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Lake browning impacts community structure and essential fatty acid content of littoral invertebrates in boreal lakes

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Abstract Many lakes in the northern hemisphere are browning due to increasing concentrations of terrestrial dissolved organic carbon (DOC). The consequences of lake browning to littoral invertebrates, however, are not fully understood. We analyzed community structure and fatty acid (FA) profiles of littoral invertebrates in humic (DOC-rich) and clear-water lakes in Eastern Finland. We found higher abundance of chironomids (Diptera: Chironomidae) in humic compared to clear-water lakes, whereas stoneflies (Plecoptera) and mayflies (Ephemeroptera: Baetidae) were more abundant in clear-water lakes. Taxon explained 65% of the differences in the FA composition of littoral invertebrates. However, the proportion and content of polyunsaturated FAs of several taxa were significantly higher in clear-water lakes compared to humic lakes. Our results reveal

differences in both community structure and nutritional quality of littoral invertebrates for fish between humic and clear-water lakes.

Keywords Polyunsaturated fatty acids · Benthic invertebrates · Terrestrial organic matter · *Asellus aquaticus* · Chironomidae

Introduction

Lake browning, due to increased concentrations of terrestrial dissolved organic carbon (DOC) and iron, has been observed in many regions in the northern hemisphere during the recent decades (e.g., Monteith et al., 2007; Weyhenmeyer et al., 2014). This phenomenon has been related to catchment soil recovery from acidification, climate change, increased precipitation and runoff, and shortened soil frost period (e.g., Hongve et al., 2004; Lepistö et al., 2014; Kritzberg, 2017). Together with anthropogenic loads of terrestrial-derived detritus via intensified land-use practices, e.g., clear-felling of forests from peatland catchments, the load of DOC is expected to rise in the future (e.g., Nieminen et al., 2015; Hayden et al., 2017).

Lake browning has many ecological consequences on lake food webs, especially via impairing the light regime for primary producers (Thrane et al., 2014;

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Bergström & Karlsson, 2019). In addition to e.g., lake size and habitat richness, environmental factors, such as water temperature, oxygen concentration, and lake trophic state, have been shown to affect the invertebrate community structure and the availability of their food sources (Lau et al., 2013; Jyväsjärvi et al., 2014; Gladyshev et al., 2016). Brown lakes (referred hereafter as humic lakes) differ from clear-water lakes in both physical and chemical characteristics. Humic lakes are generally more acidic and can suffer from wintertime oxygen deficiency (Luoto, 2013). Acidity is one of the factors affecting the littoral invertebrate assemblages. Phantom midges (Diptera: Chaoboridae) are characteristic in strongly acidic lakes, whereas gammarids (Amphipoda: Gammaridae) and some mayflies (Ephemeroptera) are limited to weakly acidic lakes (Walker et al., 1985). Oxygen deficiency is also harmful for e.g., Ephemeroptera and Plecoptera (Fochetti & Tierno De Figueroa, 2008). Some UV-sensitive species, like phantom midges from the genus *Chaoborus*, might benefit from lake browning since it shelters them from excessive UV radiation (Lindholm et al., 2018).

Certain polyunsaturated fatty acids (PUFAs) are considered essential fatty acids (EFAs) for many consumers. Traditionally, α -linolenic acid (ALA, 18:3 ω 3) and linoleic acid (LA, 18:2 ω 6) have been considered the only EFAs since they are the precursors of long-chain PUFAs (LCPUFAs, $\geq C_{20}$ PUFAs). The bioconversion efficiency from ALA to eicosapentaenoic acid (EPA, 20:5 ω 3) and docosahexaenoic acid (DHA, 22:6 ω 3) is not necessarily adequate in consumers, and thus EPA and DHA can be considered as the physiologically most EFAs (Sargent et al., 1995). Arachidonic acid (ARA, 20:4 ω 6) is important in the development of fish eggs (Sargent et al., 1999) but its importance for littoral invertebrates is unclear (Ahlgren et al., 2009). In addition to individual EFAs, the ω 3/ ω 6 ratio in consumers may be important (Ahlgren et al., 2009; Arts & Kohler, 2009), and the latter is also used to identify allochthonous vs. autochthonous food sources (Torres-Ruiz et al., 2007; Guo et al., 2016).

In aquatic ecosystems, EFAs are mainly synthesized by algae. Different algal communities have characteristic FA compositions, and EPA and DHA are synthesized by specific algal taxa. Chlorophytes contain high amounts of ALA and LA (Taipale et al., 2013), whereas diatoms, cryptophytes, and chrysophytes synthesize EPA and dinoflagellates DHA

(Ahlgren et al., 1990; Taipale et al., 2013; Galloway & Winder, 2015). Terrestrial material, e.g., plant leaves, contain mainly saturated fatty acids (SAFAs), especially $> C_{22}$ SAFAs (Brett et al., 2009; Bec et al., 2010; Taipale et al., 2014). Impacts of lake browning on EFA production in aquatic ecosystems are mostly related to the variation in algal community structure (Gutseit et al., 2007; Taipale et al., 2016; Strandberg et al., 2020b). Moreover, climate change induced increase in annual water temperature and duration of growing season are predicted to benefit cyanobacteria (Anneville et al., 2015; Senar et al., 2021), which are not a good-quality food source for consumers in terms of EFAs (Müller-Navarra et al., 2000; Martin-Creuzburg et al., 2008). Additionally, lake browning increases bacterial activity and shifts the dominance of primary production from benthic to pelagic zone via decreased light penetration (Ask et al., 2012; Vasconcelos et al., 2019).

Recently, our knowledge on FA profiles of benthic invertebrates in the northern hemisphere has increased markedly (Sushchik et al., 2003; Makhutova et al., 2011, 2016; Lau et al., 2012, 2013; Vesterinen et al., 2020). FA composition seems to be quite conserved in littoral invertebrate taxa, and phylogeny is the most important factor determining it (e.g., Sushchik et al., 2003; Makhutova et al., 2011; Lau et al., 2012; Vesterinen et al., 2020). Many littoral invertebrates are important food sources for fish (Sushchik et al., 2003; Makhutova et al., 2011). Reported favorable food sources include larvae of black flies (Diptera: Simuliidae), caddisflies (Trichoptera), and Chironomidae, and the nymphs of Plecoptera and Ephemeroptera (Khrennikov et al., 2007; Shustov et al., 2012).

In this study, we analyzed littoral invertebrate (referred hereafter as invertebrate) community data from a total of 26 lakes in Eastern Finland to investigate potential differences in the community structure of invertebrates between humic and clear-water lakes. Additionally, we analyzed the FA composition of common invertebrates from 10 lakes in Eastern Finland differing in DOC concentration. Our aim was to study the effects of lake type (humic vs. clear-water) and littoral shore type (vegetated vs. unvegetated) on the FA composition and content of invertebrates.

We hypothesized that the invertebrate communities differ between humic and clear-water lakes because of

differences in physical and chemical factors (e.g., DOC concentration, pH) and biological factors (e.g., algal community structure) between lake types. Secondly, we hypothesized that invertebrates inhabiting humic lakes have a lower PUFA content compared to the ones inhabiting clear-water lakes. Our third hypothesis was that invertebrates differ in their FA composition depending on the type of littoral shore they inhabit since different shore types harbor varying food sources (Smock & Harlowe, 1983; Jones et al., 1998).

