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Periphyton as a key diet source of essential fatty acids for macroinvertebrates across a nutrient and dissolved organic carbon gradient in boreal lakes

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Abstract

We studied how physiologically important long-chain polyunsaturated fatty acids (PUFA) in benthic macroinvertebrates (*Asellus aquaticus*, Chironomidae, and Oligochaeta) were related to those in periphyton and terrestrial organic matter (tree leaves), collected from littoral areas of 17 boreal lakes that differed in their dissolved organic carbon (DOC) and nutrient (phosphorus and nitrogen) concentrations. We also analyzed fatty acid (FA)-specific stable carbon isotopes ($\delta^{13}C_{FA}$) to investigate the dietary origin (periphyton vs. terrestrial organic matter) of PUFA in the consumers. In contrast to periphyton, terrestrial organic matter was deprived of long-chain PUFA, such as eicosapentaenoic acid (EPA), but rich in short-chain PUFA. The FA composition of macroinvertebrates was primarily taxon-specific despite the large differences in DOC and nutrient concentrations of the lakes. An increase in DOC concentration had a negative impact on the EPA content of *Asellus*, chironomids, and oligochaetes as well as the total FA content of chironomids and oligochaetes. However, the FA content of macroinvertebrates was not related to lake total phosphorus concentrations, although the total FA and EPA content of periphyton increased with the trophic status of the study lakes. The $\delta^{13}C_{PUFA}$ values of macroinvertebrates were positively related with the $\delta^{13}C_{PUFA}$ of periphyton and weakly with $\delta^{13}C_{PUFA}$ of terrestrial leaf material. The results indicate that EPA in the studied macroinvertebrate taxa was mainly derived from an algal-based diet and not via biosynthesis from allochthonous precursor FA. Thus, macroinvertebrate production in lakes may be limited by the available algae-based food sources.

The role of dietary energy and nutrients from terrestrial (allochthonous) and aquatic (autochthonous) resources to consumers in lake ecosystems is subject of ongoing debate (Pace et al. 2004; Cole et al. 2011; Brett et al. 2017). Synthesis of an extensive stable isotope data set from the northern hemisphere suggests at least 42% of allochthonous carbon contributes to

zooplankton biomass (Tanentzap et al. 2017). Until recently, the prevailing view for many decades has been that terrestrial leaf litter can be a major carbon source for macroinvertebrates in forested and sheltered headwaters, specifically in small oligotrophic lakes and shaded upland streams (Hynes 1975; Vannote et al. 1980; Wetzel 2001). However, Rasmussen (2010) presented stable isotope evidence of algae being more selectively assimilated by stream invertebrates than allochthonous sources. Allochthonous carbon sources are poor in essential biochemical compounds, especially essential fatty acids (Brett et al. 2009; Taipale et al. 2014, 2016), and thus considered low-quality food for aquatic consumers (Brett et al. 2017).

Most consumers require dietary polyunsaturated fatty acids (PUFA) to maintain somatic growth and reproduction. Docosahexaenoic acid (DHA, 22 : 6n-3), eicosapentaenoic acid (EPA, 20 : 5n-3), and arachidonic acid (ARA, 20 : 4n-6) are physiologically required for consumers as constituents of cell membranes (Vance and Vance 1996). Consumers have

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variable and often limited ability to biosynthesize these PUFA from their precursors, that is, alpha-linolenic acid (ALA, 18 : 3n-3) and linoleic acid (LIN, 18 : 2n-6) (Koussoroplis et al. 2014; Murray et al. 2014; but see Strandberg et al. 2020). Experimental studies reported that terrestrial sources are very low in omega-3 (n-3) and omega-6 (n-6) PUFA and contain only traces of EPA or DHA (Taipale et al. 2014; Hiltunen et al. 2019). Thus, even if terrestrial carbon sources are abundant in aquatic ecosystems, the concurrent presence of long-chain PUFA synthesizing algae is required for aquatic consumers, including zooplankton, benthic invertebrates, and fish (Kainz et al. 2010; Guo et al. 2016; Grieve and Lau 2018).

Boreal lakes are characterized by relatively high inputs of brown-colored, allochthonous organic matter (Algesten et al. 2004; Kortelainen et al. 2006), which is predicted to increase in the future due to recovery from acid deposition and increased precipitation, soil erosion, and terrestrial runoff induced by climate change (Monteith et al. 2007; Weyhenmeyer et al. 2014; De Wit et al. 2016). Consequently, lake browning and eutrophication will alter algal and invertebrate community composition, which has negative impacts on the availability and trophic transfer of EPA and DHA in lake food webs (Strandberg et al. 2015; Taipale et al. 2016; Kesti et al. 2021). By suppressing benthic primary and secondary productivity due to lower light and oxygen, darker water, and intensified stratification, a decline in the relative importance of benthic trophic pathways is expected (Vadeboncoeur et al. 2003; Solomon et al. 2015). The impacts of browning on PUFA occur at community levels and within species. For example, Kesti et al. (2021) found *Asellus aquaticus*, Chironomidae, and *Sida* sp. having lower PUFA contents in humic than in clear-water lakes in Eastern Finland, indicating that browning decreases the nutritional value of these invertebrates for higher consumers.

Most research on the composition of fatty acids and their trophic pathways of aquatic consumers has been conducted on pelagic organisms, such as zooplankton and fish, whereas less is known about the trophic pathways of littoral benthic macroinvertebrate fatty acids (but see Lau et al. 2014a). In this study, we examined the fatty acid composition and content of basal resources; that is, periphyton (autochthonous) and terrestrial leaf litter (allochthonous), and three commonly encountered benthic macroinvertebrate taxa with different feeding habits; that is, *Asellus aquaticus*, Chironomidae, and Oligochaeta, across 17 Finnish lakes that differ in dissolved organic carbon (DOC) and nutrient concentrations, and geographical locations. We analyzed fatty acid-specific stable carbon isotope values ($\delta^{13}C_{FA}$) of macroinvertebrates and these basal resources to test the hypothesis that macroinvertebrates in humic and eutrophic lakes have lower total fatty acid and EPA contents, but higher contents of bacterial fatty acids (BCFA) compared to those in clear and oligotrophic lakes. We based this hypothesis on the assumption that increasing nutrient and DOC concentrations lower the periphyton biomass due to shading and consequently the periphyton availability

to macroinvertebrate diets (Vadeboncoeur et al. 2003; Ask et al. 2009; Butkas et al. 2011). In eutrophic lakes, we also expected to find lower EPA content in macroinvertebrates due to higher proportions of the cyanobacteria in the water column and loss of benthic algal energy pathways (Vadeboncoeur et al. 2003). Nevertheless, we predicted strong relationships between $\delta^{13}C$ values of periphyton and invertebrate EPA, because algal-derived EPA is required and readily used for somatic growth (Karlsson 2007; Brett et al. 2017; Grieve and Lau 2018), and because motile benthic invertebrates can selectively feed on different periphyton assemblages (Devlin et al. 2013). Also, recent evidence has shown that even the dystrophic lakes may have periphyton contributing strongly to macroinvertebrate diets (Lau et al. 2014b; Vesterinen et al. 2016a,b).

