienced moderate top-down control by ciliates and protozoan flagellates in lakes with high DOC concentration. The biomass of ciliates and protozoan flagellates decreased with lake browning, possibly because of a strong top-down control by zooplankton (possibly because zooplankton was not able to efficiently graze on *Gonyostomum*). The strong top-down control by zooplankton on ciliates and protozoan flagellates may have diminished the top-down control of these heterotrophs on bacteria. This heterotrophic pathway may compensate for the reduced trophic coupling between phytoplankton and small-sized zooplankton that cannot efficiently feed on *Gonyostomum* (Johansson et al. 2013b, 2016). Increased heterotrophy at the base of the food web may decrease the availability of PUFA in the food web (Trigal et al. 2011, Johansson et al. 2013a, 2016, Strandberg et al. 2015a). The consequences of increased heterotrophy to secondary production are most likely taxa-specific and related to the physiological requirements of PUFA and possible biochemical modifications in the consumer (Perhar et al. 2013, Strandberg et al. 2014). Our previous related study (Strandberg et al. 2016) showed that the amount of EPA and DHA in piscivorous perch (*Perca fluviatilis*, collected from the same lakes as seston in this study) decreased with increasing DOC concentrations, suggesting a decreased trophic transfer

of PUFA in these *Gonyostomum*-dominated lakes. However, the low EPA and DHA content in perch in high DOC lakes may also result from an overall shift of the diet sources or decreased growth in turbid conditions. Lake browning and decreased visibility may alter foraging behavior of fish (Estlander et al. 2010). Compared to crustaceans, insect larvae have lower EPA and DHA content, and thus, increased feeding on insect larvae (or terrestrial insects at the surface) can decrease EPA and DHA levels in fish (Koizumi et al. 2018). Effects of increased DOC concentrations on fish productivity are not fully understood; most studies suggest a negative or unimodal response (Finstad et al. 2014, Benoit et al. 2016), but a whole-lake experiment found no net effect in fish productivity or growth (Koizumi et al. 2018).

In conclusion, browning of boreal lakes with peatland-dominated watersheds increased the concentration of PUFA (i.e., quantity), but not the content of PUFA (i.e., quality) in bioeston. The increased concentration of PUFA in bioeston was mainly due to the dominance of *Gonyostomum* in the phytoplankton community. These findings also indicate that bioeston PUFA concentration may be a more informative variable than PUFA content when studying the availability and trophic transfer of PUFA in lake food webs. In addition, some seston community structure data, particularly that of phytoplankton, are needed to evaluate the potential for efficient trophic transfer of PUFA to upper trophic level consumers. The results also indicate that increasing *Gonyostomum* biomass in browning boreal lakes may promote heterotrophy at the base of the food web, which in turn may be linked with decreased PUFA content in predatory fish (see Strandberg et al. 2016).

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SUPPORTING INFORMATION

Additional Supporting Information may be found online at: <http://onlinelibrary.wiley.com/doi/10.1002/ecs2.3189/full>