Materials and methods

Invertebrate community analyses

We retrieved data on invertebrate community structure from the Finnish Environment Institute (SYKE) HERTTA-database (www.syke.fi/avoindata). The data has been collected as a part of monitoring for the Water Framework Directive (WFD) by the Centre for Economic Development, Transport, and the Environment (ELY Centre) of North Karelia. We retrieved data for a total of 26 lakes located in Eastern Finland (16 humic and 10 clear-water lakes). We selected our lakes based on WFD lake type (humus-rich and humus-poor lakes), size ($\geq 1 \text{ km}^2$), and sampling method (handnet). Clear-water lakes had low concentrations of total organic carbon (TOC), total nitrogen (TN), and total phosphorus (TP) (range, TOC = 2.9–8.9 mg l^{-1} , TN = 160.0–461.0 $\mu\text{g l}^{-1}$, TP = 2.1–9.4 $\mu\text{g l}^{-1}$) whereas humic lakes had higher TOC, TN, and TP (range, TOC = 12.5–25.0 mg l^{-1} , TN = 314.9–768.2 $\mu\text{g l}^{-1}$, TP = 9.2–40.0 $\mu\text{g l}^{-1}$), respectively. Eutrophic lakes (TP > 50 $\mu\text{g l}^{-1}$) were excluded from the analyses. Invertebrate data was retrieved from the database in December 2018, and physical and chemical characteristics were retrieved in November 2019. Lake characteristics are presented in Supplementary Information (SI), Table S1. We compared the average abundance of different invertebrate taxa between the lake types. Only those taxa with an average abundance of $\geq 0.5\%$ of the total invertebrates in either of the lake types were included.

Lakes for the fatty acid analyses

Terrestrial DOC influence on FA composition and content in different food web compartments was studied in 10 lakes in the same region studied for the invertebrate community composition (see above). The selected lakes consisted of four clear-water lakes with low concentrations of DOC, TN, and TP (range, DOC = 2.8–7.9 mg l^{-1} , TN = 169.3–365.3 $\mu\text{g l}^{-1}$, and TP = 1.6–4.9 $\mu\text{g l}^{-1}$), and six humic lakes with higher concentrations of DOC, TN, and TP (range, DOC = 11.9–18.7 mg l^{-1} , TN = 285.5–629.2 $\mu\text{g l}^{-1}$, and TP = 11.1–28.8 $\mu\text{g l}^{-1}$), respectively. Lake characteristics are presented in SI, Table S2. For more detailed description for catchment characteristics, lake water chemistry and for results on phytoplankton and predatory fish see Strandberg et al. (2016, 2020b).

Invertebrate sampling and selection

Invertebrates were collected with a standardized kick-net sampling method from two different shore types, vegetated and unvegetated, in July–September 2013. Coordinates of the sampling sites are presented in SI, Table S3. Invertebrates were collected on a 0.5 mm mesh-sized sieve in the field and transferred to buckets. Afterwards the samples were sorted in the laboratory and stored in Eppendorf tubes at $-18 \text{ }^\circ\text{C}$ until further analyses in 2017. Identification of the taxa was done to the genus or species level, when possible. In the laboratory, samples were briefly thawed, rinsed with MilliQ, and frozen and freeze-dried with Christ ALPHA 1-4 LDplus (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode, Germany). Seven different taxa were selected for the FA analyses (SI, Table S4): water mites (Acari: Hydracarina), spread-winged damselflies (Zygoptera: *Lestes*), Ephemeroptera (*Heptagenia*), Chironomidae, cladocera (Cladocera: *Sida*), *Asellus* (Crustacea: *Asellus aquaticus*; Linnaeus, 1758, referred hereafter as *Asellus*), and amphipods (Amphipoda: *Pallasea quadrispinosa*; Sars, 1867, referred hereafter as *Pallasea*), present only in clear-water lake samples. When present in the samples, 1–4 subsamples of each taxa were analyzed for both shore types in each lake, consisting of several individuals (2–20) to get the desired sample weight (1–2 mg dry weight). Mean values for specific taxa in each lake and/or shore type were used in statistical

analyses. The invertebrates selected for the FA analyses represent different functional feeding guilds. Hydracarina is a taxonomically diverse group, and their adult forms are mainly predatory (Di Sabatino et al., 2000). *Lestes*, like other dragonfly larvae, are also predators. Chironomids are opportunistic feeders, many are detritivores, ingesting different kinds of particles from the sediments, but a wide variety of feeding guilds are found in this family (Reuss et al., 2013). A previous study has indicated that the community structure of Chironomidae differs between the clear-water and humic lakes (Luoto, 2013), which is also reflected in the feeding guilds (Kivilä et al., 2019). In our study, it was necessary to pool several chironomid individuals for FA analyses, and thus, these could be identified only at family level. Heptageniidae are classified as collectors/scrapers, and they utilize surface periphyton as their main food source (Cummins, 1973), but species from the genus *Heptagenia* perform mixed feeding, categorizing them as shredders/grazers. *Asellus* are shredders/grazers who utilize detritus and epilithic algae (Moog, 2002), but they are also generalists (Lau et al., 2013). *Sida* are filter feeders, who attach to the surfaces of aquatic vascular plants (Fairchild, 1981). *Pallasea* are omnivorous, feeding on both algae and smaller invertebrates (Hill, 1988).

Fatty acid analysis

Freeze-dried invertebrates were pulverized with mortar and pestle and 1–2 mg of pulverized tissue were weighed for the FA extraction. The extraction was done by extracting the lipids twice with a 2:1 (volume) chloroform:methanol following Folch et al. (1957). For gas chromatography (GC), the FAs were turned into FA methyl esters (FAMES) using an acid-catalyzed transesterification reaction with 1% H₂SO₄ in methanol (Christie, 1993). FAMES were dissolved in hexane and analyzed with a gas chromatograph (Agilent 6890N, Agilent Technologies, Wilmington, DE, USA) equipped with a mass selective spectrometer (Agilent 5973N). The column we used was an Agilent DB-23 (0.25 mm × 30 m × 0.25 μm). Helium was used as a carrier gas and the flow rate was 37 cm s⁻¹. The GC temperature program was as follows: The starting temperature was 50 °C for 1 min, after which raised from 50 to 150 °C at 15 °C min⁻¹, then to 190 °C at 1.5 °C min⁻¹, then

to 210 °C at 2 °C min⁻¹. The final temperature was held for 15 min. The total running time for samples was 59.33 min. SAFA 21:0 (heneicosanoic acid) (Nu-Chek Prep., Elysian, MN, USA) was used as an internal standard, and GLC-68E (Nu-Chek Prep.) was used for the calibration. Mass spectra and GLC-68E were used for the identification of FAMES.

Statistical analyses

Non-metric multidimensional scaling (NMDS) was used to visualize the variation in invertebrate community structure between humic and clear-water lakes and the differences in the FA composition between invertebrates according to three factors: species identity (taxon), lake type (humic vs. clear-water), and shore type (vegetated vs. unvegetated). NMDS ordinations were based on Euclidean distance. We used stress values to describe how well the ordination described our original data. Stress values < 0.2 were considered acceptable, whereas stress values > 0.2 were considered random ordinations (Clarke, 1993). Abundances of invertebrate taxa in a lake community and the FA proportions of invertebrates were arcsine square-root transformed. We performed permutational multivariate analysis of variance (PERMANOVA) to investigate the effect of lake type on the invertebrate community structure. Lake-type was treated as fixed factor, with two levels: clear-water and humic lakes. Additionally, we used PERMANOVA to analyze the taxon, lake type-, and shore type-specific differences in the FA composition of invertebrates. Taxon and lake type were treated as fixed factors. Taxon had seven levels, i.e., taxonomic groups: Hydracarina, *Heptagenia*, Chironomidae, *Lestes*, *Sida*, *Asellus*, and *Pallasea*. Lake-type had two levels: humic and clear-water lakes. Shore-type was nested in lake type. We ran an unconstructed permutation of raw data using type III sum of squares. We also ran a pair-wise test to investigate the differences for each taxon between lake types and shore types and possible interactions. Similarity percentage (SIMPER) was used to see more specifically which FAs were responsible for the differences in the FA composition.

We further investigated the effects of specific environmental factors on the invertebrate FA composition. We used distance-based linear modeling (DistLM) to evaluate how much of the variation in the FA composition of invertebrates could be

explained by different lake characteristics. These characteristics included e.g., lake area, mean depth, concentrations of Chl *a*, TP, TN, and DOC, and percentage of peatland in the lake catchment area. A full list of the analyzed lake characteristics for the FA composition is presented in SI, Table S2. We used a stepwise selection of environmental factors with adjusted R^2 as model selection criteria.