Material and methods

Study lakes and the sampling

We investigated littoral food webs in 17 lakes, of which 3 were located in northern, 3 in central, 2 in eastern, and 9 in southern Finland (Fig. 1). The lakes ranged in size from 0.9 to 8551 ha (mean lake size: 361 ha), differed in water color (4.5–447.3 mg Pt L^{-1}) and total phosphorus (TP 3.0–80.0 μg L^{-1}) and total nitrogen (TN) concentrations (220.0–1090.0 μg L^{-1}). Water quality parameters (Table 1) were obtained from regional monitoring programs from the Finnish Environmental Administration monitoring database HERTTA (https://www.syke.fi/en-US/Open_information) and are means ($\pm$ SD) for epilimnetic water (0–1 m) from four summer sampling occasions during the period 2000–2019. Most of the parameter values were from 2016 to 2019, but some of the lakes had been monitored less frequently. DOC concentrations were estimated from water color values using the linear relationship of water color and total organic carbon (TOC = 0.0872 color + 3.55; Kortelainen 1993). Because in boreal waters > 90% of DOC consists of terrestrial organic carbon (Mattsson et al. 2005), we applied the color-based TOC estimates to assess terrestrial DOC concentrations. According to the trophic classification by Carlson (1977) and TP concentrations, six of the lakes were oligotrophic, four mesotrophic, and seven eutrophic. However, the lakes Horkkajärvi, Majajärvi, and Valkea-Kotinen can also be classified dystrophic with high DOC concentrations and low productivity. These three lakes were stratified during the open-water season and had relatively high nutrient concentrations below the thin euphotic zones. Lake Syrjänalunen was groundwater-fed, while the other lakes got their water predominately from surface runoff and inflowing rivers.

We sampled macroinvertebrates from the subclass Oligochaeta (Annelida), family Chironomidae (Insecta), and the species *Asellus aquaticus* (hereinafter referred to as *Asellus*) (Crustacea) from four littoral sites at each of the study lakes, from 17 July 2018 to 06 September 2018. We used both qualitative kick-net sampling and scraped substrates from rocks to
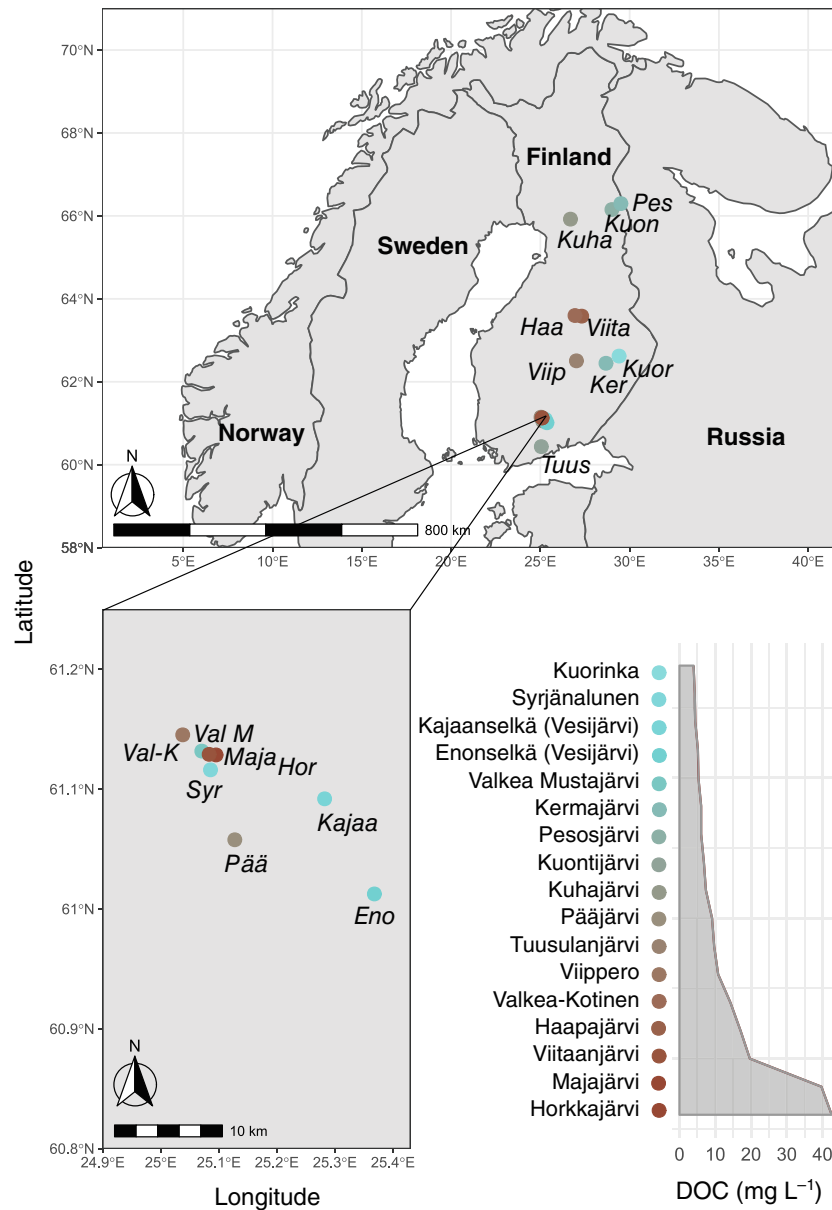
Fig. 1. Locations of the 17 study lakes together with their DOC concentrations. Color gradient from turquoise to brown illustrates the water color from clear to stained.

collect benthic macroinvertebrates. Kick-net samples were collected from the shoreline to the depth that was reachable with waders. From each of the sites, periphyton was collected by scraping off the plant, wood, and/or rock substrata into cryovials. Samples from different sites were pooled to reduce the workload of sample processing since we were not looking at site-specific differences. Also, fallen tree leaves, representing the allochthonous food source for the littoral macroinvertebrates, were collected randomly from the land near the shoreline from each sampling site. The leaves on land were used instead of those in the water to avoid biofilm (and algal fatty acids) entering our samples. To get enough freeze-dried

material of these groups for fatty acid analyses from each lake, it was not possible to separate individuals at a lower taxonomic level using time-consuming dissecting microscopy. Therefore, macroinvertebrates were identified to the level Oligochaeta, Chironomidae, and *Asellus aquaticus* (within ca. 2 h under a lamp on a white tray) the same day and thereafter frozen at -20°C. Chironomids were first separated according to their color to green and red specimens, which was thought to refer to their habitat oxygen concentrations and possibly different resource pools (red referring to high hemoglobin and low O_2) (Lancaster and Downes 2013). However, a latter comparison of their fatty acids revealed no statistically significant

Table 1. Physico-chemical characteristics of the study lakes (TP, TN, DOC). The values are means ($\pm$ SD) of epilimnetic water (0–1 m) from four summer sampling occasions during the years 2000–2019. Lake types are the typologies for the EU Water Framework Directive, classified by the Finnish Environmental Institute.

