

This is a self-archived version of an original article. This version may differ from the original in pagination and typographic details.

Author(s): Jones, Roger; Kankaala, Paula; Nykänen, Hannu; Peura, Sari; Rask, Martti; Vesala, Sami

Title: Whole-Lake Sugar Addition Demonstrates Trophic Transfer of Dissolved Organic Carbon to Top Consumers

Year: 2018

Version: Accepted version (Final draft)

Copyright: © Springer Science+Business Media, LLC 2017

Rights: In Copyright

Rights url: <http://rightsstatements.org/page/InC/1.0/?language=en>

Please cite the original version:

Jones, R., Kankaala, P., Nykänen, H., Peura, S., Rask, M., & Vesala, S. (2018). Whole-Lake Sugar Addition Demonstrates Trophic Transfer of Dissolved Organic Carbon to Top Consumers. *Ecosystems*, 21(3), 495-506. <https://doi.org/10.1007/s10021-017-0164-6>

Whole-lake sugar addition demonstrates trophic transfer of dissolved organic carbon to top consumers

Roger I. Jones^{1*}, Paula Kankaala², Hannu Nykänen^{1,3}, Sari Peura^{1,4}, Martti Rask⁵, Sami Vesala⁶

5

¹University of Jyväskylä, Department of Biological and Environmental Science, PO Box 35, FI-40014 University of Jyväskylä, Finland.

²Department of Environmental and Biological Sciences, University of Eastern Finland, PO Box 111, FI-80101 Joensuu, Finland

10 ³Department of Environmental and Biological Sciences, University of Eastern Finland, FI-70211 Kuopio, Finland

⁴SLU, Department of Forest Mycology and Plant Pathology, Science for Life Laboratories, Box 7026, 750 07 Uppsala, Sweden

⁵Natural Resources Institute Finland, Surfontie 9 A, FI-40500 Jyväskylä, Finland

15 ⁶Natural Resources Institute Finland, PL 2, FI-00791 Helsinki, Finland

Running title: Trophic transfer of DOC in lakes

*Corresponding author; email: roger.i.jones@jyu.fi

20

Author contribution

RIJ conceived and planned the study with advice from MR, obtained funding, led the data analysis and wrote the first draft of the manuscript; PK, HN, SP and SV collected data; all authors contributed substantially to the final manuscript.

25 **Abstract**

Terrestrial dissolved organic carbon (DOC) provides an external carbon source to lake ecosystems. However, there is ongoing debate about whether external DOC that enters a lake can pass up the food web to support top consumers. We show, from experimental manipulation of a whole lake, that externally loaded DOC can contribute appreciably to fish biomass. Monthly
30 additions of cane sugar with a distinct carbon stable isotope value during two years rapidly enriched the ^{13}C content of zooplankton and macroinvertebrates, with a more gradual ^{13}C enrichment of fish. After sugar addition stopped the ^{13}C content of consumers reverted towards original values. A simple isotope mixing model indicated that by the end of the sugar addition almost 20% of fish carbon in the lake was derived from the added sugar. Our results provide the
35 first direct experimental demonstration at relevant ecological spatial and temporal scales that externally loaded DOC to lakes can indeed transfer to top consumers.

Key words: lakes, fish, macroinvertebrates, zooplankton, food webs, allochthony, cane sugar, stable isotopes

40

Introduction

45 Since Elton (1927), ecologists have viewed ecosystems as fuelled by solar energy mobilised via
photosynthetic fixation of inorganic carbon within the ecosystem (*autochthonous production*).
However, freshwater systems also receive chemical energy in the form of detrital organic matter
from terrestrial plants within the surrounding catchment area (*allochthonous production*).
Recognition of the large allochthonous loading of organic matter into lakes and its domination of
50 lake carbon pools has stimulated a profound reappraisal of lake ecosystem processes (see Cole
2013). However, the extent to which allochthonous organic matter can enter lake food webs and
make an appreciable contribution to consumer biomass (*consumer allochthony*), and especially to
that of top consumers, remains controversial (Berggren and others 2010; Brett and others; Cole
2013; Mehner and others 2016; Brett and others 2017; Tanentzap and others 2017).

55 Compelling evidence for consumer allochthony has come from stable isotope analysis, mainly of
carbon (C) and more recently of hydrogen (H), using both natural abundance isotope ratios (e.g.
Grey and others 2001; Karlsson and others 2003, 2007, 2012; Cole and others 2011; Tanentzap
and others 2017) and experimental additions of ^{13}C -labelled dissolved inorganic carbon (DIC) (e.g.
60 Pace and others 2004, 2007; Carpenter and others 2005; Taipale and others 2008). Many stable
isotope studies of consumer allochthony in lakes have focused on direct consumption by
zooplankton of terrestrial particulate detritus originating from fallen tree leaves (e.g. Solomon and
others 2008; Weidel and others 2008; Bartels and others 2012; Scharnweber and others 2014).
However, other authors have highlighted the lack of essential fatty acids in terrestrial detritus and
65 have argued that it therefore represents a poor or even unsuitable food for aquatic consumers like
Daphnia (e.g. Brett and others 2009, 2017), although recent experiments have confirmed that

zooplankton can benefit energetically from access to the carbohydrates from terrestrial detritus during phytoplankton deficiency (McMeans and others 2015; Taipale and others 2016a).

70 In fact, dissolved organic carbon (DOC), mostly of terrestrial origin (Wilkinson and others 2013a), is generally an order of magnitude more abundant than particulate organic carbon (POC) in lakes (e.g. Jones and others 1997; Wetzel 2001). Although largely refractory, a proportion (<20%) of this detrital DOC is known to be readily biodegradable (Tranvik 1988; Hulatt and others 2014). Given the dominance of allochthonous DOC in the carbon pool of lakes, even this small proportion
75 of labile material can represent a substantial potential resource to aquatic microbes. Indeed, metabolic measurements and lake carbon budgets have shown that many lakes are “net heterotrophic” (e.g. Salonen and others 1983; del Giorgio and Peters 1994; Cole and others 1994; Kankaala and others 1996; Jones and others 2001) due to mineralization of allochthonous organic matter. A part of the allochthonous DOC thereby becomes incorporated into microbial biomass
80 (Guillemette and others 2015), and through this microbial link (Jones 1992; Jansson and others 2007) becomes available to higher trophic levels.

However, empirical evidence for transfer of allochthonous DOC to higher consumers in lakes mainly derives from studies of pelagic food webs in small-scale enclosures (Karlsson and others
85 2007; Kankaala and others 2010a; Faithfull and others 2012), which inevitably lack ecological realism due to their small spatial and temporal scale, failure to encompass all lake habitats, and hydrological isolation. Moreover, results derived from manipulations of natural humic dissolved organic matter (DOM), either in enclosures (e.g. Arvola and others 1996) or at the whole-lake scale (e.g. Kelly and others 2016), can be ambiguous because natural chromophoric DOM affects

90 light penetration and primary production, and also contains key nutrients such as nitrogen (N) and
phosphorus (P) which affect ecosystem function (Jones 1992, 1998). To obviate these problems
we therefore performed a whole-lake experimental manipulation encompassing pelagic, littoral
and benthic habitats, in which we added cane sugar as an allochthonous source of DOC monthly
during two successive open water periods. Of course, sugar cannot accurately mimic natural DOM.
95 However, it can be considered to represent the labile fraction of natural DOM as a potential organic
substrate for microbes, while it does not affect water colour and contains no mineral nutrients.
Moreover, the carbon stable isotope composition of the added cane sugar (from a C₄ plant, $\delta^{13}\text{C}$
value around -12‰) was distinct from that of the “local” DOC (derived from C₃ plants, $\delta^{13}\text{C}$ value
around -26 to -28‰), which allowed us to track the fate of the added DOC through the food web
100 (c.f. Kankaala and others 2010a; Peura and others 2014). We expected that added sugar DOC
transferred up through the lake food web would be detectable as increased $\delta^{13}\text{C}$ values first in the
primary consumers, especially pelagic zooplankton, and then in fish secondary consumers. We
also expected that after sugar additions ceased the $\delta^{13}\text{C}$ values of primary consumers would rapidly
return to pre-sugar addition values and that those of fish would return more slowly. Changes
105 in $\delta^{13}\text{C}$ values should enable estimation of the contribution of added DOC to fish biomass in the
lake.

Material and methods

The whole-lake manipulation was conducted in Alinen-Mustajärvi (henceforth AM), a small
110 boreal humic lake in southern Finland (61°12'N, 25°06'E) with an area of 0.007 km², a
maximum depth of 6.5 m and an estimated volume of 31x10³ m³. The lake is typically ice-
covered from around late November to late April, and during summer develops a very steep and

stable stratification with an epilimnion depth of around 2 m and an anoxic hypolimnion. The DOC concentration at the start of the experiment was around 10 mg L⁻¹ in the epilimnion and 20
115 mg L⁻¹ in the hypolimnion. A nearby (61°15'N, 25°04'E) lake, Valkea-Kotinen (henceforth VK), served as a partial reference. This lake has an area of 0.04 km², a maximum depth of 6.5 m and an estimated volume of 103x10³ m³. VK has similar duration of ice cover and also develops a very steep and stable stratification during summer with an epilimnion depth of around 2 m and an anoxic hypolimnion. The DOC concentration in the epilimnion is 13-18 mg L⁻¹. Littoral
120 macrophyte vegetation is restricted in both lakes due to the poor light penetration; however, floating littoral vegetation mats with associated periphyton can be highly productive in such lakes (Vesterinen and others 2016) and can harbour abundant macroinvertebrates. Both lakes contain populations of European perch (*Perca fluviatilis*) and northern pike (*Esox lucius*). Further information about the two lakes can be found elsewhere (e.g. Kankaala and others 2010a;
125 Rask and others 2010; Peura and others 2014).