We also compared the proportion and content of FAs between humic and clear-water lakes in our invertebrates. In addition, we calculated the $\omega 3/\omega 6$ and DHA/ARA ratio of invertebrates, as their optimal ratios are important for normal growth and development of invertebrates (Makhutova et al., 2011). We used Student's *t*-test and ANOVA (Tukey post hoc test) to test the differences between FA composition, and $\omega 3/\omega 6$ and DHA/ARA ratio of invertebrates. NMDS, PERMANOVA, SIMPER, and DistLM analyzes were done with Primer 6 and Permanova+ program package (PRIMER-E Ltd, Plymouth, UK). Other statistical analyzes were done with IBM SPSS Statistics 25 program package (IBM Corp., Armonk, New York, USA).

Results

Invertebrate community structure

The most common invertebrates in humic lakes were Chironomidae, Asellidae, and Ephemeroptera (Leptophlebiidae), and in clear-water lakes Asellidae, Coleoptera (Elmidae), and Chironomidae, respectively (SI, Table S5). We found a significant difference in the invertebrate community structure between the two lake types at family level (PERMANOVA, Pseudo- $F = 2.4013$, $p < 0.01$) (Fig. 1). Taxa contributing the most to the differences between the two lake types were Asellidae (21%), Chironomidae (15%), and Leptophlebiidae (10%) (SIMPER). Taxa and their contribution to the differences between the two lake types are listed in Table 1. When relative abundances were compared between the lake types, Chironomidae were significantly more abundant in humic lakes compared to clear-water lakes (*t*-test, $t = 2.773$, $df = 24$, $P < 0.05$), whereas Plecoptera as a whole (*t*-test, $t = -2.263$, $df = 24$, $P < 0.05$), Ephemeroptera (Baetidae: $t = -3.943$, $df = 24$, $P < 0.05$), and Plecoptera (Capniidae: $t = -2.162$,

$df = 24$, $P < 0.05$) were significantly more abundant in clear-water lakes compared to humic lakes. We also found that certain families from Amphipoda (Pallaseidae) and Trichoptera (Hydropsychidae) were completely absent from humic lakes (SI, Table S5).

Fatty acid composition of invertebrates

We identified a total of 61 different FAs in our invertebrate samples from 10 lakes. FAs with an average proportion of $\geq 0.5\%$ of total FAs in at least one of the taxa are presented in Table 2. *Pallasea* had a significantly higher DHA/ARA ratio and proportion of DHA (Tukey post hoc, $P < 0.001$) compared to the other taxa (Table 2). There were also significant differences in the overall FA composition between invertebrates (PERMANOVA, Pseudo- $F = 33.762$, $P < 0.001$) (Fig. 2). Taxa accounted for 65% of the differences in the FA composition of invertebrates (PERMANOVA).

Effects of lake characteristics on FA composition of invertebrates

Lake type significantly affected the FA composition of Chironomidae ($t = 2.1696$, $P < 0.01$), *Sida* ($t = 2.4778$, $P < 0.05$), and *Asellus* ($t = 4.6496$, $P = 0.0001$) (PERMANOVA pair-wise tests). Lake type alone accounted for only 5% of the differences in the FA composition of invertebrates, and lake type and taxa in combination accounted for 10% (PERMANOVA). The FAs contributing the most to the differences between the two lake types in our invertebrates were: Hydracarina: LA (18%), Chironomidae: EPA (13%), *Heptagenia*: ALA (17%), *Lestes*: 16:1 ω 7 (19%), *Sida*: DHA (19%), *Asellus*: 16:1 ω 7 (57%) (SIMPER) (Table 3). FAs which contributed at least 10% of the differences in the invertebrate FA composition between humic and clear-water lakes are presented in Table 3. More complete list of the FAs is presented in SI, Table S6, respectively.

In many taxa, the relative proportions of summed monounsaturated FAs (MUFA), SAFA and PUFA differed between the two lake types (Table 2). The proportion of PUFAs was significantly higher in clear-water lakes compared to humic lakes in Chironomidae, *Sida*, *Lestes*, and *Asellus* (*t*-test, Chironomidae: $t = -2.911$, $df = 14$, $P < 0.05$. *Sida*: $t = -3.087$, $df = 8$, $P < 0.05$; *Lestes*: $t = 2.927$, $df = 18$,

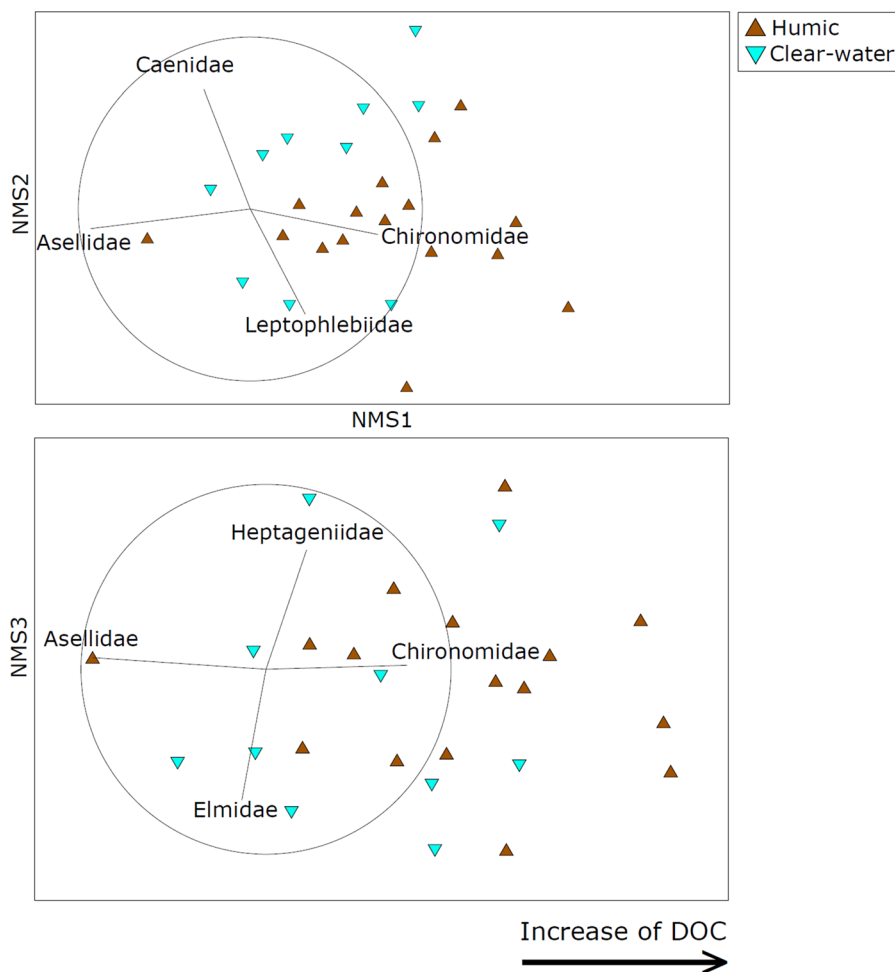


Fig. 1 Non-metric multidimensional scale (NMS) ordinations of the littoral invertebrate community composition in humic and clear-water lakes at the family level. Samples from humic lakes are presented as brown and samples from clear-water lakes as light blue triangles, respectively. The ordinations were based on

Euclidean distance. Littoral invertebrate families that correlate strongly (Pearson $r > 0.6$) with either of the axes are visualized with vectors. The 3D stress value for the ordination was 0.11. The amount of DOC increases towards the right side of the ordinations

$P < 0.01$; *Asellus*, $t = -5.032$, $df = 25$, $P < 0.001$) (Fig. 3). Lake type also had a significant effect on the $\omega 3/\omega 6$ and/or DHA/ARA ratio of these four taxa. The $\omega 3/\omega 6$ (t -test: Chironomidae, $t = -3.745$, $df = 14$, $P < 0.005$; *Lestes*, $t = -3.563$, $df = 18$, $P < 0.005$; *Asellus*, $t = -2.443$, $df = 25$, $P < 0.05$) and the DHA/ARA ratio (t -test: *Sida*, $t = -4.160$, $df = 8$, $P < 0.005$; *Asellus*, $t = -4.162$, $df = 25$, $P < 0.001$) were significantly higher in clear-water lakes (Table 2). We could not compare the FA composition or ratios of *Pallasea* between the two lake types because all samples came from clear-water lakes.