Lake	Coordinates (ETRS-TM35FIN)	Area (ha)	TP (μg L^{-1})	TN (μg L^{-1})	Water color (mg Pt L^{-1})	DOC (mg L^{-1})	Lake type
Syrjänalunen	N = 6785764, E = 400190	0.9	3.0 ± 3.0	352.0 ± 20.1	8.3 ± 3.3	4.2 ± 0.2	Small-sized humus poor
Horkkajärvi	N = 6788064, E = 401030	1.1	38.3 ± 8.4	852.0 ± 60.3	447.3 ± 52.5	42.6 ± 4.6	Humus rich
Majajärvi	N = 6788171, E = 399990	3.8	24.3 ± 4.5	735.5 ± 64.3	414.4 ± 71.8	39.7 ± 6.3	Humus rich
Valkea-Kotinen	N = 6791330, E = 396000	4.0	17.5 ± 4.4	442.5 ± 12.6	122.5 ± 28.7	14.2 ± 2.5	Humus rich
Valkea Mustajärvi	N = 6788778, E = 398682	13.0	10.0 ± 2.2	300.0 ± 16.3	20.0 ± 7.1	5.3 ± 0.6	Small-sized humus poor
Pesosjärvi	N = 7355310, E = 612515	44.0	5.0 ± 0.8	250.0 ± 25.8	28.8 ± 6.3	6.1 ± 0.5	Calcium rich
Viippero	N = 6930265, E = 501040	98.0	13.3 ± 1.2	393.3 ± 11.5	83.3 ± 25.2	10.8 ± 2.2	Shallow humic
Kuhajärvi	N = 7310250, E = 486209	305.0	34.8 ± 4.8	732.5 ± 315.3	63.8 ± 8.5	8.0 ± 2.6	Shallow humic
Viitaanjärvi	N = 7051105, E = 515295	361.0	47.3 ± 6.3	637.5 ± 142.4	185.0 ± 44.3	19.7 ± 3.9	Humus rich
Kuontijärvi	N = 7336522, E = 590607	595.0	18.5 ± 4.2	464.0 ± 72.7	37.5 ± 8.7	6.8 ± 0.8	Calcium rich
Tuusulanjärvi	N = 6702338, E = 393085	600.0	78.8 ± 22.8	1090.0 ± 290.5	44.0 ± 16.6	7.4 ± 1.4	Nutrient rich
Kuorinka	N = 6945081, E = 621686	1300.0	3.0 ± 0.0	220.0 ± 72.8	4.5 ± 1.0	3.9 ± 0.1	Medium-sized humus poor
Pääjärvi	N = 6771758, E = 399010	1344.0	8.9 ± 1.0	1250.0 ± 57.7	70.5 ± 11.0	9.7 ± 1.0	Medium-sized humic
Haapajärvi	N = 7049730, E = 498342	2588.0	80.0 ± 9.7	890.0 ± 110.2	155.0 ± 55.7	17.1 ± 4.9	Shallow humus rich
Kajaanselkä	N = 6781306, E = 403373	4271.0	19.0 ± 6.7	317.5 ± 25.0	10.0 ± 0.0	4.4 ± 0.0	Large humus poor
Enonselkä	N = 6768600, E = 423617	6470.0	31.0 ± 6.5	480.0 ± 39.2	18.3 ± 2.9	5.1 ± 0.3	Large humus poor
Kermajärvi	N = 6925631, E = 587116	8551.0	5.0 ± 1.4	380.0 ± 61.6	28.8 ± 4.8	6.1 ± 0.4	Large humus poor

differences (Pearson's correlation coefficient between the identified fatty acids of green and red specimens [both content and relative proportions] = 0.998, t = 94.39, $p < 0.0001$), so they were pooled in this study. All animals and excess plant material were removed from the periphyton samples under the microscopy and the samples were frozen at −20°C. Within a few days, all samples were transported to the laboratory, where they were stored at −80°C before freeze-drying and fatty acid analysis.

Fatty acid analysis

Prior to fatty acid analysis, samples (macroinvertebrate, periphyton, and dead leaves) were lyophilized with ALPHA 1–4 LD plus (Christ), homogenized with a mortar and pestle, and subsamples of 1.96 ± 0.46 mg DW^{-1} (mean ± SD for animals) and 4.53 ± 0.69 mg DW^{-1} (mean ± SD for periphyton and dead leaves) were analyzed for fatty acids. Lipids were extracted twice with 2 : 1 (by vol) chloroform : methanol following Folch et al. (1957) using tricosanoate acid (23 : 0) as an internal standard. Fatty acids were derivatized into fatty acid methyl esters (FAME) using an acid catalyzed transesterification reaction with 1% H_2SO_4 in methanol while heating at 90°C in a heat block for 90 min. We dissolved the produced FAME in hexane and analyzed them with a gas chromatograph attached to a mass spectrometer (GC-2010 Plus & QP-2010 Ultra, Shimadzu) with Zebron ZB-FAME column (30 m + 5 m guardian × 0.25 mm × 0.2 μm). At the beginning of each GC–MS run, the oven temperature was held at 50°C for 1 min, followed by raising with 10°C min^{-1} to 130°C, 7°C min^{-1} to 180°C, 2°C min^{-1} to 200°C and was held there for 3 min, after which the oven temperature was raised with 10°C min^{-1} to 260°C. The injection temperature was 270°C and the interface 250°C. Total

column flow was 27.5 mL min^{-1} and linear velocity 36.3 cm s^{-1}. Identification of the fatty acids was based on ion spectra, and the fatty acid content calculations were done based on four-point standard mixture calibration curves (GLC-566c, Nu-Chek Prep) with GC–MS solution version 4.42 (Shimadzu). The internal standard recovery was 86.7% ± 12.9%.