The manipulation experiment in AM consisted of three phases. The first phase was a pre-manipulation year in 2007. In the second phase, during 2008 and 2009, 66 kg of cane sugar was added monthly to the epilimnion during the open water season, equivalent to a mean daily
130 loading of 0.07 mgC L⁻¹ and an elevated epilimnetic DOC concentration of 2 mgC L⁻¹. The proportion of labile DOC in the lake was assumed to be around 15 % of the total DOC (e.g. Tranvik 1998), so that the added sugar represented an increase in labile DOC to the level expected for the fourth quartile of boreal lakes according to the Nordic Lake Survey (Henriksen and others 1998). The sugar was first dissolved in lake water and the sugar solution was then

135 distributed evenly across the lake surface from a rowing boat. In the third phase sampling was carried out during 2010-2012 to monitor recovery after sugar addition had stopped.

The sugar addition was funded and undertaken to investigate effects on lake metabolism (see Peura and others 2014). However, the opportunity was also taken to collect samples of three
140 groups of consumers from AM for stable isotope analysis (SIA) to evaluate incorporation of the added sugar carbon into the food web, and some equivalent samples were collected from VK. These samples had to be collected opportunistically according to the availability of personnel and resources, and hence the sampling regime was necessarily irregular. Perch (secondary consumers) were sampled in spring after ice melt, in summer, and in autumn before ice
145 formation, using wire traps with 1 cm square mesh, which select perch with total length around 80 mm and above. Approximately 20 perch were selected randomly for analysis on each sampling occasion. No perch samples were available from AM in 2007 as no trapping was undertaken in that year. Individual fish were patted dry and weighed, and total length was measured. Age was determined from the opercular bone according to LeCren (1947). A piece of
150 dorsal muscle was removed, placed in an Eppendorf tube, frozen at -20°C, and later freeze-dried for SIA. Littoral macroinvertebrates (mainly primary consumers, but including some secondary consumers such as odonate larvae, gerrids and notonectids) were sampled from around the shore by sweep netting (mesh size 500 µm) at the same three seasons during 2008-2012 as the perch sampling, but no macroinvertebrate samples could be collected in 2007. Macroinvertebrates were
155 sorted into clean water for gut evacuation, identified and oven-dried for 48 h at 60°C for SIA. Results are presented as annual mean values for all macroinvertebrate groups pooled together (15 to 33 analysed samples per year) and also for selected individual groups. Crustacean zooplankton

(primary consumers) was sampled from AM 4-5 times per year during 2007-2009 and twice per year during 2010-2012, but no samples were available from VK. Samples were collected from the whole water column with a net of mesh size 50 μm and, after gut evacuation in clean water, were frozen at -20°C . For SIA, individuals of the most abundant taxa on each sampling occasion were picked manually into pre-weighed tin capsules to give sufficient material for analysis, as described in Kankaala and others (2010b). Results are presented as annual mean values for cladocerans and copepods pooled together (4 to 52 analysed samples per year).

Carbon and nitrogen stable isotope ratios of perch muscle, macroinvertebrates and zooplankton were measured with a Flash 1112 Elemental Analyser connected to a DELTAplus Advantage IRMS (Thermo Finnigan, San Jose, California, USA) and run using dried and homogenized fish muscle as an internal laboratory working standard. Results are expressed as $\delta\text{‰}$ relative to the international standards Pee Dee belemnite for C and atmospheric nitrogen for N. $\delta^{13}\text{C}$ values were not corrected for lipid content in view of the low C:N ratios (Kiljunen and others 2006).

For data analyses all samples from a particular year were pooled to provide annual mean values. In cases where no data were available from 2007, the first samples collected after ice-out in 2008 and before any sugar addition were used to represent “pre-sugar-addition” values. Variation across years was evaluated using single factor ANOVA. Differences in carbon isotope values between consecutive years were tested using a paired t-test. We assumed that the increase in perch $\delta^{13}\text{C}$ during the period of sugar addition above the $\delta^{13}\text{C}$ value before sugar addition commenced was due to incorporation of sugar carbon and calculated the percentage of perch carbon biomass comprised of sugar carbon ($\%\text{Biomass}_{\text{sugar}}$) as

$$\% \text{Biomass}_{\text{sugar}} = \frac{\delta^{13}\text{C}_{\text{post}} - \delta^{13}\text{C}_{\text{pre}}}{\delta^{13}\text{C}_{\text{sugar}} + (\text{TL}-1) \times \text{TF} - \delta^{13}\text{C}_{\text{pre}}} \times 100$$

where $\delta^{13}\text{C}_{\text{pre}}$ was the value for perch in May 2008 before the first sugar addition, $\delta^{13}\text{C}_{\text{post}}$ was
 185 the value for perch in May 2010 at the start of the first open water season after sugar addition
 stopped, and $\delta^{13}\text{C}_{\text{sugar}}$ was the measured value for the cane sugar used in the addition (-11.93‰).
 Trophic level (TL) of perch was estimated at 3.5 given that perch in the lake feed on a mixture of
 primary consumers and some predatory macroinvertebrates. The isotopic fractionation of carbon
 between trophic levels (TF) was assumed to be 0.4 (Post 2002). We also tested the sensitivity of
 190 model estimates of %Biomass_{sugar} to different values of TL (3.0, 3.5, 4.0) and of TF (0.4, 1.0).

Results

Crustacean zooplankton

Sampled zooplankton comprised cladocerans (*Daphnia*, *Bosmina* and *Holopedium*) and
 195 copepods in various proportions on different sampling occasions. Isotope values reported here
 are annual mean values of all the groups analysed. Addition of cane sugar to AM during 2008
 and 2009 produced a striking increase in mean annual $\delta^{13}\text{C}$ of crustacean zooplankton with the
 highest value in 2009, the second year of sugar addition (Fig. 1). After sugar addition stopped,
 $\delta^{13}\text{C}$ values declined but did not return to the pre-addition value. The variation in $\delta^{13}\text{C}$ among
 200 years was highly significant ($F_{126, 5}$, $p < 0.001$). Differences between successive years were
 significant for 2007-2008 ($p = 0.002$) and 2008-2009 ($p = 0.018$) but not for other paired years.

Littoral macroinvertebrates

Littoral macroinvertebrate taxa found consistently and abundantly in samples included *Asellus*

205 and larvae of Ephemeroptera, Chironomidae and Odonata; other taxa were found less consistently or in only small numbers. Isotope values reported here are annual mean values of all the taxa analysed. Addition of cane sugar to AM during 2008 and 2009 produced a striking increase in mean annual $\delta^{13}\text{C}$ of littoral macroinvertebrates with the highest value in 2009, the second year of sugar addition (Fig. 2). After sugar addition stopped, $\delta^{13}\text{C}$ values declined but did not quite return to the pre-addition value. The variation in $\delta^{13}\text{C}$ among years was highly significant ($F_{131, 5}$, $p < 0.001$). Differences between successive years were significant for all paired years (p between < 0.001 and 0.026) except for paired years 2008-2009 and 2011-2012. Variation in $\delta^{13}\text{C}$ among years was also significant in VK ($F_{121, 5}$, $p < 0.001$) but did not relate to the period of sugar addition. The difference in macroinvertebrate $\delta^{13}\text{C}$ values between AM and 215 VK was high in the years of sugar addition and the year afterwards but otherwise was negligible (Fig. 2).

The pattern of $\delta^{13}\text{C}$ response to sugar addition exhibited by the overall macroinvertebrate community in AM was also evident in most taxonomic groups, including both primary 220 consumers and predators (Fig. 3). However, the small number of samples for many individual taxa meant that $\delta^{13}\text{C}$ variation among years was only significant in some cases, such as *Asellus* ($F_{8, 5}$, $p = 0.031$) and Odonata larvae ($F_{18, 5}$, $p < 0.001$). In contrast, neustonic Gerridae showed no significant $\delta^{13}\text{C}$ variation among years ($F_{8, 4}$, $p = 0.652$).

225 *Fish*

Approximately 20 perch were analysed from each sampling occasion from each lake (Supplementary Information 1). As there were no gender-related differences in stable isotope values, individuals of both sexes and the few unsexed immature individuals were pooled for data analysis. Fish from both lakes were of approximately equivalent length, weight and age (from 1 to 12 years). The size and age and data indicate that the perch were poorly nourished with stunted growth. This was reflected in the low C:N ratio of perch muscle tissue (mean \pm SE of all analyses 3.29 ± 0.02), which indicates negligible lipid content in the muscle and hence no need to correct $\delta^{13}\text{C}$ values for lipid content (Kiljunen and others 2006).