Shore type did not have significant effects on the FA composition of invertebrates, except for *Heptagenia* (PERMANOVA Pseudo- $F = 22.638$, $P < 0.001$). This difference, however, was statistically significant only in humic lakes (PERMANOVA pair-wise tests, $t = 4.9824$, $P < 0.01$). The FAs contributing the most to the differences between the two shore types in humic lakes in *Heptagenia* were ALA (18%), 16:1 ω 7 (14%), and 16:0 (12%) (SIMPER). ALA was generally more common in unvegetated shores, whereas 16:1 ω 7 and 16:0 were more common in vegetated shores.

In combination, different physical and chemical parameters explained 37–92% of the FA composition

Table 1 Proportions and standard deviations of different invertebrate families and their contributions to the dissimilarities of invertebrate communities between humic and clear-water lakes (SIMPER)

Taxa	Proportion (%)		Contribution to dissimilarity (%)	Cumulative contribution (%)
	Humic	Clear-water		
Asellidae	17.0 ± 14.2	22.7 ± 15.8	21	21
Chironomidae	22.2 ± 10.8	9.4 ± 7.4	15	36
Leptophlebiidae	11.9 ± 8.2	6.2 ± 8.2	10	45
Elmidae	5.4 ± 6.0	10.0 ± 8.2	9	54
Heptageniidae	9.6 ± 10.0	6.9 ± 5.6	6	61
Caenidae	4.7 ± 4.0	7.4 ± 5.7	6	66
Ceratopogonidae	4.8 ± 3.7	5.9 ± 4.2	5	72
Capniidae	0.0 ± 0.1	1.9 ± 3.5	4	76
Pallaseidae	0.0 ± 0.0	1.8 ± 2.5	4	79
Corixidae	1.6 ± 4.3	0.3 ± 0.5	3	82
Baetidae	0.2 ± 0.2	1.8 ± 2.2	3	85
Lepidostomatidae	2.1 ± 3.0	1.8 ± 1.3	3	87
Psychomyiidae	1.8 ± 2.1	1.8 ± 1.5	2	90
Limnephilidae	0.6 ± 1.2	1.2 ± 2.1	2	92

of our invertebrates (DistLM). The most important parameters to explain the FAs were: for Hydracarina and Asellus: organic carbon to total nitrogen (C/N) ratio of the lake water (Hydracarina 17%, Asellus 56%), for Chironomidae: Secchi depth (25%), for *Heptagenia*: lake area as percentage of catchment (15%), for *Sida* and *Lestes*: Mean depth of the lake (D_{mean}) (*Sida* 53%, *Lestes* 21%) (DistLM). Statistically significant factors and their proportion explaining the FA composition of invertebrates are presented in Table 4, and a more complete list of the factors is presented in SI, Table S7, respectively.

Effects of lake characteristics on FA content of invertebrates

The total FA content (per dry weight, DW) of invertebrates ranged from 29.5 to 234.9 $\mu\text{g mg DW}^{-1}$. On average, the total FA content ($\mu\text{g mg DW}^{-1}$) was: Hydracarina: 132.2 ± 70.7 , *Heptagenia*: 140.0 ± 42.5 , *Lestes*: 153.4 ± 68.1 , Chironomidae: 72.4 ± 32.1 , *Sida*: 80.7 ± 20.9 , *Asellus*: 132.5 ± 69.4 , and *Pallasea*: 133.8 ± 49.2 . The average FA content of invertebrates in humic and clear-water lakes is presented in Table 2 and lake specific FA content is presented in SI, Table S8, respectively. Lake type significantly influenced the total FA content of *Heptagenia* and *Asellus*. In *Heptagenia*, the FA

content was significantly higher in clear-water lakes (t -test, $t = -2.581$, $df = 14$, $P < 0.05$). In *Asellus* the total FA content was significantly higher in humic lakes (t -test, $t = 3.499$, $df = 25$, $P < 0.005$), which was mainly due to their high MUFA and SAFA content. In Chironomidae and *Sida* the PUFA content was significantly higher in clear-water lakes (t -test, Chironomidae: $t = -2.910$, $df = 14$, $P < 0.05$. *Sida*: $t = -2.965$, $df = 8$, $P < 0.05$) (Table 2).

Discussion

Our results show that lake browning influences both the community structure and the FA composition and content of invertebrates. The invertebrate community structure was different between the two lake types, supporting our first hypothesis. Lake type also affected the FA composition and content of *Asellus*, *Sida*, *Heptagenia*, *Lestes*, and Chironomidae. Shore type, however, had only a small influence on the FA composition of invertebrates.

Invertebrate community structure

Community structure of invertebrates in boreal lakes is shaped by many factors, e.g., lake area, total phosphorous (Heino, 2008) and DOC concentrations

Table 2 The average proportions (% of total FAs), content ($\mu\text{g mg DW}^{-1}$), and standard deviations (\pm) of FAs, and the $\omega 3/\omega 6$ and DHA/ARA ratios of littoral invertebrates in humic and clear-water lakes