Compound-specific stable isotope analysis

The ^{13}C/^{12}C (δ^{13}C) values of dominant fatty acids present in macroinvertebrates, periphyton, and dead terrestrial leaves were determined using gas chromatography–combustion–isotope ratio mass spectrometry (THERMO™,) with helium as the carrier gas and coupled to a GC Isolink 2™ (Thermo) where the separated fatty acids were combusted at 1000°C. The generated CO_2 was transported with the carrier gas (helium) to the Conflo IV universal continuous flow interface (Thermo), where each sample was diluted with the carrier gas helium and connected with the reference CO_2 gas. Finally, all CO_2 molecules were analyzed in the Delta V™ Advantage Isotope Ratio Mass Spectrometry (Thermo). The δ^{13}C value of each fatty acid ($\delta^{13}C_{FA}$) was corrected to account for a single carbon atom from the methanol (− 27.5 ± 0.0‰) that was added during fatty acid transmethylation using the following formula:

$$\delta^{13}C_{FA} = \frac{(\text{number of C in FAME} + \delta^{13}C_{FAME}) - (\delta^{13}C_{methanol})}{\text{number of C in FA}}.$$

Data analysis

The relationships between multivariate fatty acid data of the macroinvertebrates and their food sources and water

quality parameters of the lakes, which represent the predictor variables, were analyzed with distance-based linear model (DistLM) and distance-based redundancy analysis (dbRDA) routine with PRIMER 6 (version 6.1.15) & PERMANOVA (version 1.0.5) (PRIMER-E Ltd). TP, TN, and DOC concentrations were used as continuous and quantitative predictor variables, and the group of organisms was used as a categorical (nominal) predictor variable. The analyzed fatty acid data were reported a mass fractions (μg fatty acid mg dry weight^{-1}) or relative values (%), and we operated the multivariate statistical methods on Euclidean distances without transforming the data (Happel et al. 2017). We used a step-wise procedure for building the DistLM model and adjusted R^2 for the selection criterion for the model.

Because the samples consisted of periphyton, dead leaves, and animals (highly different organisms), we expected that the differences in fatty acid composition between groups are very large, which prevent us from observing finer-scale differences within taxa and relationships between single fatty acid groups and water quality. Hence, we studied Pearson's correlation coefficients between single macroinvertebrate fatty acid groups and water quality parameters. The examined fatty acid groups were total fatty acids, EPA, and saturated *iso-* and *anteiso*-methyl-branched fatty acids (i.e., BCFA; Kaneda 1991). The examined water quality parameters were TP and DOC concentrations of the lakes, of which the first represents the nutrient commonly limiting algal growth in Finnish lakes and the latter the parameter strongly affecting the light climate of lakes. Pearson's correlation coefficients were calculated using the cor.test() function in R version 4.0.5. (R Core Team 2021). Before Pearson's correlation, we ln(log)-transformed the data to satisfy the assumption of data being normally distributed and to improve data visualization.

The correlation between δ^{13}C values of macroinvertebrate LIN, ALA, and EPA, and δ^{13}C values of LIN, ALA, and EPA in the two potential food sources, periphyton, and dead terrestrial leaf were examined using Pearson's correlation. We examined only these three PUFA since they could be found from all the samples, unlike ARA, stearidonic acid, and DHA.

Graphs were established using PRIMER 6 (version 6.1.15) and *ggplot2* (Wickham 2016) in R, version 4.0.5. (R Core Team 2021).

Results

Fatty acid composition, content, and variation of macroinvertebrates and their food sources

Chironomids were caught from each of the 17 lakes, *Asellus* from 15 lakes, except the highly humic Lake Horkkajärvi and clear-water Lake Pesosjärvi. Oligochaetes were missing in two of the most humic lakes, Lake Horkka and Majajärvi, and also in the rather humic Lake Viippero.

In *Asellus*, the most abundant fatty acid (mean relative content of total fatty acids; %) was 16 : 1n-7 (18%), while EPA was

the most abundant fatty acid in chironomids (13%; Supporting Information Table S1). Oligochaetes were richer in EPA (25%), but had less ARA than *Asellus* and chironomids (Supporting Information Table S1). The most abundant fatty acid in periphyton was 16 : 1n-7 (18%; Supporting Information Table S1), while the most abundant fatty acids in the dead leaves were 16 : 0 (12%), the long-chain saturated fatty acids 26 : 0 (11%) and 28 : 0 (9%), but also ALA (11%) and LIN (9%) (Fig. 2; Supporting Information Table S1). Chironomids and *Asellus* had the highest total fatty acid contents (mean $\pm$ SD) (53.5 $\pm$ 18.1 and 40.5 $\pm$ 16.5 μg mg^{-1}, respectively), followed by oligochaetes (28.5 $\pm$ 9.7 μg mg^{-1}), while dead leaves and periphyton had the lowest total fatty acid contents (7.2 $\pm$ 1.6 and 8.3 $\pm$ 3.3 μg mg^{-1}, respectively). Periphyton had slightly higher omega-3 : omega-6 (n-3 : n-6) ratios (mean $\pm$ SD) (2.0 $\pm$ 0.6) than chironomids and oligochaetes (1.5 $\pm$ 0.7 and 1.5 $\pm$ 0.4, respectively), and *Asellus* and dead leaves had the lowest n-3 : n-6 ratios (1.1 $\pm$ 0.3 and 1.1 $\pm$ 1.1, respectively). The BCFA contents were highest in oligochaetes and chironomids (1.3 $\pm$ 0.6 and 1.2 $\pm$ 0.7 μg mg^{-1}, respectively), followed by *Asellus* (0.8 $\pm$ 0.5 μg mg^{-1}), periphyton (0.1 $\pm$ 0.1 μg mg^{-1}) and dead leaves (0.04 $\pm$ 0.01 μg mg^{-1}).

The DistLM and dbRDA showed that taxon primarily explained the fatty acid composition of macroinvertebrates instead of environmental characteristics. The adjusted R^2 of our DistLM model, including TP, TN, and DOC concentrations, and five groups of organisms (*Asellus*, Chironomidae, Oligochaeta, periphyton, and dead terrestrial leaf) as explanatory variables, was 0.52. Marginal tests, which test the relationship between a response variable and an individual variable, showed that the groups of organisms were the only significant explanatory variable in the data cloud when taken alone and ignoring other variables (Supporting Information Table S2). Sequential (conditional) tests with more than one explanatory variable showed that the three macroinvertebrate taxon groups together explained 88% of the model R^2 variance, while the environmental variables explained only 6.3%. Based on their fatty acid composition, taxon groups (including the groups of periphyton and dead leaves) clustered into separate groups (Fig. 2). The data mostly clustered along the dbRDA1 axis, which explained 75.6% of the fitted variation and 41.7% of the total variation. Vector correlation of TP, TN, and DOC concentrations was low, < 0.2 (Fig. 2).