Perch from AM showed a clear increase in mean annual $\delta^{13}\text{C}$ in response to the addition of cane sugar during 2008 and 2009 (Fig. 4). The highest value was recorded for 2010, the first year after sugar addition was stopped, after which $\delta^{13}\text{C}$ declined but did not return to the pre-addition value. The variation in $\delta^{13}\text{C}$ among years was highly significant ($F_{301, 5}$, $p < 0.001$). Differences between successive years were significant for all paired years (all p values < 0.001) except 2011-2012 ($p = 0.985$). The variation in $\delta^{13}\text{C}$ among years was marginally significant for perch from VK ($F_{310, 5}$, $p = 0.049$), but showed no relation to the period of sugar addition.

The perch sampled in each year were separated into year classes (2005 to 2012) and the response of mean annual $\delta^{13}\text{C}$ to the sugar addition was examined for each year class (Fig. 5). Reflecting the typical strong interannual variability in perch recruitment, some year classes provided only few individuals for stable isotope analysis. Year classes 2005-2007, which were recruited to the population prior to the start of sugar addition, showed a clear response of $\delta^{13}\text{C}$, and values remained above pre-addition levels even in 2012. Year class 2008, recruited during the first year

of sugar addition, showed the most pronounced response in $\delta^{13}\text{C}$. Year class 2009, recruited during the second year of sugar addition, appeared to show a reduced response, although individuals of that year class were only captured in 2011 and 2012. Individuals of year classes 2010-2012, recruited after sugar addition had stopped, all yielded $\delta^{13}\text{C}$ values that were closely comparable with pre-sugar values and hence showed no indication of influence from the ^{13}C -enriched sugar (Fig. 5).

The simple isotope mixing model estimated the percentage of perch carbon biomass (individuals of all year classes combined) comprised of sugar carbon (%Biomass_{sugar}) at the end of the two years of sugar addition to be 18.7%. Different combinations of values of TL and TF used in the model yielded a range of %Biomass_{sugar} values from 17.1% (TL = 4, TF = 1.0) to 18.8% (TL = 3, TF = 0.4), so in practice our estimates of %Biomass_{sugar} were rather insensitive to the values used in the model.

Nitrogen stable isotope values

Mean annual AM zooplankton $\delta^{15}\text{N}$ values varied significantly among years ($F_{126, 5}$, $p < 0.001$) but the variation showed no relation to the period of sugar addition (Fig. 6). Mean annual total macroinvertebrate and perch $\delta^{15}\text{N}$ values from AM also varied significantly among years ($F_{129, 5}$, $p = 0.022$ and $F_{301, 5}$, $p = 0.002$ respectively) but the variation did not show any obvious relation to the period of sugar addition (Fig. 6). Mean annual total macroinvertebrate $\delta^{15}\text{N}$ did not vary significantly in VK ($F_{121, 5}$, $p = 0.197$), whereas variation in mean annual $\delta^{15}\text{N}$ of perch from VK was significant ($F_{310, 5}$, $p = 0.003$) but the variation did not show any relation to the period of sugar addition (Fig. 6). Values of $\delta^{15}\text{N}$ were slightly lower from AM than from VK for both

macroinvertebrates and perch but the difference between the two lakes was consistent and showed no inter-annual pattern (Fig. 6).

275 **Discussion**

Our results from the whole-lake sugar addition provide the first direct experimental demonstration at relevant ecological spatial and temporal scales for trophic transfer of externally loaded DOC to top consumers. The two main categories of food for perch in the lake, 280 zooplankton and littoral macroinvertebrates, both exhibited a marked and highly significant increase in $\delta^{13}\text{C}$ during sugar addition, with values declining immediately after sugar addition stopped. The same pattern was exhibited by the perch in the lake but, as expected for these larger and longer-lived top consumers, both the increase in $\delta^{13}\text{C}$ in response to sugar addition and the subsequent decline after sugar addition was stopped showed a lag relative to the responses of the 285 smaller and shorter-lived primary consumers. Although $\delta^{13}\text{C}$ values in the reference lake also varied significantly during the study, the variation was less pronounced, showed no relation to the period of sugar addition, and presumably reflects stochastic year-to-year variation in $\delta^{13}\text{C}$ related to differences in zooplankton and macroinvertebrate community composition and in the relative contributions of different primary carbon sources to the food web (Taipale and others 290 2008, 2009; Berggren and others 2015). Similar stochastic year-to-year variation in $\delta^{13}\text{C}$ could be expected in the manipulated lake, but the effect of sugar addition was great enough to be clearly evident above any background variability, as seen in the differences between $\delta^{13}\text{C}$ values for primary and secondary consumers from the two lakes.

295 This kind of stochastic year-to-year variation is also evident from the significant $\delta^{15}\text{N}$ variation
in both lakes, which showed no relation to the period of sugar addition. The added sugar
contained no N so would not have directly affected $\delta^{15}\text{N}$ values in AM. The variation in $\delta^{15}\text{N}$
probably reflects year-to-year variation in the relative contributions of different N sources in the
lakes, perhaps related to annual variation in precipitation and hydrology, which appears to have
300 affected both lakes similarly, as the differences in $\delta^{15}\text{N}$ of both macroinvertebrates and perch
between the two lakes were consistent throughout the period of study. However, $\delta^{15}\text{N}$ values
were consistently lower in AM than in VK indicating different “baseline” $\delta^{15}\text{N}$ values for the two
lakes. Perch $\delta^{15}\text{N}$ values in both lakes were consistently around 4 to 4.5 ‰ above the values for
their primary consumer prey. This is more than the often quoted difference of 3.5 ‰ between
305 trophic levels (e.g. Post 2002) and probably reflects an element of cannibalism in the perch.
However, as $\delta^{15}\text{N}$ is strongly related to trophic level (e.g. Post 2002) the absence of any
systematic response in the $\delta^{15}\text{N}$ of zooplankton, macroinvertebrates or fish provides evidence
that sugar addition did not alter the food web structure in the lake.

310 The added sugar carbon could enter the food web via microbial use of the sugar as a substrate – a
‘microbial link’ (Jones 1992; Tranvik 1992; Jansson and others 2007). Addition of sugar
increased bacterial production and led to a sharp increase in $\delta^{13}\text{C}$ of particulate organic matter
(POM) in both the epilimnion and metalimnion of the lake, but only a slow rise in the
hypolimnion (Peura and others 2014), which is consistent with incorporation into bacterial
315 biomass in the upper water layers. It is also possible that some added sugar flocculated to form
particles with higher $\delta^{13}\text{C}$ (von Wachenfeldt and Tranvik 2008) which might be directly
consumed; however, if flocculation was a major process the resultant sedimentation of the floc

particles should have produced a more rapid rise in $\delta^{13}\text{C}$ of POM in the hypolimnion than was actually recorded (Peura and others 2014). Therefore direct bacterial incorporation of DOM was probably the most important mechanism for uptake of added sugar.

An alternative mechanism for entry of added sugar to the food web could be microbial mineralization of the sugar to yield ^{13}C -enriched CO_2 which was then taken up by photosynthetic phytoplankton and periphyton, and transmitted to the primary consumers. However, respiration of sugar with $\delta^{13}\text{C}$ of around -12‰ would be expected to yield CO_2 with $\delta^{13}\text{C}$ of around -13‰. In the epilimnion of Alinen Mustajärvi the existing DIC had $\delta^{13}\text{C}$ of around -14‰, and as the pH was around 5.1 (Peura and others 2014) effectively all this DIC would be in the form of CO_2 so that the $\delta^{13}\text{C}$ of DIC can be considered to represent the $\delta^{13}\text{C}$ of free CO_2 . Therefore any CO_2 derived from respiration of the added cane sugar would actually have had $\delta^{13}\text{C}$ very similar to that of the ambient CO_2 . In fact, no change in either concentration or $\delta^{13}\text{C}$ of DIC in Alinen Mustajärvi in the years of sugar addition was observed (Peura and others 2014), which would appear to exclude this alternative explanation. Moreover, Kankaala and others (2010a) reported an increase in zooplankton $\delta^{13}\text{C}$ in mesocosms with added sugar even though the $\delta^{13}\text{C}$ of DIC decreased by around 4% during the experiment. Thus we are confident that the transfer of the ^{13}C -enriched signature of the added cane sugar from DOC to primary consumers was primarily by direct uptake of DOC by bacteria and onward transfer through the food web of the resultant ^{13}C -enriched bacterial carbon. However, as we have no isotope analysis of the microbial components the true pathway by which the DOC became incorporated into higher trophic levels cannot be confirmed from our data.

In fact, transfer of allochthonous DOC via microbes to zooplankton has been reported for lakes (e.g. Jansson and others 2007; Berggren and others 2010) and has been demonstrated experimentally in mesocosms with added cane sugar (Kankaala and others 2010a; Faithfull and others 2012), so we expected this to be the main route for trophic transfer of added sugar to fish.