SAFAs	Hydracarina		Lestes		Heptagenia		Chironomidae	
	Humic	Clear-water	Humic	Clear-water	Humic	Clear-water	Humic	Clear-water
12:00	1.9 ± 1.9	0.4 ± 0.3	t.d	t.d	0.6 ± 0.5	1.0 ± 0.5	t.d	t.d
14:00	4.7 ± 1.9	3.0 ± 0.5	1.8 ± 0.9	2.1 ± 0.8	2.8 ± 1.6	3.5 ± 0.9	5.0 ± 2.0	3.7 ± 1.0
15:00	t.d	t.d	0.6 ± 0.4	0.4 ± 0.1	t.d	t.d	1.2 ± 0.8	0.3 ± 0.1
16:00	17.9 ± 4.6	16.1 ± 2.4	18.8 ± 4.7	18.8 ± 2.6	26.1 ± 5.4	27.0 ± 3.3	31.1 ± 6.8	21.5 ± 1.5
17:00	1.3 ± 0.7	1.5 ± 0.5	1.4 ± 0.4	1.3 ± 0.4	0.8 ± 0.3	0.7 ± 0.1	2.5 ± 1.1	1.0 ± 0.6
18:00	17.3 ± 5.8	20.3 ± 4.2	9.1 ± 2.5	8.2 ± 1.2	8.7 ± 1.8	7.7 ± 0.5	11.1 ± 2.3	6.9 ± 2.9
20:00	0.8 ± 0.2	0.7 0.1	0.6 ± 0.4	0.6 ± 0.3	0.0	0.0	1.1 ± 0.5	0.9 ± 0.2
22:00	t.d	t.d	t.d	t.d	0.6 ± 0.2	0.4 ± 0.1	t.d	t.d
Total (%)	43.9 ± 9.3	41.9 ± 4.4	32.3 ± 3.8	31.3 ± 2.9	39.7 ± 6.2	40.3 ± 4.7	52.5 ± 10.5	34.4 ± 3.1
SAFA ($\mu\text{g mg DW}^{-1}$)	57.9 ± 21.1	48.8 ± 26.5	52.3 ± 22.4	47.5 ± 22.8	49.4 ± 19.0	70.6 ± 13.5	32.2 ± 9.9	32.8 ± 11.6
MUFAs								
14:1	1.3 ± 1.1	0.3 ± 0.2	t.d	t.d	0.5 ± 0.3	0.7 ± 0.1	0.3 ± 0.7	1.4 ± 0.6
16:1 ω 9	t.d	t.d	t.d	t.d	t.d	t.d	0.4 ± 0.2	0.6 ± 0.2
16:1 ω 7	16.0 ± 4.4	13.3 ± 3.8	9.1 ± 3.5	6.6 ± 2.0	18.8 ± 5.0	17.9 ± 0.5	9.4 ± 3.0	12.0 ± 4.2
16:1 ω 5	t.d	t.d	t.d	t.d	0.6 ± 0.3	0.7 ± 0.3	0.5 ± 0.3	0.5 ± 0.2
18:1 ω 9	15.5 ± 3.7	15.5 ± 4.3	10.5 ± 1.8	10.4 ± 0.7	7.2 ± 1.2	9.2 ± 0.5	10.6 ± 2.6	9.0 ± 2.2
18:1 ω 7	5.3 ± 1.1	6.1 ± 1.4	12.3 ± 2.7	11.1 ± 1.1	13.5 ± 2.9	13.7 ± 0.6	4.6 ± 2.3	3.7 ± 1.7
Total (%)	38.1 ± 4.6	35.2 ± 7.2	31.9 ± 3.8	28.1 ± 1.6	40.5 ± 2.1	42.2 ± 0.5	26.5 ± 3.4	27.3 ± 2.6
MUFA ($\mu\text{g mg DW}^{-1}$)	53.9 ± 24.5	44.9 ± 31.5	53.6 ± 26.9	43.8 ± 21.8	50.1 ± 17.6	73.1 ± 9.7	17.3 ± 7.6	26.9 ± 12.0
PUFAs								
16:2 ω 4	t.d	t.d	t.d	t.d	0.4 ± 0.2	0.7 ± 0.2	0.4 ± 0.5	1.3 ± 0.9
16:3 ω 3	t.d	t.d	t.d	t.d	0.0	0.0	0.2 ± 0.4	2.2 ± 0.8
16:3 ω 4	t.d	t.d	t.d	t.d	t.d	t.d	0.1 ± 0.3	0.6 ± 0.5
18:2 ω 6	6.7 ± 6.9	7.9 ± 3.1	7.6 ± 4.3	6.6 ± 1.1	3.1 ± 1.4	2.8 ± 1.4	6.9 ± 3.6	8.9 ± 3.2
18:3 ω 3	1.6 ± 1.2	4.8 ± 3.0	5.5 ± 1.5	6.0 ± 1.2	3.9 ± 2.7	2.5 ± 1.1	3.9 ± 2.1	9.8 ± 2.6
18:3 ω 4	0.0	0.0	t.d	t.d	t.d	t.d	t.d	t.d
18:4 ω 3	t.d	t.d	0.6 ± 0.3	1.4 ± 0.7	t.d	t.d	0.2 ± 0.2	1.4 ± 1.1
20:4 ω 6	1.0 ± 1.2	1.3 ± 0.9	5.5 ± 1.5	5.1 ± 1.1	2.6 ± 1.3	2.0 ± 0.8	1.4 ± 1.5	2.0 ± 0.8
20:5 ω 3	2.7 ± 3.5	3.8 ± 1.6	12.0 ± 3.0	16.0 ± 2.5	6.3 ± 2.5	5.5 ± 2.0	3.9 ± 3.6	8.8 ± 1.6
22:6 ω 3	0.0	0.0	t.d	t.d	t.d	t.d	t.d	t.d
Total (%)	12.0 ± 11.3	17.8 ± 7.7	31.1 ± 6.4	35.0 ± 4.2	16.2 ± 7.4	13.5 ± 5.4	17.4 ± 9.8	35.2 ± 4.7
PUFA ($\mu\text{g mg DW}^{-1}$)	27.0 ± 37.6	18.7 ± 7.6	51.2 ± 23.9	52.2 ± 18.7	20.6 ± 11.5	25.9 ± 8.3	13.2 ± 11.7	35.4 ± 14.0
Other								
i13:0	0.9 ± 1.5	0.0	t.d	t.d	t.d	t.d	0.0	0.0
i15:0	0.0	0.5 ± 0.4	t.d	t.d	t.d	t.d	0.8 ± 0.4	0.3 ± 0.2
Phytanate	t.d	t.d	t.d	t.d	t.d	t.d	t.d	t.d
i17:0	t.d	t.d	t.d	t.d	t.d	t.d	0.6 ± 0.5	0.3 ± 0.3
ai17:0	t.d	t.d	t.d	t.d	t.d	t.d	0.5 ± 0.6	0.5 ± 0.5
Total (%)	1.9 ± 1.7	1.5 ± 1.0	0.9 ± 0.3	1.2 ± 0.4	0.8 ± 0.4	0.7 ± 0.3	2.0 ± 1.2	1.1 ± 0.9
Total FAs ($\mu\text{g mg DW}^{-1}$)	141.9 ± 74.9	114.5 ± 58.2	158.7 ± 70.6	145.5 ± 63.4	121.3 ± 40.3	171.2 ± 23.6	64.5 ± 26.3	96.1 ± 36.2
$\omega 3/\omega 6$	0.7 ± 0.5	0.9 ± 0.2	1.5 ± 0.4	2.0 ± 0.3	1.7 ± 0.4	1.8 ± 0.2	0.9 ± 0.3	2.2 ± 0.9
DHA/ARA	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
		<i>Sida</i>			<i>Asellus aquaticus</i>			<i>Pallasea quadrispinosa</i>
SAFAs		Humic	Clear-water		Humic	Clear-water		Clear-water
12:00		0.0	0.0		t.d	t.d		t.d
14:00		4.9 ± 0.7	5.1 ± 0.7		2.3 ± 0.8	1.6 ± 0.6		3.2 ± 2.1
15:00		1.7 ± 0.2	0.8 ± 0.4		t.d	t.d		t.d
16:00		25.3 ± 1.5	21.2 ± 2.1		19.5 ± 1.7	19.9 ± 1.6		17.9 ± 2.1
17:00		1.1 ± 0.1	1.0 ± 0.5		t.d	t.d		t.d

Table 2 continued

SAFAs	<i>Sida</i>		<i>Asellus aquaticus</i>		<i>Pallasea quadrispinosa</i>
	Humic	Clear-water	Humic	Clear-water	Clear-water
18:00	4.2 ± 0.9	3.6 ± 0.4	3.8 ± 1.5	4.9 ± 0.9	2.2 ± 0.5
20:00	0.0	0.0	t.d	t.d	t.d
22:00	t.d	t.d	t.d	t.d	t.d
Total (%)	37.1 ± 2.6	31.6 ± 2.1	25.5 ± 1.9	26.5 ± 1.5	23.4 ± 4.5
SAFA (µg mg DW ⁻¹)	25.4 ± 5.6	30.0 ± 5.0	44.7 ± 16.7	23.8 ± 11.1	30.6 ± 8.6
MUFAs					
14:1	0.0	0.0	t.d	t.d	t.d
16:1ω9	1.2 ± 0.2	1.1 ± 0.1	t.d	t.d	t.d
16:1ω7	6.6 ± 1.8	3.9 ± 1.0	33.4 ± 11.3	13.4 ± 5.6	14.4 ± 4.9
16:1ω5	0.5 ± 0.1	0.5 ± 0.2	0.6 ± 0.2	0.8 ± 0.2	t.d
18:1ω9	7.4 ± 1.4	7.8 ± 0.9	11.6 ± 2.3	16.5 ± 1.9	19.3 ± 1.5
18:1ω7	7.8 ± 0.7	7.1 ± 0.6	6.1 ± 1.9	8.3 ± 1.1	4.8 ± 1.0
MUFA (µg mg DW ⁻¹)	16.2 ± 3.9	19.6 ± 3.6	91.1 ± 43.7	34.3 ± 16.7	55.1 ± 22.2
PUFAs					
16:2ω4	t.d	t.d	1.3 ± 0.6	0.5 ± 0.5	t.d
16:3ω3	t.d	t.d	t.d	t.d	t.d
16:3ω4	0.0	0.0	t.d	t.d	t.d
18:2ω6	5.7 ± 0.5	5.8 ± 0.4	4.4 ± 1.5	8.5 ± 1.7	5.7 ± 1.4
18:3ω3	8.9 ± 2.2	8.1 ± 0.3	2.6 ± 1.4	6.1 ± 2.1	3.2 ± 1.0
18:3ω4	4.3 ± 1.6	6.4 ± 0.6	t.d	t.d	t.d
18:4ω3	0.0	0.0	t.d	t.d	0.7 ± 0.4
20:4ω6	5.0 ± 0.6	6.2 ± 0.7	4.0 ± 1.9	5.5 ± 1.7	4.3 ± 0.5
20:5ω3	12.7 ± 1.6	16.4 ± 1.4	4.2 ± 2.1	7.0 ± 1.2	14.1 ± 0.6
22:6ω3	0.2 ± 0.2	1.2 ± 0.5	0.4 ± 0.3	0.8 ± 0.2	4.6 ± 0.4
Total (%)	36.8 ± 4.4	44.1 ± 1.5	16.8 ± 6.3	28.3 ± 4.1	32.6 ± 1.4
PUFA (µg mg DW ⁻¹)	26.2 ± 7.7	43.9 ± 9.1	26.7 ± 8.6	25.1 ± 14.4	46.7 ± 18.1
Other					
i13:0	0.0	0.0	t.d	t.d	0.0
i15:0	0.6 ± 0.1	0.5 ± 0.1	t.d	t.d	t.d
Phytanate	t.d	t.d	0.9 ± 0.4	0.3 ± 0.2	t.d
i17:0	0.0	0.0	t.d	t.d	t.d
ai17:0	0.0	0.0	t.d	t.d	t.d
Total (%)	0.6 ± 0.1	0.5 ± 0.1	0.9 ± 0.4	0.3 ± 0.2	t.d
Total FAs (µg mg DW ⁻¹)	67.9 ± 16.1	93.5 ± 16.9	165.6 ± 65.1	84.5 ± 42.1	133.8 ± 49.2
ω3/ω6	2.1 ± 0.3	2.2 ± 0.2	0.8 ± 0.2	1.0 ± 0.1	2.3 ± 0.5
DHA/ARA	0.0 ± 0.0	0.2 ± 0.1	0.1 ± 0.0	0.2 ± 0.0	1.1 ± 0.2