In *Asellus*, there was a significant negative correlation between EPA content and lake DOC concentrations (Pearson's $r = -0.56$, $t = -2.55$, $p < 0.05$), but the other tested fatty acid groups were not significantly related to DOC or TP concentrations (Fig. 3a–c). In chironomids, both total fatty acid content (Pearson's $r = -0.48$, $t = -3.15$, $p < 0.01$) and EPA content (Pearson's $r = -0.48$, $t = -3.13$, $p < 0.01$) were negatively correlated with DOC concentrations (Fig. 3d–f). In oligochaetes, there were significant and strong negative correlations between DOC concentration and all the tested fatty acid groups (Pearson's r ranging from -0.60 to -0.74, $p < 0.05$;
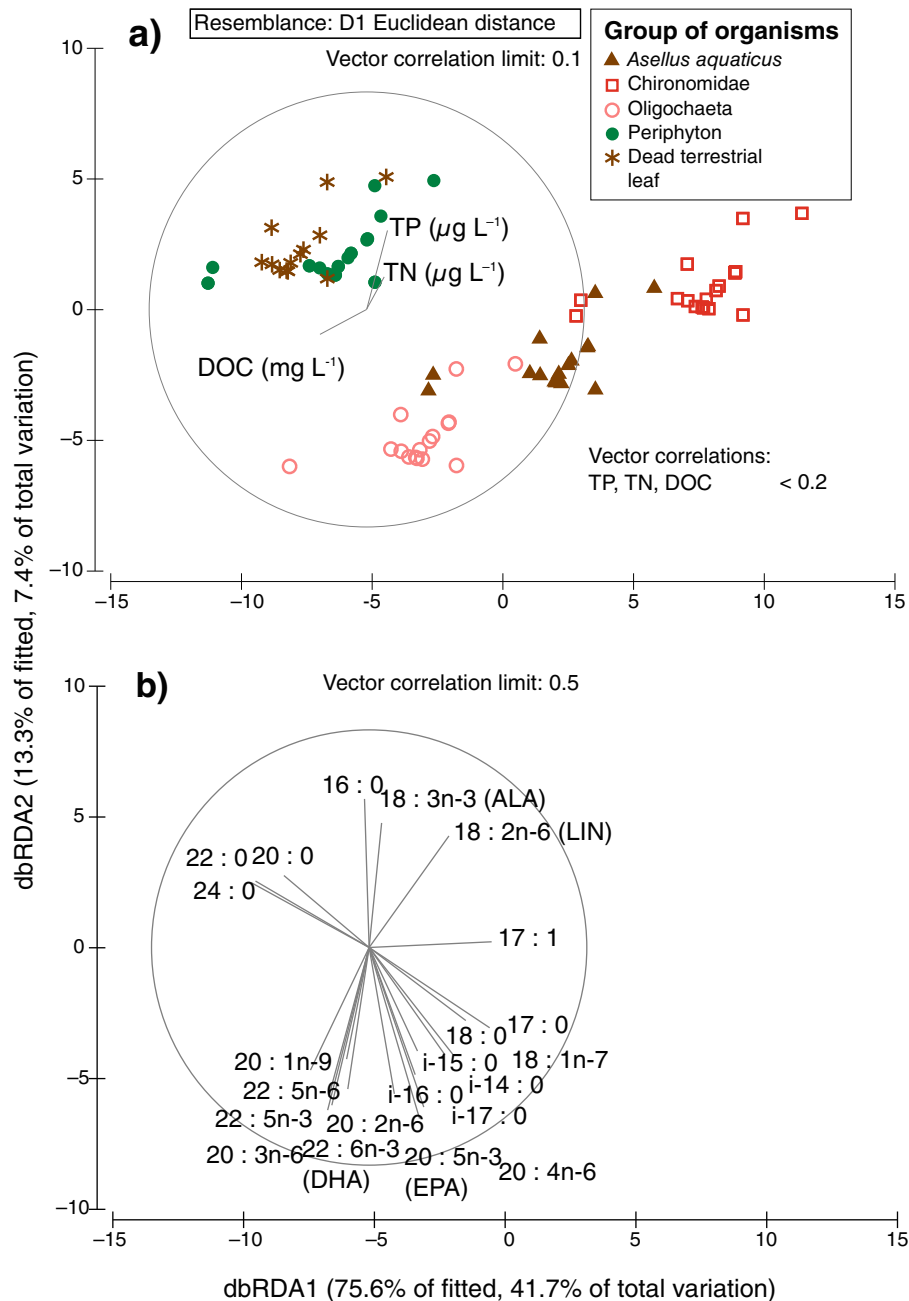
Fig. 2. dbRDA ordination describing the relationship between multivariate FA data of the macroinvertebrates and their food sources and water quality parameters of the lakes. Water quality parameters, TP, TN, and DOC concentration, representing the vectors in (**a**), had < 0.2 correlation with the axes, whereas the vector fatty acids in (**b**) had ≥ 0.5 correlation with the axes.

Fig. 3g–i). In periphyton (Fig. 3j–l), there was a significant positive correlation between TP concentrations and total fatty acid content (Pearson's $r = 0.45$, $t = 2.32$, $p < 0.05$) as well as between TP concentrations and EPA content (Pearson's $r = 0.48$, $t = 2.53$, $p < 0.05$). Moreover, in periphyton BCFA content was significantly correlated with TP (Pearson's $r = 0.63$, $t = 3.36$, $p < 0.01$) and DOC concentrations (Pearson's $r = 0.55$, $t = 3.01$, $p < 0.01$).