345 Much less is known about the role of allochthonous DOC for benthic communities (Dodds and Cole 2007), for which the focus has been more on the direct use of particulate terrestrial detritus by certain feeding guilds of macroinvertebrates (Solomon and others 2008; Bartels and others 2012). The strong response of the littoral macroinvertebrate community in AM to the added sugar was therefore somewhat unexpected. However, bacterial production in benthic biofilms
350 can be substantial and supported to a considerable extent by allochthonous organic matter (Ask and others 2009), and it appears that in AM part of the added sugar was taken up into littoral benthic biofilms and thence into macroinvertebrate primary consumers and predators (cf. Premke and others 2010; Karlsson and others 2012). In AM there was no clear difference in the response of feeding guilds to added sugar; ‘grazers’ like ephemeropteran larvae and ‘detritivores’ like
355 *Asellus* and chironomid larvae, as well as predators like odonate larvae all showed a similar pattern of change in $\delta^{13}\text{C}$. A notable exception was the lack of response in Gerridae, which feed largely on terrestrial insects fallen onto the water surface (Spence and Anderson 1994), and thus would not be expected to show any change in $\delta^{13}\text{C}$ associated with sugar addition to the lake water column. In fact, the $\delta^{13}\text{C}$ of the pooled macroinvertebrates increased more with sugar
360 addition relative to the pre-sugar value than did the $\delta^{13}\text{C}$ of zooplankton, and also reverted more slowly after sugar addition had stopped, presumably reflecting that these littoral consumers are longer-living with a slower turnover of biomass than zooplankton. These differences suggest that the littoral food web was particularly important for trophic transfer of DOC in the lake.

365 Perch from AM showed a clear pattern of change in $\delta^{13}\text{C}$ in response to the sugar addition
reflecting (but with a time lag) the changes in $\delta^{13}\text{C}$ of their principle prey, zooplankton and
littoral macroinvertebrates. No such pattern was seen in perch from VK. Perch in AM showed
the poor growth (low length and weight per unit age) typical of fish in unproductive small boreal
lakes (Rask 1983, 1984; Rask and Arvola 1985) and can be assumed to be food-limited. These
370 stunted fish are very rarely piscivorous (other than feeding on fish fry) and their diet consists
mainly of zooplankton (especially early in the open water season) and various
macroinvertebrates, especially *Asellus*, odonates, ephemeropterans and chironomids (Rask 1983,
1984; Rask and Arvola 1985). Thus both pelagic and littoral food webs offer potential pathways
for trophic transfer of added DOC to perch. Bartels and others (2016) argued that higher DOC
375 leads to perch feeding more on pelagic resources and reduces habitat coupling, but their results
reflect altered competition between perch and roach (*Rutilus rutilus*) and would not be applicable
in AM where perch is the only fish species apart from a few pike.

None of the consumer groups studied showed a return of $\delta^{13}\text{C}$ values to the pre-addition value
380 even three years after sugar addition stopped. One explanation for this could be that some added
sugar continued to enter the lake food web long after addition of sugar had stopped. This seems
unlikely because such a highly labile substrate should be metabolised rapidly if not flushed from
the lake by hydrological water renewal. Both AM and VK are headwater lakes with no defined
inflow. The long-term mean water residence time for VK is 16 months and varied from 14
385 months in a wet year to 25 months in a dry year (Einola and others 2011). No hydrological data
are available for AM but, given its smaller volume, water residence times are likely to be

somewhat shorter than for VK. Moreover, $\delta^{13}\text{C}$ values did not differ significantly between 2011 and 2012 for any of the consumer groups studied; if the impact of added sugar was persisting but progressively diminishing in the years after addition stopped, differences would also have been expected between $\delta^{13}\text{C}$ in 2011 and 2012. In fact, perch recruited to the population in the years after sugar addition had stopped showed stable $\delta^{13}\text{C}$ values that were similar to pre-sugar values and significantly lower than those of fish that had lived through the period of sugar addition. Therefore, the relatively high $\delta^{13}\text{C}$ that persisted in AM perch of earlier year classes probably reflects prolonged retention of sugar carbon in the tissue of these long-lived but slow-growing organisms in the lake. Reported rates of carbon isotope turnover in fish muscle tissue vary widely depending on size and growth conditions (see Weidel and others 2011). Juvenile fish with higher mass-specific growth rates show faster isotopic turnover than more slowly growing adult fish, which is consistent with the most pronounced response to sugar addition in AM shown by year classes recruited during the years of sugar addition and with the absence of a sugar effect in year classes recruited in the years after sugar addition had stopped. Weidel and others (2011) reported carbon isotope half-lives for yellow perch (*Perca flavescens*), a species very similar to European perch, of 8 days for 0+ fish and 116 days for age 2 fish, but these values were obtained during the main growing season and values representing an entire year including winter ice cover, when effectively there is no growth, are likely to be longer. Moreover, older perch in AM have very slow growth and presumably very low metabolic rates which will also reduce rates of isotope turnover, so it would not be surprising if fish in the lake from earlier year classes retained a detectable isotopic signal from the sugar addition for some years after the addition had stopped. We believe that a more likely general explanation for the $\delta^{13}\text{C}$ values of all the consumer groups remaining somewhat higher than the pre-addition values is the evident stochastic inter-annual

variation in $\delta^{13}\text{C}$ in these lakes, which was also apparent in perch $\delta^{13}\text{C}$ values reported for earlier years from VK and for another nearby small lake, Halsjärvi (Rask and others 2010). We had only a single pre-sugar-addition $\delta^{13}\text{C}$ value for each consumer group, and if that value happened to derive from a phase with particularly low $\delta^{13}\text{C}$ it would not be surprising if the post-addition values did not return to that level.

The simple isotope mixing model indicated that by the start of the first growing season after sugar addition had stopped sugar carbon (i.e. carbon derived from DOC) comprised almost 20% of perch carbon when the DOC concentration in the epilimnion produced by each monthly sugar addition was 2 mgC L^{-1} . Different combinations of values of TL and TF tested in the model demonstrated that estimates of %Biomass_{sugar} were rather insensitive to variability in the trophic fractionation parameters. Estimates of %Biomass_{sugar} will also be affected by any change in baseline consumer ^{13}C values which would affect the ‘natural’ perch values represented by $\delta^{13}\text{C}_{\text{pre}}$ in the model. We cannot determine any such change for AM perch because of the sugar addition, but in the reference lake VK the mean perch $\delta^{13}\text{C}$ value had decreased by 0.52 by May 2010. If this same decrease representing a possible baseline shift is assumed for $\delta^{13}\text{C}$ values of AM perch, then the estimate of %Biomass_{sugar} increases slightly from 18.7% to 20.6%, suggesting that any small shift in baseline $\delta^{13}\text{C}$ values of AM perch would have rather little impact on our estimate of %Biomass_{sugar}.

The natural DOC concentration in the epilimnion of AM at the start of the experiment was around 10 mg L^{-1} . Assuming 15-20% of this was sufficiently labile to behave similarly to the added sugar (see review by Tranvik 1998), this would mean a natural labile DOC availability of

around 1.5 to 2.0 mgC L⁻¹. This suggests that under ambient DOC concentrations, if the natural labile DOC was incorporated into the food web in the same way as sugar, around 15-20% of the carbon biomass of perch in AM might derive from trophic transfer of DOC. In practice total consumer allochthony of AM perch is likely to be higher than this, as terrestrial POC can also make an important contribution to pelagic and benthic food webs in lakes (e.g. Cole and others 2006; Mehner and others 2016). Based on combined H and C stable isotope analysis, values for total fish allochthony of from 20% to >50% have been reported from a variety of lakes (Weidel and others 2008; Karlsson and others 2012; Mehner and others 2016).

Labile DOC in lakes can also be of autochthonous origin. Phytoplankton production in AM during the ice-free period was around 100 mgC m⁻³ d⁻¹ (Peura and others 2014), and release of DOC from phytoplankton is typically around 10% of production (e.g. Baines and Pace 1991). Hence phytoplankton might generate around 5.5 µgC L⁻¹ d⁻¹ in the epilimnion of AM. We did not measure littoral primary production, but recent work on similar small boreal lakes suggests it can be around 4-5 times that of phytoplankton (Vesterinen and others in press). Assuming a similar proportion exuded, the total autochthonous generation of DOC in the epilimnion might then be around 30 µgC L⁻¹ d⁻¹. This would be a little less than half our mean daily sugar addition of 70 µgC L⁻¹ and substantially less than the estimated pool of labile DOC in the lake (15-20% of the natural DOC concentration of 10 mg L⁻¹; Tranvik 1998). These rough calculations suggest that DOC in AM during the ice-free period is mainly of allochthonous origin, as is generally the case in north temperate lakes (e.g. Wilkinson and others 2013) with the labile fraction presumably being renewed frequently by hydrological input and photochemical conditioning

455 (Tranvik 1998), but with an appreciable contribution from autochthonous DOC which
presumably has rapid turnover.

Our results from AM are likely to be widely applicable to the small, DOC-rich lakes that are so
numerous around the boreal zone, but caution should be applied before extending them to larger
460 lakes where the role of allochthonous organic matter appears to be lower (e.g. Wilkinson and
others 2013b; Taipale and others 2016b; Tanentzap and others 2017). In lakes with lower
loadings of allochthonous DOC or in more eutrophic lakes with higher generation of
autochthonous DOC, the mix of DOC can be expected to have a higher percentage of algal DOC
(e.g. Bade and others 2007) and greater overall lability, so that a higher proportion of total DOC
465 is likely to be passed up the food chain.