Student's *t*-test results for the statistically significant ($P \leq 0.05$) results are highlighted

t.d. Trace amounts

(Johnson & Goedkoop, 2002; Aroviita et al., 2019). Both *Asellus* and Chironomidae were common taxa in our lakes, and together contributed 36% of the differences in the community structure between humic and clear-water lakes. This is no surprise considering the generalist nature and adaptability of *Asellus* (Sket, 1994) and the vast variability of the members of the family Chironomidae. Chironomidae is one of the most abundant and widely distributed insect families in freshwater ecosystems, and members of this family

differ in their tolerance to extreme environmental conditions, e.g., acidity, salinity, and low oxygen concentrations (Pinder, 1986), which leads to differences in the chironomid assemblages between clear-water and humic lakes (Luoto, 2013). Chironomidae was the only family that was significantly more abundant in humic lakes. Chironomids in humic lakes might be detritivores since they prefer shallow water systems and high amount of organic matter. Conversely, filter feeders prefer clear-water conditions

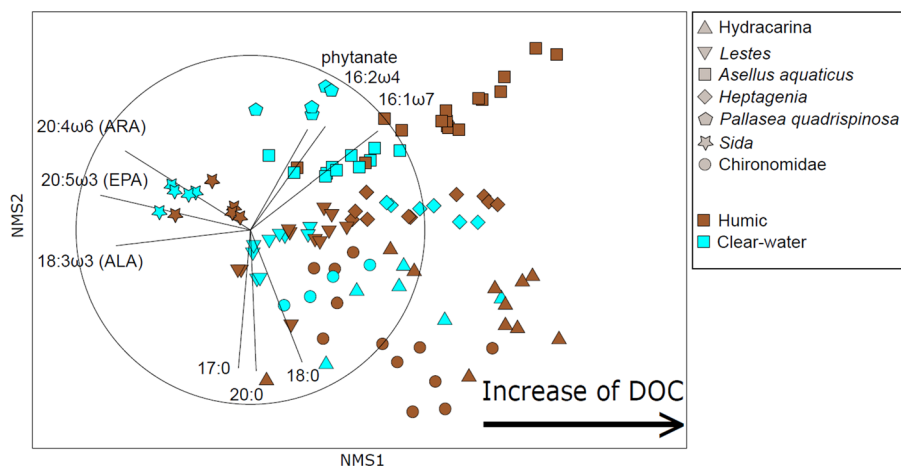


Fig. 2 Non-metric multidimensional scale (NMS) ordination of the FA composition of littoral invertebrates. Littoral invertebrate taxa are presented with different markers. Samples from humic lakes are presented as brown and samples from clear-water lakes as light blue, respectively. The ordination was

based on Euclidean distance. FAs that correlate strongly (Pearson $r > 0.6$) with either of the axes are visualized with vectors. The 2D stress value for the ordination was 0.15. The amount of DOC increases towards the right side of the ordination

Table 3 Proportions and standard deviations of different FAs and their contributions to the dissimilarities of the invertebrate FA composition between humic and clear-water lakes (SIMPER)

Taxa	FA	Proportion (%)		Contribution to dissimilarity (%)	Cumulative contribution (%)
		Humic	Clear-water		
Hydracarina	18:2ω6	6.7 ± 6.9	7.9 ± 3.1	18	18
	18:3ω3	1.6 ± 1.2	4.8 ± 3.0	14	31
	20:5ω3	2.7 ± 3.5	3.8 ± 1.6	11	42
Chironomidae	20:5ω3	3.9 ± 3.6	8.8 ± 1.6	13	13
	18:3ω3	3.9 ± 2.1	9.8 ± 2.6	11	24
	16:3ω3	0.2 ± 0.4	2.2 ± 0.8	10	34
<i>Heptagenia</i>	18:3ω3	3.9 ± 2.7	2.5 ± 1.1	17	17
	16:0	26.1 ± 5.4	27.0 ± 3.3	13	30
	20:5ω3	6.3 ± 2.5	5.5 ± 2.0	12	42
<i>Lestes</i>	16:1ω7	18.8 ± 5.0	17.9 ± 0.5	10	52
	16:1ω7	9.1 ± 3.5	6.6 ± 2.0	19	19
	20:5ω3	12.0 ± 3.0	16.0 ± 2.5	16	35
<i>Sida</i>	18:2ω6	7.6 ± 4.3	6.6 ± 1.1	13	48
	16:0	18.8 ± 4.7	18.8 ± 2.6	12	59
	22:6ω3	0.2 ± 0.2	1.2 ± 0.5	19	19
<i>Asellus aquaticus</i>	16:1ω7	6.6 ± 1.8	3.9 ± 1.0	17	36
	18:3ω4	4.3 ± 1.6	6.4 ± 0.6	13	49
	20:5ω3	12.7 ± 1.6	16.4 ± 1.4	11	60
	16:0	25.3 ± 1.5	21.2 ± 2.1	10	70
<i>Asellus aquaticus</i>	16:1ω7	33.4 ± 11.3	13.4 ± 5.6	58	58

FAs with a contribution $\geq 10\%$ are listed here

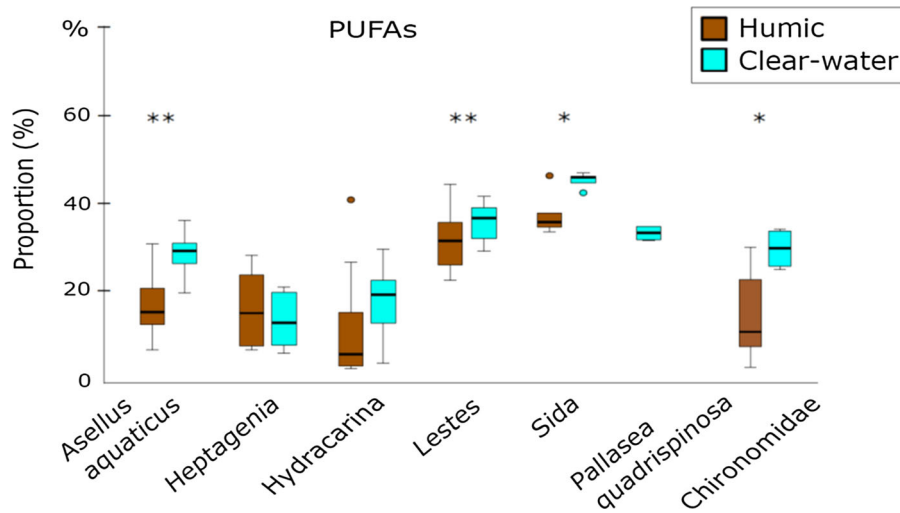


Fig. 3 Proportion of PUFAs relative to the total FAs of littoral invertebrates. Statistically significant differences have been marked with big asterisks, so that $*P < 0.05$, and $**P < 0.01$, respectively. Proportions from humic lakes are presented as brown and the proportions from clear-water lakes as light blue, respectively. The boxes represent the median, and 25th and 75th quantiles, and the whiskers represent the smallest observation

greater than or equal to lower hinge $- 1.5 \times$ interquartile range and the largest observation less than or equal to upper hinge $+ 1.5 \times$ interquartile range. Circles represent the smallest observations less than lower hinge $- 1.5 \times$ interquartile range and the biggest observations greater than upper hinge $+ 1.5 \times$ interquartile range (outlier)