δ^{13}C values of LIN, ALA, and EPA of macroinvertebrates and their food sources

Values of $\delta^{13}C_{LIN}$ in *Asellus* ranged from -22.9‰ to -40.2‰ between clear oligotrophic Lake Kuorinka and Lake Haapajärvi with high DOC concentrations. Similarly, the $\delta^{13}C_{ALA}$ and $\delta^{13}C_{EPA}$ in *Asellus* were more enriched in ^{13}C in clear-water lakes (from -29.3 to -16.0‰) than in lakes with high DOC concentrations (from -44.1 to -27.4‰;
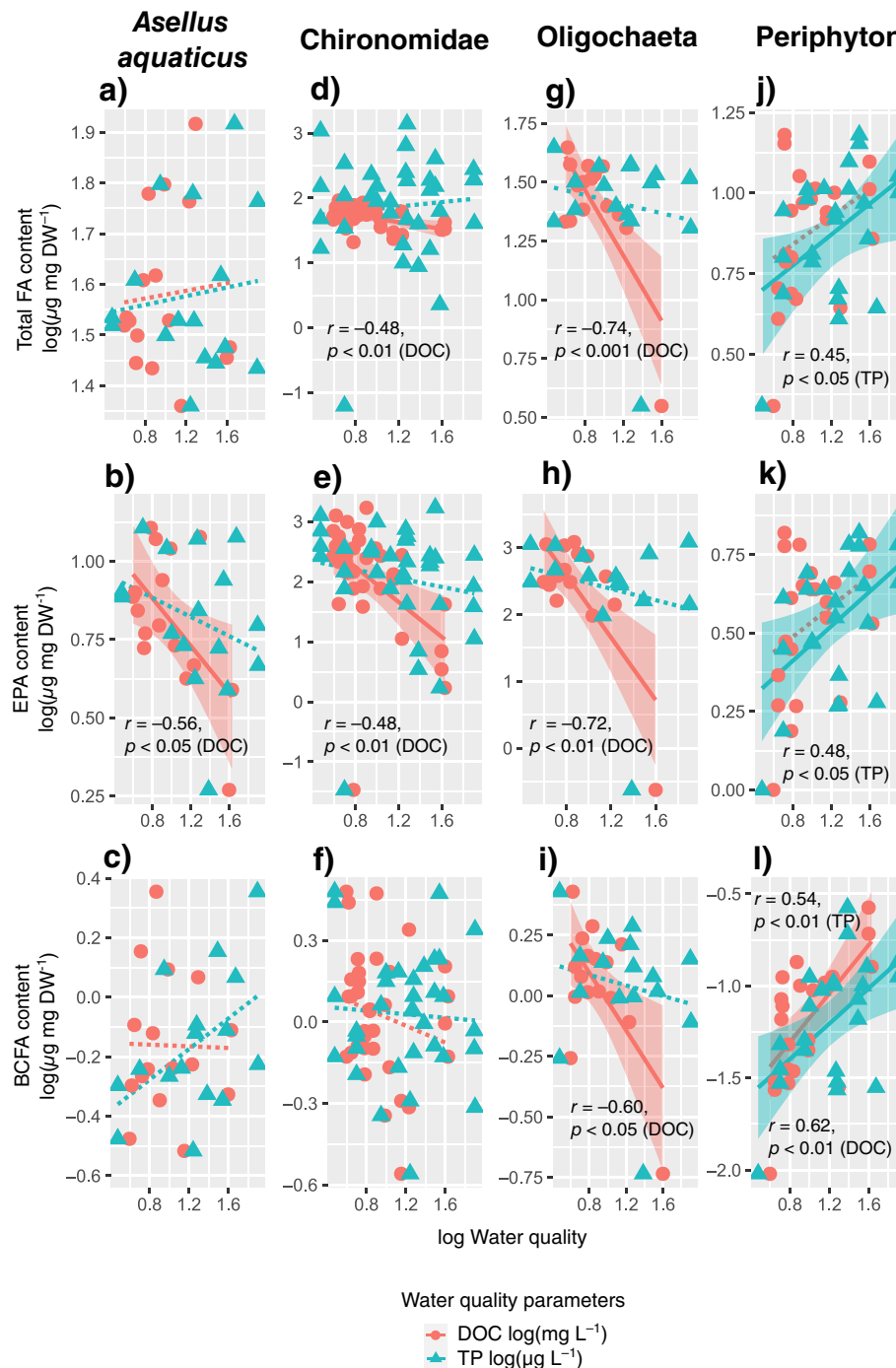
Fig. 3. Total fatty acid, EPA and BCFA content (μg fatty acid mg dry weight^{-1} [DW]) of macroinvertebrates and periphyton related to lake DOC and TP concentrations (Pearson's correlation). Total fatty acid content is the sum of all the identified fatty acids, and BCFA content is the sum of all the odd-chain, saturated fatty acids including their *iso-* and *anteiso-*branched homologs. Data are log-transformed and only significant correlations (straight lines and *r* values) are presented. The bands represent 95% confidence intervals.

Supporting Information Table S2). Nevertheless, highly negative δ^{13}C values of *Asellus* LIN, ALA, and EPA were found in clear-water groundwater-fed Lake Syrjänalunen (Supporting Information Table S3). Chironomids also showed similar patterns in the δ^{13}C values of LIN, ALA, and

EPA as *Asellus*; with less negative values in clear water compared to more stained lakes, except Lake Syrjänalunen (Supporting Information Table S3). Similar patterns were also seen in oligochaetes δ^{13}C values (Supporting Information Table S3).
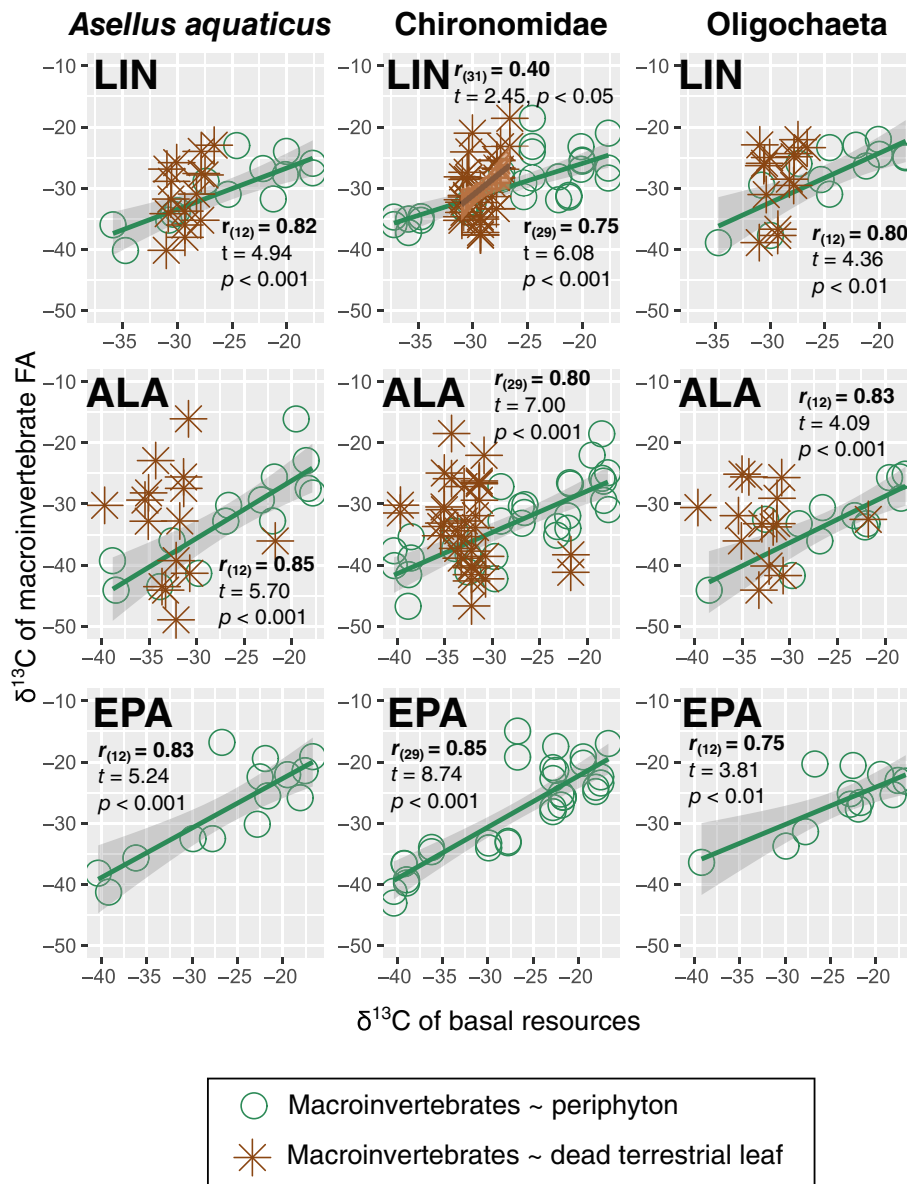
Fig. 4. Pearson's correlation between the macroinvertebrate (dependent variable) and periphyton and terrestrial leaf litter (independent variables) $\delta^{13}C$ of LIN ($\delta^{13}C_{LIN}$), ALA ($\delta^{13}C_{ALA}$), and EPA ($\delta^{13}C_{EPA}$). Only significant correlation coefficients are presented. The bands represent 95% confidence intervals. The number of observations for Chironomidae is larger due to combination of green and red specimens.