We recognise that our study suffers from a number of shortcomings imposed by the framework
of available funding and resources. The baseline or pre-manipulation data derive from only a
short period, and this brief snapshot may not encompass the possible variability in pre-
470 manipulation conditions. The inherent variability between even geographically close lakes means
that VK cannot be considered a true “control” for AM. And the experimental manipulation could
not be performed with replicate lakes. These shortcomings mean that the results must be
interpreted with caution. Nevertheless, this kind of whole-lake experimental approach offers a
level of spatial and temporal realism that can never be achieved with small-scale mesocosm
475 experiments, however highly replicated and controlled. We believe that the very clear responses
of $\delta^{13}\text{C}$ (but not of $\delta^{15}\text{N}$) in all groups of organisms following sugar addition provide some

unique insights into trophic transfer of DOC at the ecosystem scale despite the limitations of the study.

480 Our results demonstrate that simple DOC compounds like sugars can be quantitatively important in lake food webs via trophic transfer as far as mature top consumers, despite containing no key mineral nutrients or essential biochemicals. Although much natural allochthonous DOC is recalcitrant, a proportion (<20%) of this detrital DOC is known to be readily biodegradable (Tranvik 1988; Hulatt and others 2014) and can be expected to behave similarly to the sugar
485 added in our experiment. Our finding of appreciable trophic transfer of DOC is consistent with recent experimental evidence that terrestrial organic matter can substantially support growth and reproduction of freshwater zooplankton when algal resources are deficient (McMeans and others 2015; Taipale and others 2016a). However, there is evidence that allochthonous support of lake food webs does not compensate for decreased primary production arising from reduced light
490 penetration in the presence of coloured organic matter (Karlsson and others 2009, 2015; Jones and Lennon 2015). Therefore the documented increase in DOM loading from catchments to boreal freshwater ecosystems (e.g. Roulet and Moore 2006; Vuorenmaa and others 2006; Monteith and others 2007) is likely to decrease overall fish productivity, as well as altering phytoplankton community structure with adverse effects on availability and transfer of essential
495 fatty acids through food webs to top consumers (Taipale and others 2016b).

Finally, our results demonstrate the value in ecology of experimental manipulations at the whole-ecosystem scale, despite the inevitable absence of replication and true control treatments. Short-term and small-scale enclosure experiments of the kind widely used in studies of the role of

500 allochthonous organic matter in lakes (e.g. Kankaala and others 1996, 2010; Faithfull and others
2012) would not have revealed the importance of the benthic pathway, the importance to mature
fish and the long-term pattern of response and return that were evident from our multi-year
whole-lake experiment.

505

Acknowledgements

This work was funded by Academy of Finland grants 114604 and 137671 to RJ. We are grateful
for the support from staff and facilities at Lammi Biological Station, University of Helsinki and
the Evo Station of the Natural Resources Institute Finland.

510

References

- Ask, J., Karlsson, J., Persson, L., Ask, P., Bystrom, P. & Jansson, M. 2009. Whole-lake estimates of carbon flux through algae and bacteria in benthic and pelagic habitats of clearwater lakes. *Ecology* 90: 1923–1932.
- 515 Arvola, L., Kankaala, P., Tulonen, T. & Ojala, A. 1996. Effects of phosphorus and allochthonous humic matter enrichment on the metabolic processes and community structure of plankton in a boreal lake. *Canadian Journal of Fisheries and Aquatic Sciences* 53: 1646–1662.
- Bade, D.L., Carpenter, S.R., Cole, J.J., Pace, M.L., Kritzberg, E., Van de Bogert, M.C., Cory, R.M. & McKnight, D.M. 2007. Sources and fates of dissolved organic carbon in lakes as
520 determined by whole-lake carbon isotope additions. *Biogeochemistry* 84: 115–129.
- Baines, S.B. & Pace, M.L. 1991. The production of dissolved organic matter by phytoplankton and its importance to bacteria – patterns across marine and freshwater systems. *Limnology and Oceanography* 36: 1078–1090.
- Bartels, P., Cucherousset, J., Gudas, C., Jansson, M., Karlsson, J., Persson, L., Premke, K.,
525 Rubach, A., Steger, K., Tranvik, L.J. & Eklöv, P. 2012. Terrestrial subsidies to lake food webs: an experimental approach. *Oecologia* 168: 807–818.
- Bartels, P., Hirsch, P.E., Svanbäck, R. & Peter Eklöv, P. 2016. Dissolved organic carbon reduces habitat coupling by top predators in lake ecosystems. *Ecosystems* 19: 955–967.
- Berggren, M., Strom, L., Laudon, H. Karlsson, J., Jonsson, A., Giesler, R., Bergström, A.-K. &
530 Jansson, M. 2010 Lake secondary production fueled by rapid transfer of low molecular weight organic carbon from terrestrial sources to aquatic consumers. *Ecology Letters* 13: 870–880.

Berggren M., Bergström, A.-K. & Karlsson, J. 2015. Intraspecific autochthonous and allochthonous resource use by zooplankton in a humic lake during the transitions between winter, summer and fall. *PLoS ONE* 10(3): e0120575. doi:10.1371/journal.pone.0120575

Brett, M.T., Arhonditsis, G.B., Chandra, S. & Kainz, M.J. 2012. Mass flux calculations show strong allochthonous support of freshwater zooplankton production is unlikely. *PLoS ONE* 7(6): e39508. doi:10.1371/journal.pone.0039508

Brett, M.T., Bunn, S.E, Chandra, S., Galloway, A.W.E., Guo, F., Kainz, M.J., Kankaala, P., Lau, D.C.P., Moulton, T.P., Power, M.E., Rasmussen, J.B., Taipale, S.J., Thorp, J.H. & Wehr, J.D 2017. How important are terrestrial organic carbon inputs for secondary production in freshwater ecosystems? *Freshwater Biology* DOI: 10.1111/fwb.12909.

Brett, M.T., Kainz, M.J., Taipale, S.J. & Seshan, H. 2009. Phytoplankton, not allochthonous carbon, sustains herbivorous zooplankton production. *Proceedings of the National Academy of Sciences USA* 106: 21197–21201.

Carpenter, S.R., Cole, J.J., Pace, M.L., Van de Bogert, M., Bade, D.L., Bastviken, D. and others 2005. Ecosystem subsidies: terrestrial support of aquatic food webs from C-13 addition to contrasting lakes. *Ecology* 86: 2737–2750.

Cole, J.J. 2013. *Freshwater Ecosystems and the Carbon Cycle*. Excellence in Ecology 18. International Ecology Institute, Oldendorf/Luhe, pp. 1-125.

Cole, J.J., Caraco, N.F., Kling, G.W. & Kratz, T. (1994). Carbon dioxide supersaturation in the surface waters of lakes. *Science* 165, 1568–1570.

Cole, J.J., Carpenter, S.R., Kitchell, J., Pace, M.L., Solomon, C.T., & Weidel B. 2011. Strong evidence for terrestrial support of zooplankton in small lakes based on stable isotopes of

555 carbon, nitrogen, and hydrogen. *Proceedings of the National Academy of Sciences USA* 108:
1975-1980.

Cole, J.J., Carpenter, S.C., Pace, M.L., Van de Bogert, M.C., Kitchell, J.L. & Hodgson, J.R.
2006. Differential support of lake food webs by three types of terrestrial organic carbon.
Ecology Letters 9: 558–568.

560 del Giorgio, P.A., Peters, R.H. 1994. Patterns in planktonic P:R ratios in lakes: influence of lake
trophism and dissolved organic C. *Limnology and Oceanography* 39: 772–787.

Dodds, W.K. & Cole, J.J. 2007. Expanding the concept of trophic state in aquatic ecosystems :
it's not just the autotrophs. *Aquatic Sciences* 69: 427–439.

Einola, E., Rantakari, M., Kankaala, P., Kortelainen, P., Ojala, O., Pajunen, H., Mäkelä, S. &
565 Arvola, L. 2011. Carbon pools and fluxes in a chain of five boreal lakes: A dry and wet year
comparison. *Journal of Geophysical Research* 116: G03009, doi:10.1029/2010JG001636.

Elton, C.S. 1920. *Animal Ecology*. MacMillan, New York, pp. 1-260.

Faithfull, C.L., Huss, M., Vrede, T., Karlsson, J., Bergström, A.-K. 2012. Transfer of bacterial
production based on labile carbon to higher trophic levels in an oligotrophic pelagic system.
570 *Canadian Journal of Fisheries and Aquatic Sciences* 69: 85–93.

Grey, J., Jones, R.I., & Sleep, D. 2001. Seasonal changes in the importance of the source of
organic matter to the diet of zooplankton in Loch Ness, as indicated by stable isotope
analysis. *Limnology and Oceanography* 46: 505–513.

Guillemette, F., McCallister, L. & del Giorgio, P.A. 2015. Selective consumption and metabolic
575 allocation of terrestrial and algal carbon determine allochthony in lake bacteria. *ISME
Journal* 10: 1373-1382.