Table 4 Contributions of different variables to the differences in the FA composition between humic and clear-water lakes in invertebrates (DistLM)

Taxa	Variable	SS	Pseudo- <i>F</i>	<i>P</i>	Proportion explained (%)
Hydracarina	C/N lake water	0.16	3.14	< 0.05	17
Chironomidae	Secchi depth (m)	0.29	4.70	0.005	25
<i>Lestes</i>	Mean depth of the lake (m)	0.06	7.51	< 0.001	21
	Total N ($\mu\text{g l}^{-1}$)	0.06	4.03	< 0.05	19
	Peatland (%)	0.05	4.18	< 0.01	17
	Secchi depth (m)	0.04	5.30	0.001	12
	DOC ($\mu\text{g l}^{-1}$)	0.10	6.65	< 0.05	26
<i>Sida</i>	Mean depth of the lake (m)	0.06	9.07	0.0001	53
	Agriculture (%)	0.01	4.93	< 0.05	13
	Lake area (km^2)	0.01	2.34	< 0.05	12
<i>Asellus aquaticus</i>	C/N lake water	0.74	31.54	0.0001	56
	Total P ($\mu\text{g l}^{-1}$)	0.13	7.18	< 0.01	9

P-values for the statistical significance of each variable are presented. Only statistically significant variables ($P \leq 0.05$) are presented here. For variables, see SI, Table S2

SS sum of squares

(Kivilä et al., 2019). This demonstrates the need for a higher level of taxonomic resolution in determining the effects of lake browning on littoral invertebrate community structures.

Plecoptera as a whole and families from Plecoptera (Capniidae) and Ephemeroptera (Baetidae) were significantly more common in clear-water lakes. Moreover, Pallaseidae and Hydropsychidae were completely absent in humic lakes. Pallaseidae are

mostly found in large, clear-water lakes (Särkkä et al., 1990). This is most likely related to their intolerance to acidic conditions (Økland & Økland, 1986). Humic lakes have a lower calcium availability due to lower pH (Drzymulska et al., 2015). Amphipods need calcium during ecdysis (Zehmer et al., 2002), and the rate of calcium uptake during this process is dependent on calcium levels (Alstad et al., 1999) and pH (Malley, 1980). Altogether, pH of the lake water positively correlates with invertebrate species richness on a pH range of 4–7 (Hämäläinen & Huttunen, 1990). Other acid-sensitive taxa include gastropods (Gastropoda) and Ephemeroptera (Økland & Økland, 1986).

Oxygen concentration of lake water is also one of the most important factors shaping invertebrate community structure. Humic and other organic substances increase the consumption of oxygen in lakes due to increased bacterial activity (Jones, 1992; Ask et al., 2012). Decreased oxygen concentration will be particularly harmful for less tolerant taxa, such as Plecoptera, Ephemeroptera, and Amphipoda (Fochetti & Tierno De Figueroa, 2008). We suggest that both the lower pH and decreased oxygen concentration in humic lakes are the reason for the lower abundance of Plecoptera and Baetidae and absence of Pallaseidae and Hydropsychidae. There are, however, variations within the members of the family Hydropsychidae for oxygen and pollution sensitivity (Vuori & Parkko 1996; Stuijzand et al. 1999; Savic et al., 2013).

Humic lakes are generally smaller and shallower than clear-water lakes (Nürnberg & Shaw, 1999). Lake size has been shown to affect the invertebrate community structure because larger lakes offer a wider selection of habitats (Heino, 2008). Habitat type has been shown to affect the invertebrate community composition more than water chemistry. Smaller lakes offer less variation in habitat types and are therefore expected to be dominated by generalists (Tolonen, 2004). The high percentage of Chironomidae in humic lake invertebrate community partly supports this.

Fatty acid composition of invertebrates

Proportions of FAs showed different patterns in the sampled invertebrates. When moving up the food chain, the proportion of SAFAs tends to decrease, whereas the proportion of C_{20–22} PUFAs increases (Lau et al., 2012). This is partly demonstrated in our

data as well because predatory *Lestes* had a relatively low proportion of SAFAs and a high proportion of PUFAs. This is also supported by the results of Makhutova et al. (2016). However, Hydracarina, which were likely predators, showed a completely opposite trend, having a high proportion of SAFAs and a low proportion of PUFAs. Unfortunately, studies about the FA composition of Hydracarina are scarce, so we have little to compare with. We also found hardly any differences in the FA composition of Hydracarina between the two lake types, so it might indicate a strong phylogenetic control on their FA composition.

There is evidence that the FA composition of Chironomidae follows that of their diet, but they also actively modify dietary FAs (Strandberg et al., 2020a). They contained a lot of SAFAs, especially the ones sampled from humic lakes. This supports the findings of Kara et al. (2012), who found high levels of SAFAs from Chironomidae during all seasons. According to Makhutova et al. (2017) the PUFA content of Chironomidae is variable, and it is controlled by both phylogeny and feeding strategy.

Sida had a high proportion of PUFAs with EPA being the most common one. FA composition of Cladocera has been found to reflect that of their diet (Brett et al., 2006). They also retain dietary EPA and ARA (Taipale et al., 2011) and actively biosynthesize C₂₀ PUFAs from C₂₂ PUFAs (Strandberg et al., 2014). EPA has been shown to support the growth and reproduction of *Daphnia* (Müller-Navarra et al., 2000; Persson & Vrede, 2006; Ravet & Brett, 2006) so it might hold importance for *Sida* as well. *Sida* also contained ARA, but the studies about its necessity, at least in *Daphnia* reproduction, have yielded varying results (Becker & Boersma, 2005; Ravet et al., 2012).

Pallasea had a significantly higher proportion of DHA compared to the other taxa. In general, predatory invertebrate larvae contain higher amounts of DHA compared to ARA (Ahlgren et al., 2009), but it should be noted that insects contain less or completely lack DHA, compared to crustaceans (Guo et al., 2016). This pattern is clearly visible in our data as well. DHA could be related to the nervous system, which is rich in it (Lauritzen et al., 2001) and may also indicate active synthesis of DHA from its shorter chain analogs (Maazouzi et al., 2007). DHA is a limiting FA in fish growth (Sargent et al., 1999; Ballantyne et al., 2003), so DHA-rich *Pallasea* can be considered a high-

quality food source for them. Amphipods can be an important food source for many fish species, e.g., brown trout (*Salmo trutta*; Linnaeus, 1758), also several macroinvertebrate predators prefer them (Macneil et al., 1999). *Pallasea* also contained a lot of EPA. Their diets consist of algae and small invertebrates (Hill, 1988), which are probably the sources of it. Also, Gammaridae selectively utilize EPA-rich particles from the sediments and water column (Makhutova et al., 2003), so same might be true for *Pallasea*.

Asellus contained a lot of MUFAs and relatively low amounts of PUFAs. Lau et al. (2012) showed that *Asellus* were rich in biomarker FAs of terrestrial-derived detritus (18:1 ω 9, 18:2 ω 6), bacteria (18:1 ω 7), and diatoms (16:1 ω 7). These FAs were also common in our *Asellus*. They also had a low ω 3/ ω 6 ratio, which might indicate the use of terrestrial-derived organic matter or other low-quality food sources.