The $\delta^{13}C$ values of LIN, ALA, and EPA in periphyton showed similar patterns as the macroinvertebrates with more enriched in ^{13}C in clear water compared to lakes with high DOC concentrations (Supporting Information Table S3). Unfortunately, periphyton samples from the groundwater-fed Lake Syrjänalunen were lost during the sample processing. In dead leaves onshore, the variation in $\delta^{13}C$ values of fatty acids was lower compared to macroinvertebrates and periphyton (Supporting Information Table S3). The mean ($\pm$ SD) $\delta^{13}C_{LIN}$ values in dead leaves were isotopically enriched (-29.3 ± 1.4‰) than $\delta^{13}C_{ALA}$ (-33.2 ± 2.2‰). There was no EPA detected in dead leaves.

In all three macroinvertebrate taxa, $\delta^{13}C_{LIN}$ was strongly correlated with that in periphyton (Pearson's $r = 0.75$–0.82, $p < 0.01$). Only in chironomids, $\delta^{13}C_{LIN}$ was also significantly correlated with that in terrestrial leaves (Pearson's $r = 0.4$, $p < 0.05$, Fig. 4), whereas in the other taxa the relationship was weak. Also, strong, significant positive correlations (Pearson's $r = 0.80$–0.85, $p < 0.001$) were found between the $\delta^{13}C_{ALA}$ of macroinvertebrates and periphyton (Fig. 4), but there was no significant relationship between the $\delta^{13}C_{ALA}$ of dead leaves and the three invertebrate taxa. Similarly, there was a strong, significant correlation (Pearson's $r = 0.75$–0.85, $p < 0.01$) between the $\delta^{13}C_{EPA}$ of macroinvertebrates and periphyton (Fig. 4).

Discussion

This field study demonstrates that the fatty acid composition of the tested benthic invertebrates, *Asellus*, chironomids, and oligochaetes, are primarily taxon-specific despite the highly variable environmental conditions of these study lakes. Moreover, our compound-specific stable isotope results indicate that macroinvertebrates obtained their physiologically essential fatty acid, EPA, from algal-based food rather than bioconversion from precursors in terrestrial leaf material, emphasizing the importance of periphyton in these lakes. The responses of total fatty acid, EPA, and BCFA content to the selected environmental drivers were variable and differed among taxa. In general, the increase in the lake DOC concentration was associated with a lower EPA content in all of the three macroinvertebrate taxa, and also the total fatty acid content of chironomids and oligochaetes was lower in lakes with higher DOC concentrations, thus partly supporting our hypothesis. Yet, an increase in TP concentration had no similar effect on the fatty acid content of macroinvertebrates as we hypothesized. Instead, the periphyton total fatty acid, EPA and BCFA content increased along the TP gradient. Also, increasing BCFA content in periphyton coincided with lake DOC and TP concentrations, but DOC and TP concentrations had no relationship with macroinvertebrate BCFA contents. This indicates periphyton presumably benefitted more from high nutrient concentrations than was constrained by poor light conditions. Also, the increased BCFA content in the periphyton may be the result of increased overall productivity and decomposition within the periphytic mats.

There are two potential pathways for EPA in macroinvertebrates: directly from their algal diet or by elongating and desaturating dietary ALA, which is also found in terrestrial leaves. In these boreal lakes, the $\delta^{13}C_{LIN}$, $\delta^{13}C_{ALA}$, and $\delta^{13}C_{EPA}$ values of macroinvertebrates were positively related to periphyton values, whereas macroinvertebrate $\delta^{13}C_{LIN}$ and $\delta^{13}C_{ALA}$ values were moderately or poorly related to those in dead terrestrial leaves, indicating that littoral macroinvertebrates received the majority of their LIN, ALA, and EPA from periphytic algae in all studied lake types. However, some of the LIN in macroinvertebrates may have originated from terrestrial sources, although in our study, only in chironomids the $\delta^{13}C_{LIN}$ value was significantly correlated with that in terrestrial LIN. This may be the result of a larger sample size and also a narrower confidence interval among chironomids. There is experimental evidence (Goedkoop et al. 2007; Strandberg et al. 2020) that *Chironomus riparius* can synthesize ARA and EPA from precursor fatty acids and sustain viable populations in the presence of poor-quality food. This capacity probably differs among species and may, hypothetically, be well-developed with species such as *C. riparius*, which are adapted to low-oxygenated and poorly lit environments, where the access to dietary ALA and EPA is limited. Strandberg et al. (2020) and Goedkoop et al. (2007) also demonstrate remarkably higher growth and viability of *C.*

riparius when algal-based food was available. Similarly, Grieve and Lau (2018) reported higher somatic growth of *Asellus aquaticus* when they were fed with both leaf litter and algae, and, surprisingly, when leaf litter was predominant. Altogether, these studies emphasize the importance of algal-based food for macroinvertebrates in various lakes with different relative proportions of allochthonous and autochthonous resources. Our study supports previous findings, suggesting that periphyton contributes strongly to macroinvertebrate diets even in highly humic lakes (Lau et al. 2014b; Vesterinen et al. 2016b). Mixing-model results based on bulk stable carbon, nitrogen, and/or hydrogen isotopes, suggest that in many brown-water lakes the proportion of terrestrial resources could be high in consumer diets (Cole et al. 2011; Karlsson et al. 2015; Tanentzap et al. 2017), but experimental studies have shown that consumers poorly assimilate terrestrial organic matter and they always need algal-origin PUFA for supporting somatic growth and reproduction (Brett et al. 2009; Taipale et al. 2014; McMeans et al. 2015).