- Henriksen, A., Skjelvåle, B.L., Mannio, J., Wilander, A., Harriman, R., Curtis, C., Jensen, J.P.,
Fjeld, E. & Moiseenko, T. 1998. Northern European lake survey, 1995. *Ambio* 27: 80–91.
- Hulatt, C.J., Kaartokallio, H., Asmala, E., Autio, R., Stedmon, C.A., Sonninen, E., Oinonen, M.
580 & Thomas, D.T. 2014. Bioavailability and radiocarbon age of fluvial dissolved organic matter
(DOM) from a northern peatland-dominated catchment: effect of land-use change. *Aquatic
Sciences* 76: 393–404.
- Jansson, M., Persson, L., DeRoos, A.M., Jones, R.I. & Tranvik, L.J. 2007. Terrestrial carbon and
intraspecific size-variation shape lake ecosystems. *Trends in Ecology and Evolution* 22: 316–
585 322.
- Jones, R.I. 1992. The influence of humic substances on lacustrine planktonic food chains.
Hydrobiologia 229: 73–91.
- Jones, R.I. 1998. Chapter 7: Phytoplankton, primary production and nutrient cycling. In: Hessen,
D.O. & Tranvik, L. (Editors) *Aquatic Humic Substances: Ecology and Biogeochemistry*.
590 *Ecological Studies* 133, Springer-Verlag, pp 145-175.
- Jones, R.I., Laybourn-Parry, J., Walton, M.C. & Young, J.M. 1997. The forms and distribution
of carbon in a deep, oligotrophic lake (Loch Ness, Scotland). *Verhandlungen der
Internationalen Vereinigung für theoretische und angewandte Limnologie* 26: 330-334.
- Jones, R.I., Grey, J., Quarmby, C. & Sleep, D. 2001. Sources and fluxes of inorganic carbon in a
595 deep, oligotrophic lake (Loch Ness, Scotland). *Global Biogeochem Cycles* 15: 863–870.
- Jones, S.E. & Lennon, J. 2015. A test of the subsidy–stability hypothesis: the effects of terrestrial
carbon in aquatic ecosystems. *Ecology* 96: 1550–1560.

- Kankaala, P., Arvola, L., Tulonen, T. & Ojala, A.(199). Carbon budget for the pelagic food web of the euphotic zone in a boreal lake (Lake Pääjärvi). *Canadian Journal of Fisheries and Aquatic Sciences* 5: 1663–1674.
- 600 Kankaala, P., Peura, S., Nykänen, H., Sonninen, E., Taipale, S., Tirola, M. & Jones, R.I. 2010a. Impacts of added dissolved organic carbon on boreal freshwater pelagic metabolism and food webs in mesocosm experiments. *Fundamental and Applied Limnology* 177: 161–176.
- Kankaala, P., Taipale, S., Li, L., & Jones, R.I. 2010b. Diets of crustacean zooplankton, inferred from stable carbon and nitrogen isotope analyses, in lakes with varying allochthonous dissolved organic carbon content. *Aquatic Ecology* 44: 781–79.
- 605 Karlsson, J., Bergström, A.-K., Byström, P., Gudas, C., Rodriguez, P. & Hein, C. 2015. Terrestrial organic matter input suppresses biomass production in lake ecosystems. *Ecology* 96: 2870–2876.
- 610 Karlsson, J., Byström, P., Ask, J., Ask, P., Persson, L. & Jansson, M. 2009. Light limitation of nutrient-poor lake ecosystems. *Nature* 460: 506–509.
- Karlsson, J., Jonsson, A., Meili, M. & Jansson, M. 2003. Control of zooplankton dependence on allochthonous organic carbon in humic and clear-water lakes in northern Sweden. *Limnology and Oceanography* 48: 269–276.
- 615 Karlsson, J., Lymer, D., Vrede, K., & Jansson, M. 2007. Differences in efficiency of carbon transfer from dissolved organic carbon to two zooplankton groups: an enclosure experiment in an oligotrophic lake. *Aquatic Sciences* 69: 108–114.
- Karlsson, J., Berggren, M., Ask, J., Byström, P., Jonsson, A., Laudon, H. & Jansson, M. 2012. Terrestrial organic matter support of lake food webs: Evidence from lake metabolism and stable hydrogen isotopes of consumers. *Limnology and Oceanography* 57: 1042–1048.
- 620

Kelly, P.K., Craig, N., Solomon, C.T., Weidel, B.C., Zwart, J.A. & Jones, S.E. 2016.

Experimental whole-lake increase of dissolved organic carbon concentration produces unexpected increase in crustacean zooplankton density. *Global Change Biology* 22: 2766–2775.

625 Kiljunen, M., Grey, J., Sinisalo, T., Harrod, C., Immonen, H. & Jones, R.I. 2006. A revised model for lipid-normalizing delta C-13 values from aquatic organisms, with implications for isotope mixing models. *Journal of Applied Ecology* 43: 1213–1222.

LeCren, E.D. 1947. The determination of age and growth of the perch (*Perca fluviatilis*) from the opercular bone. *Journal of Animal Ecology* 16: 188-204.

630 McMeans, B.G., Koussoropolis, A.-M., Arts, M.T. & Kainz, M.J. 2015. Terrestrial dissolved organic matter supports growth and reproduction of *Daphnia magna* when algae are limiting, *Journal of Plankton Research* 37: 1201-1209.

Mehner, T., Attermeyer, K., Brauns, M., Brothers, S., Diekmann, J., Gaedke, U., Grossart, H.-P., Köhler, J., Lischke, B., Meyer, N., Scharnweber, K., Syväranta, J., Vanni, M.J. & Hilt, S.

635 2016. Weak response of animal allochthony and production to enhanced supply of terrestrial leaf litter in nutrient-rich lakes. *Ecosystems* 19: 311–325

Monteith, D.T., Stoddard, J.L., Evans, C.D., de Wit, H.A., Forsius, M., Hogasen, T. and others 2007. Dissolved organic carbon trends resulting from changes in atmospheric deposition chemistry. *Nature* 450: 537–540.

640 Pace, M.L., Cole, J.J., Carpenter, S.R., Kitchell, J.F., Hodgson, J.R., Van de Bogert, M.C. and others 2004. Whole-lake carbon-13 additions reveal terrestrial support of aquatic food webs. *Nature* 427: 240–243.

- Pace, M., Carpenter, S.C., J. Cole, J.J., Coloso, J., Kitchell, J. Hodgson, J., Middelburg, J.,
Preston, N., Solomon, C.T. & Weidel, B. 2007. Does terrestrial organic carbon subsidize the
645 planktonic food web in a clear-water lake? *Limnology and Oceanography* 52: 2177–2189.
- Peura, S., Nykänen, H., Kankaala, P. Eiler, A., Tirola, M. & Jones, R.I.(2014. Enhanced
greenhouse gas emissions and changes in plankton communities following an experimental
increase in organic carbon loading to a humic lake. *Biogeochemistry* 118: 177–194.
- Post, D.M. 2002. The long and short of food-chain length. *Trends in Ecology and Evolution* 17:
650 269–277.
- Premke, K., Karlsson, J., Steger, K., Gudas, C., von Wachenfeldt, E. & Tranvik L.J. 2010.
Stable isotope analysis of benthic fauna and their food sources in boreal lakes. *Journal of the
North American Benthological Society* 29:1339–1348.
- Rask, M. 1983. Differences in growth of perch (*Perca fluviatilis* L.) in two small forest lakes.
655 *Hydrobiologia* 101: 139-144.
- Rask, M. 1984. The effect of low pH on perch, *Perca fluviatilis* L. III. The perch population in a
small, acidic, extremely humic forest lake. *Annales Zoologica Fennici* 2: 15-22.
- Rask, M. & Arvola, L. 1985. The biomass and production of pike, perch and whitefish in two
small lakes in southern Finland. *Annales Zoologica Fennici* 22: 129-136.
- 660 Rask, M., Verta, M., Korhonen, M., Salo, S., Forsius, M., Arvola, L., Jones, R.I. & Kiljunen, M.
2010. Does lake thermocline depth affect methyl mercury concentrations in fish?
Biogeochemistry 101: 311–322.
- Roulet, N. & Moore, T.R. 2006. Environmental chemistry – browning the waters. *Nature* 444:
283-284.

- 665 Salonen, K., Kononen, K. & Arvola, L. 1983. Respiration of plankton in two small, polyhumic lakes. *Hydrobiologia* 101: 65-70.
- Scharnweber, K., Syväranta, J., Hilt, S., Brauns, M., Vanni, M.J., Brothers, S., Köhler, J., Knezevic-Jaric, J. & Mehner, T. 2014. Whole-lake experiments reveal the fate of terrestrial particulate organic carbon in benthic food webs of shallow lakes. *Ecology* 95: 1496–1505.
- 670 Solomon, C.T., Carpenter, S.R., Cole, J.J. & Pace, M.L. 2008. Support of benthic invertebrates by detrital resources and current autochthonous primary production: results from a whole-lake C-13 addition. *Freshwater Biology* 53: 42–54.
- Spence, J.R., & Andersen, N.M. 1994. Biology of water striders: interactions between systematics and ecology. *Annual Review of Entomology* 39:101–128.
- 675 Taipale, S., Kankaala, P., Tirola, M. & Jones, R.I. (2008). Whole-lake dissolved inorganic C-13 additions reveal seasonal shifts in zooplankton diet. *Ecology*, 89, 463–74.
- Taipale, S., Kankaala, P., Hämäläinen, H. & Jones, R.I. 2009. Seasonal shifts in the diet of lake zooplankton revealed by phospholipid fatty acid analysis. *Freshwater Biology* 54: 90–104.
- Taipale, S.J., Galloway, A.W.E., Aalto, S.L., Kahilainen, K.K., Strandberg, U. & Kankaala, P. 680 2016a. Terrestrial carbohydrates support freshwater zooplankton during phytoplankton deficiency. *Scientific Reports* 6:30897 DOI: 10.1038/srep30897.
- Taipale, S., Vuorio, K. Strandberg, U., Kahilainen, K.K., Järvinen, M., Hiltunen, M., Peltomaa, E. & Kankaala, P. 2016b. Lake eutrophication and brownification downgrade availability and transfer of essential fatty acids for human consumption. *Environment International* 96: 156–
- 685 166.
- Tanentzap, A.J., Kielstra, B.W., Wilkinson, G.M., Berggren, M., Craig, N., del Giorgio, P.A., Grey, J., Gunn, J.M., Jones, S.E., Karlsson, J., Solomon, C.T. & Pace, M.L. 2017. Terrestrial

support of lake food webs: Synthesis reveals controls over cross-ecosystem resource use.
Science Advances 3: e1601765.