Heptagenia had a relatively low ω 3/ ω 6 ratio (\sim 2), with reported ratios of Ephemeroptera being as high as 6 (Bell et al., 1994; Sushchik et al., 2003; Torres-Ruiz et al., 2007), which suggests differences in the FA composition of different members of Ephemeroptera. The ω 3/ ω 6 ratios have been used to identify allochthonous vs. autochthonous food sources, with ratios $>$ 1 usually indicative of predominantly autochthonous food sources (Torres-Ruiz et al., 2007; Guo et al., 2016) and ratios $<$ 1 allochthonous food sources and poor nutritional condition (Taipale et al., 2014).

The ω 3/ ω 6 ratios were relatively low (\sim 1–2) in the invertebrate taxa analyzed here, but within the range ($<$ 1 to 7) generally found in invertebrate taxa (Ahlgren et al., 2009). *Asellus* have been shown to selectively scrape the surface of submerged leaves, ingesting fungal mycelia (Bloor, 2011). Typically, the most abundant PUFA in fungi is 18:2 ω 6, and thus their ω 3/ ω 6 ratio is also low (Stahl & Klug, 1996). Certain littoral resources, specifically leaf litter of terrestrial plants, also contain a high amount of ω 6-PUFAs, and thus have a low ω 3/ ω 6 ratio (Harris & James, 1965; Torres-Ruiz & Wehr, 2010). Invertebrates rely on benthic algae as their main food source in low-DOC lakes, whereas in high-DOC lakes they may utilize relatively more phytoplankton and allochthonous organic carbon (Premke et al., 2010). However, leaf litter is not nutritionally adequate for all benthic invertebrates, e.g., Gammaridae excreted ingested

fragments of the leaf litter and did not assimilate them (Bogatov et al., 2021).

Fatty acids in humic and clear-water lake invertebrates

In this study, taxon explained 65% of the variation in FA composition, which supports previous findings (Lau et al., 2012; Vesterinen et al., 2020). Despite the strong phylogenetic control, lake type significantly influenced the FA composition of *Lestes*, Chironomidae, *Sida*, and *Asellus*. Lake type also significantly influenced the FA content of *Heptagenia* and *Asellus*. Finally, we found only small differences in the FA composition of invertebrates between vegetated and unvegetated shores, challenging our third hypothesis.

The proportions of several individual and total PUFAs were significantly affected by the lake type. When significant differences were found, the proportion of PUFAs was always lower in samples from humic lakes compared to samples from clear-water lakes. Only exception was the FA 16:2 ω 4 in *Asellus*. The proportion of MUFAs was significantly different in *Asellus* between the two lake types, which is largely explained by higher proportion of 16:1 ω 7 in humic lakes compared to clear-water lakes. It also explained most of the differences in the FA composition of *Asellus* between the two lake types. High amounts of C₁₆ MUFAs and low amounts of PUFAs have been connected to the consumption of bacteria (Kiyashko et al., 2004). According to the results by Pond et al. (1997) 16:2 ω 4 might also be of bacterial origin, so *Asellus* most likely utilized bacteria in humic lakes. The C/N ratio of lake water explained most of the variation in the FA composition of *Asellus* between the two lake types. Our previous study found that lake water chemistry was strongly correlated with lake catchment characteristics in our study lakes; lakes with high C/N ratios were strongly correlated with high DOC values and proportion of peatland in the catchment area (Strandberg et al., 2016). Thus, the lower proportion of PUFAs and lower ω 3/ ω 6 ratio in humic lakes compared to clear-water lakes is probably related to the utilization of terrestrial-derived organic matter or other low-quality food source.

Lake type had significant effects on the FA composition and content of Chironomidae in our study. Our results showed that the proportions of SAFAs and PUFAs and the ω 3/ ω 6 ratio differed

between the two lake types. This could indicate that detrital allochthonous matter is a more common food source for chironomids in humic lakes, whereas in clear-water lakes their diets might consist more of algae. Makhutova et al. (2017) also found that the FA composition of Chironomidae differs between different species. Since lake type also affects the community structure of Chironomidae (Luoto, 2013), we realize that lake type specific differences in FA profiles in Chironomidae may result from different community composition and may not be directly due to differences between the lake types.

Together with Chironomidae, lake type significantly influenced the proportion of PUFAs and the $\omega 3/\omega 6$ ratio of *Sida*. Environmental factors accounting the most to the differences in the FA composition between the two lake types in these two taxa (Secchi depth, mean depth) had a strong negative correlation with the phosphorous and DOC concentration of lake water (Strandberg et al., 2016). The FA explaining most of the differences between the two lake types was EPA in Chironomidae and DHA in *Sida*. The differences in the FA composition of Chironomidae and *Sida* between the two lake types might be related to the changes in the algal communities between the two lake types. Chironomidae had a significantly higher percentage of markers for green algae (16:3 $\omega 3$ and 16:4 $\omega 3$, Makhutova et al., 2016) in clear-water lakes compared to the humic lakes. In Chironomidae, the explanation for the higher proportion of SAFAs in humic lakes might be related to the utilization of relatively more abundant allochthonous leaf litter compared to clear lakes, since leaf litter mainly contains SAFAs (Brett et al., 2009; Bec et al., 2010; Taipale et al., 2014). Also, the $\omega 3/\omega 6$ ratios support the idea of higher proportions of allochthonous food items in the diets of Chironomidae in humic lakes. Some feeding guilds might obtain EFAs more efficiently, depending on the available food items. Filter feeders, for example, might suffer from the increased abundance of poor-quality food sources (regarding EFAs), since they do not select their food items. This might explain the differences in the FA composition of *Sida* between the two lake types.

In *Lestes*, the differences in the FA composition between the two lake types were mainly found in PUFAs and $\omega 3/\omega 6$ ratio. Mean depth of the lake contributed the most to the differences between the two lake types. This factor also had a strong negative

correlation with the phosphorous and DOC concentration. Differences in the $\omega 3/\omega 6$ ratio between the two lake types might be related to the quality of prey organisms. Zygoptera have broad diets, so changes in relative abundance of poor- and high-quality food items might reflect their FA composition.

DOC concentration was the only significant factor explaining the differences in the FA composition of *Heptagenia*. However, since there were only small differences in the FA composition of *Heptagenia* between humic and clear-water lakes, it indicates that phylogeny is more important driver in establishing the FA composition of *Heptagenia*. Effects of shore type did not affect the FA composition of invertebrates, besides *Heptagenia*. We hypothesized that different habitats contain different food sources, leading to differences in the FA compositions of invertebrates. Most invertebrate taxa are considered opportunistic regarding their food sources (Tierno de Figueroa et al., 2019) and can utilize several types of food (Friberg & Jacobsen, 1994), which might lead to differences in their FA composition. It is possible that the shore types in our study contained similar food sources, and thus clear differences in the FA compositions were not detectable. Vegetated shores generally contain a wider range of potential food sources, including epiphytic algae (Jones et al., 1998), decaying macrophytes (Smock & Harlowe, 1983), and allochthonous leaf litter (Weatherhead & James, 2001). Why the differences in the FA composition between the shore types were only present in *Heptagenia*, remains unclear. One possible explanation could be, that there are taxon-specific habitat preferences in different species within the genus *Heptagenia*.

Conclusions

With intensified land-use practices and ongoing climate change, the amount of terrestrial DOC in lake waters is expected to rise. Our study indicates that lake browning may alter the littoral food webs in two ways, (1) by changing invertebrate community structure and (2) by decreasing the proportion and content of PUFAs in several invertebrate taxa. Our results on invertebrates are supported by the observed significantly lower EFA content of predatory perch (*Perca fluviatilis*; Linnaeus, 1758, length > 250 mm) in humic lakes compared with those in clear-water lakes

(Strandberg et al., 2016). Thus, lake browning may lower the amount of PUFAs in fish used in human consumption. Our results indicate that lake browning might negatively influence the transfer and/or accumulation of EFAs in boreal lake food webs, all the way up to humans.

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Data availability The littoral macroinvertebrate community data that support the findings of this study are openly available in the Finnish Environment Institute (SYKE) HERTTA-database at www.syke.fi/avoindata. The fatty acid data that support the findings of this study are available from the corresponding author upon reasonable request. Other data is given as Supplementary Information.

Declarations

Conflict of interest The authors declare no conflict of interest.

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