The pattern of decreasing fatty acid-specific $\delta^{13}C$ values of periphyton and macroinvertebrates with increasing lake DOC concentration corresponds with that observed in the bulk $\delta^{13}C$ values of pelagic particulate organic matter (mainly phytoplankton) and zooplankton (del Giorgio and France 1996; Jones et al. 1999; Karlsson et al. 2003). This widely observed trend is explained to result from bacterial respiration using allochthonous DOC as their carbon source, uptake of this depleted dissolved inorganic carbon (DIC) by phytoplankton, which is then grazed by zooplankton (Jones et al. 1999; Lennon et al. 2006). We found rather negative $\delta^{13}C$ values of LIN, ALA, and EPA also in periphyton, which can be interpreted to be due to uptake of heterotrophically respired DIC and the subsequent synthesis of n-3 and n-6 PUFA by periphyton (Wetzel 1993; Nakano 1996). Alternatively, isotopic fractionation may be greater between intracellular environment and cell matrix when periphyton growth rate is low due to reduced light availability, thus, affecting the $\delta^{13}C$ values of periphyton cells (Finlay 2004; Hill and Middleton 2006; Hill et al. 2008). Although not measured, potentially very low periphyton production in the dystrophic lakes in our study may explain the low $\delta^{13}C_{FA}$ values. Vesterinen et al. (2017) reported low periphyton primary production in Lake Horkkajärvi, the most stained study lake. A possibility that macroinvertebrates could get high EPA and DHA contents from bacterial-dominated heterotrophic biofilms or methane-oxidizing bacteria is very unlikely since these bacteria do not contain n-3 or n-6 PUFA (Taipale et al. 2012; Brett et al. 2017). Therefore, it is obvious, as supported by our data, that LIN, ALA, and EPA in macroinvertebrates are mostly of algal origin. However, the more depleted $\delta^{13}C_{FA}$ values of periphyton in the lakes of high DOC concentrations compared with the clear-water lakes may indicate that recycling of heterotrophically respired allochthonous carbon and subsequently taken up by periphyton may be an alternative nutritional carbon pathway.

When considering the effect of lake eutrophication and browning on the fatty acid content of macroinvertebrates, an important aspect is the availability and transfer of fatty acids, especially EPA and DHA, to higher trophic levels. The pool of EPA and DHA in the littoral habitats is largely affected by the magnitude of periphyton primary production, which can be strongly suppressed by shading of phytoplankton in eutrophic lakes or increased browning effect by terrestrial DOC (Vadeboncoeur and Lodge 2000; Vadeboncoeur et al. 2003; Ask et al. 2009). Eutrophication and browning both can severely reduce macroinvertebrate production (Jones et al. 2014), but also fish production in lakes (Karlsson et al. 2009). However, the effects of increased nutrient and DOC concentrations on consumer production are complex and habitat-specific (Jones et al. 2014). For example, Vesterinen et al. (2016a, 2017) found rather highly productive periphyton attached to floating-leaved or submerged vegetation in highly humic lakes, indicating that periphyton contributed strongly to macroinvertebrate diets (Vesterinen et al. 2016b). In this study, we did not measure periphyton and macroinvertebrate biomass and production, as the samples were collected for diet quality purposes. However, a strong productivity gradient along the lakes from highly oligotrophic to highly humic and eutrophic can be assumed. It appears that fatty acid content and composition of macroinvertebrates, although being rather taxon-specific, are also affected by environmental characteristics, such as DOC concentrations.

It is important to consider that eutrophication and browning can both shape the community structures of benthic macroinvertebrates and favor different feeding habits (Vander Zanden and Vadeboncoeur 2020; Kesti et al. 2021). In our study, oligochaetes were less frequent in the humic lakes than in the other types of lakes. They were particularly missing from steep-sided, strongly stratified, and highly humic lakes Majajärvi and Horkkajärvi. In such lakes, both periphyton and macroinvertebrate production is limited to a narrow margin and within the aquatic vegetation, where several macroinvertebrate taxa, including chironomids and *Asellus*, can succeed (Kairesalo et al. 1992; Vesterinen et al. 2016b, 2017). Oligochaetes, however, are commonly found in benthic sediments or other relatively flat surfaces (Särkkä and Aho 1980; Särkkä 1982). Potential ineptitude to attach to macrophyte or bryophyte substrata may explain why oligochaetes were not found in the lakes most rich in DOC. Generally, Oligochaeta species seem to cope with eutrophication, darker water color, and low oxygen concentrations found in Finland (Särkkä and Aho 1980), but their surprisingly high EPA content in our study lakes is a strong indication of algal-based diet and/or efficient bioconversion ability.

Due to the fact, that eutrophication and browning shape the macroinvertebrate community structures (Kesti et al. 2021), there are probably marked differences in the species composition within our study lake set. This is likely to have had an impact on the results when comparing taxa at the level of family or subclass (Chironomidae and Oligochaeta). Also, chironomids and oligochaetes may have included both detritivore and predatory taxa, having a closer or more distant trophic link to algae. This, however, does not have a significant impact on the interpretation of the compound-specific stable isotope results because the $\delta^{13}C_{FA}$ values of macroinvertebrates and periphyton were closely related to each other. The fact that there were such clear correlations, despite the lower taxonomic resolution, indicates that these are ecologically highly relevant results. If there were more distant trophic links between algae and some macroinvertebrate species, this would indicate that these essential fatty acids pass through the food chains unmodified. However, a more accurate taxonomic resolution is recommended in similar studies in the future, since this could probably also reveal more specific and stronger fatty acid relationships between certain macroinvertebrate taxa and their food sources.

In conclusion, while the fatty acid compositions of benthic macroinvertebrates were mostly taxon-specific and not strongly influenced by physico-chemical characteristics of lakes, the total fatty acid and EPA contents were affected by water quality. Although this study did not report on biomass of periphyton and macroinvertebrates across this lake gradient, it indicates that the macroinvertebrate production in lakes may be partly limited by algal-derived PUFA, specifically EPA. This field study supports the recent findings that even the most stained lakes may have remarkable periphyton production contributing to macroinvertebrate diets.

Data availability statement

The authors confirm that the data supporting the findings of this study are available within the article its supplementary materials.

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Conflict of Interest

None declared.