- 690 Tranvik, L.J. 1988. Availability of dissolved organic carbon for planktonic bacteria in
oligotrophic lakes of differing humic content. *Microbial Ecology* 16: 311–322.
- Tranvik, L.J. 1992. Allochthonous dissolved organic matter as an energy source for pelagic
bacteria and the concept of the microbial loop. *Hydrobiologia* 229: 107–114.
- Tranvik, L.J. 1998. Chapter 10: Degradation of dissolved organic matter in humic waters by
695 bacteria. In: Hessen, D.O. & Tranvik, L. (Editors) *Aquatic Humic Substances: Ecology and
Biogeochemistry*. Ecological Studies 133, Springer-Verlag, pp 145-175.
- Vesterinen, J., Devlin, S.P., Syväranta, J. & Jones, R.I. 2016. Accounting for littoral primary
production by periphyton shifts a highly humic boreal lake towards net autotrophy.
Freshwater Biology 61: 265–276.
- 700 Vesterinen, J., Devlin, S.P., Syväranta, J. & Jones, R.I. 2016. Influence of littoral periphyton on
whole-lake metabolism relates to littoral vegetation in humic lakes. *Ecology* in press.
- von Wachenfeldt, E. & Tranvik, L.J. 2008. Sedimentation in boreal lakes- the role of flocculation
of allochthonous dissolved organic matter in the water column. *Ecosystems* 11: 803–814.
- Vuorenmaa, J., Forsius, M. & Mannio, J.(200). Increasing trends of total organic carbon
705 concentrations in small forest lakes in Finland from 1987 to 2003. *Science of the Total
Environment* 36: 47–65.
- Weidel, B., Carpenter, S.R., Cole, J.J., Hodgson, J., Kitchell, J., Pace, M.L. & Solomon, C.T.
2008. Carbon sources supporting fish growth in a north temperate lake. *Aquatic Sciences* 70:
446–458.

- 710 Weidel, B.C., Carpenter, S.R., J.F. & Vander Zanden, M.J. 2011. Rates and components of
carbon turnover in fish muscle: insights from bioenergetics models and a whole-lake ^{13}C
addition. *Canadian Journal of Fisheries and Aquatic Sciences* 68: 387–399.
- Wetzel, R.G. 2001. 3rd edn. Academic Press, San Diego, pp. 1-1006.
- 715 Wilkinson, G.M., Pace, M.L. & Cole, J.J. 2013a. Terrestrial dominance of organic matter in
north temperate lakes. *Global Biogeochemical Cycles* 27: 1-9.
- Wilkinson, G.M., Carpenter, S.C., Cole, J.J., Pace, M.L. & Yang, C. 2013b. Terrestrial support
of pelagic consumers: patterns and variability revealed by a multilake study. *Freshwater
Biology* 58: 2037-2049.

720 **Figure legends**

Figure 1. Changes in $\delta^{13}\text{C}$ (annual mean values \pm SE) of crustacean zooplankton in the manipulated lake Alinen Mustajärvi. Sugar additions to the lake were made during 2008 and 2009.

725

Figure 2. Changes in $\delta^{13}\text{C}$ (annual mean values \pm SE) of littoral macroinvertebrates in the manipulated lake Alinen Mustajärvi (AM) and the reference lake Valkea-Kotinen (VK). Upper panel shows differences in annual mean $\delta^{13}\text{C}$ between AM and VK. Sugar additions to AM were made during 2008 and 2009.

730

Figure 3. Changes in $\delta^{13}\text{C}$ (annual mean values) of selected littoral macroinvertebrate taxa in the manipulated lake Alinen Mustajärvi. Sugar additions to the lake were made during 2008 and 2009.

735 **Figure 4.** Changes in $\delta^{13}\text{C}$ (annual mean values \pm SE) of all analysed perch in the manipulated lake Alinen Mustajärvi (AM) and the reference lake Valkea-Kotinen (VK). Upper panel shows differences in annual mean $\delta^{13}\text{C}$ between AM and VK. Sugar additions to AM were made during 2008 and 2009.

740 **Figure 5.** Changes in $\delta^{13}\text{C}$ (annual mean values \pm SE) of perch from different year classes (2005-2012) in Alinen Mustajärvi. The range of number of individuals analysed per year (n) is shown for each year class. Sugar additions to the lake were made during 2008 and 2009.

Figure 6. Changes in $\delta^{15}\text{N}$ (annual mean values \pm SE) of perch (circles), macroinvertebrates
745 (diamonds) and zooplankton (squares) in the manipulated lake Alinen Mustajärvi (AM, filled
symbols) and the reference lake Valkea-Kotinen (VK, open symbols). Upper panel shows
differences in annual mean $\delta^{15}\text{N}$ between AM and VK for perch and macroinvertebrates. Sugar
additions to AM were made during 2008 and 2009.

750 Fig 1

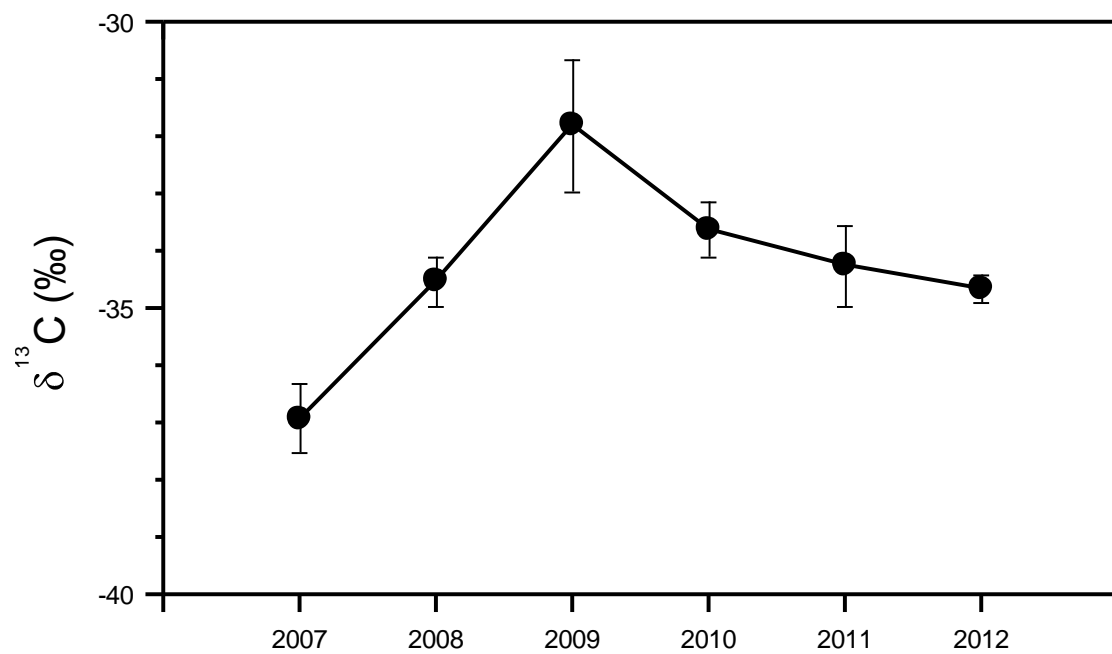
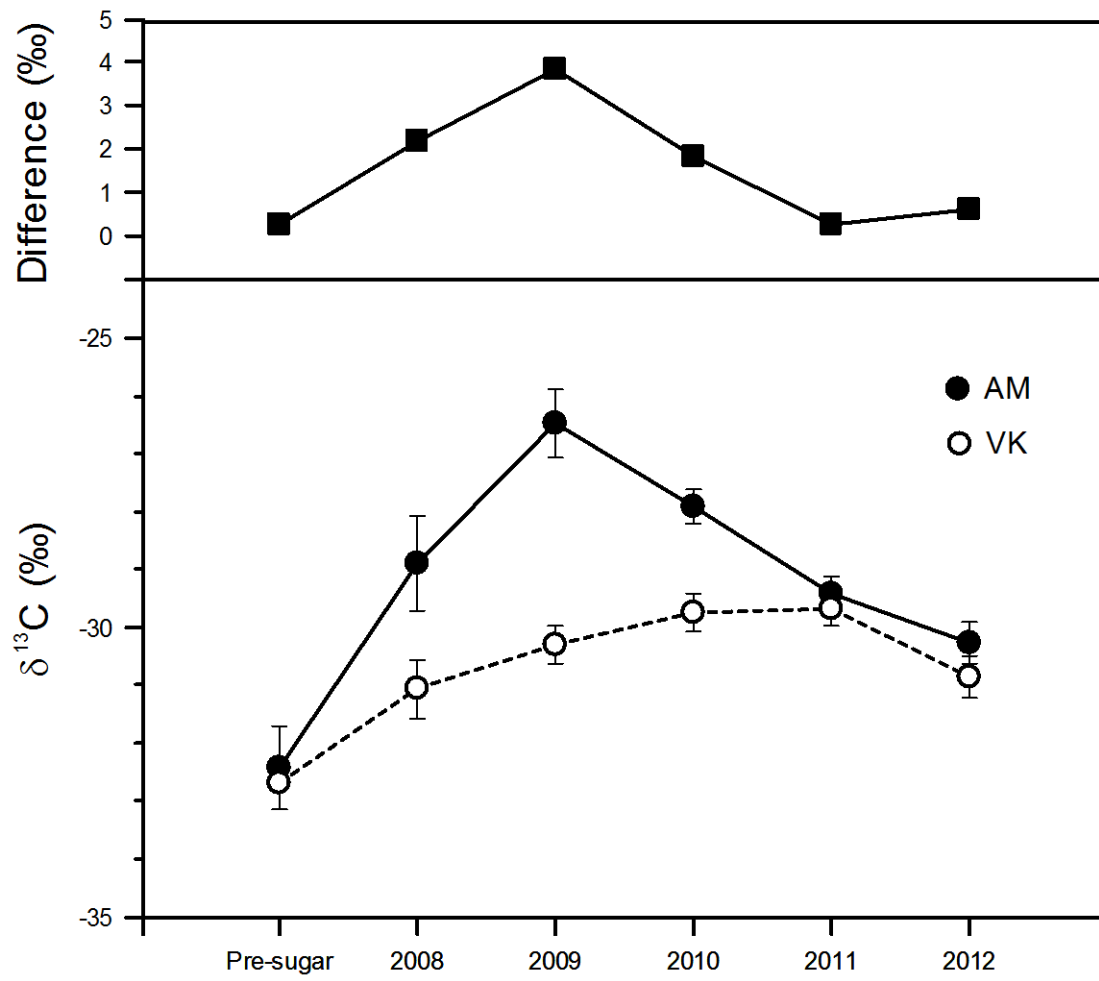


Fig 2



755

Fig 3

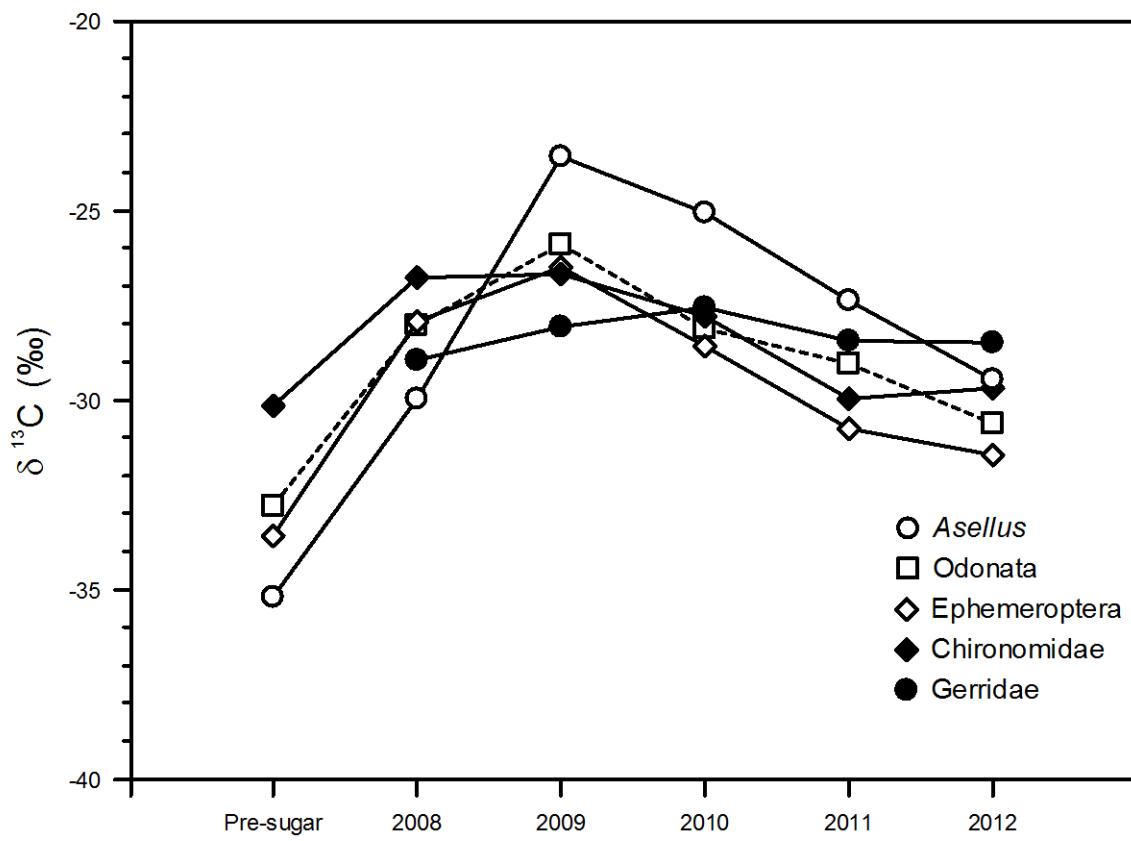


Fig 4

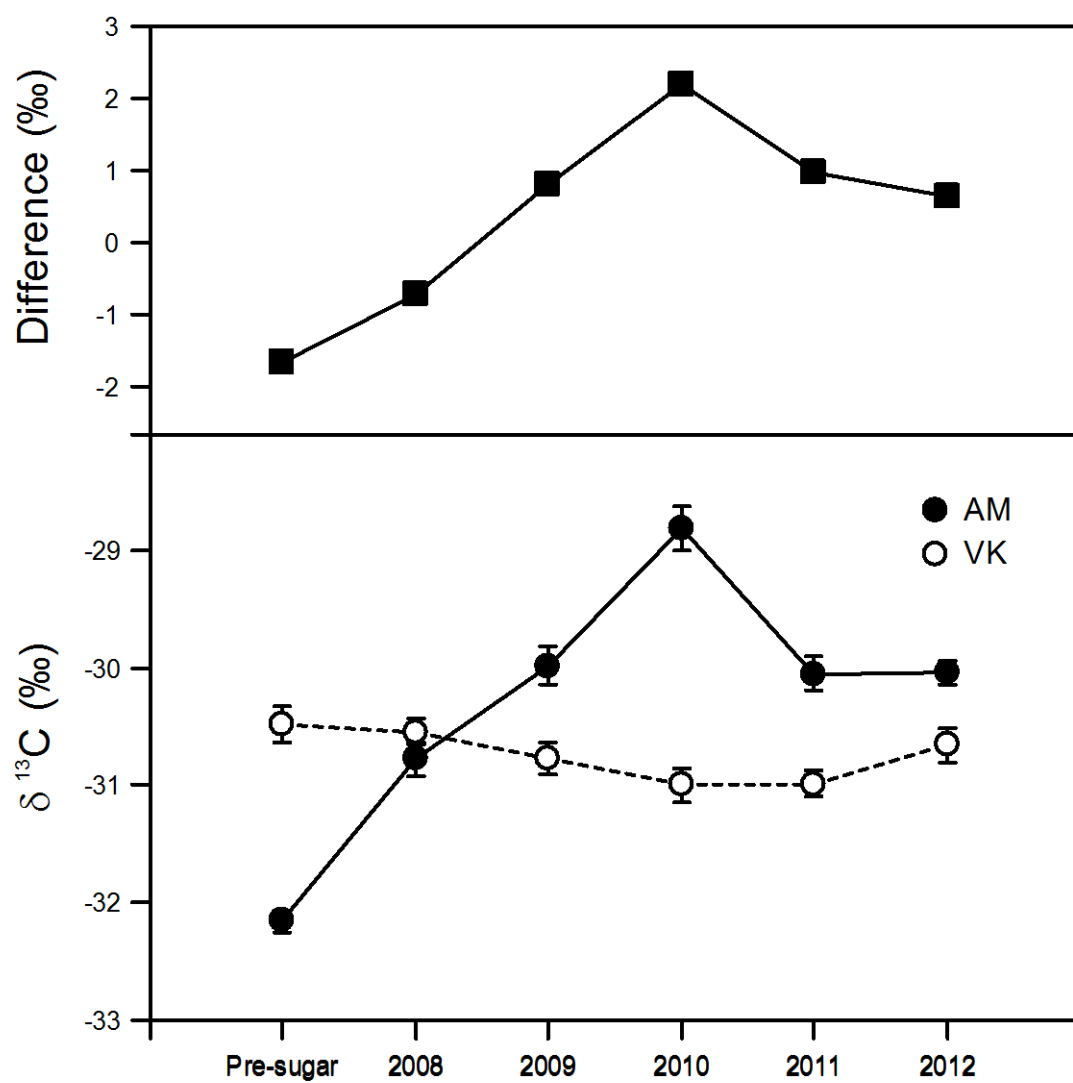


Fig. 5

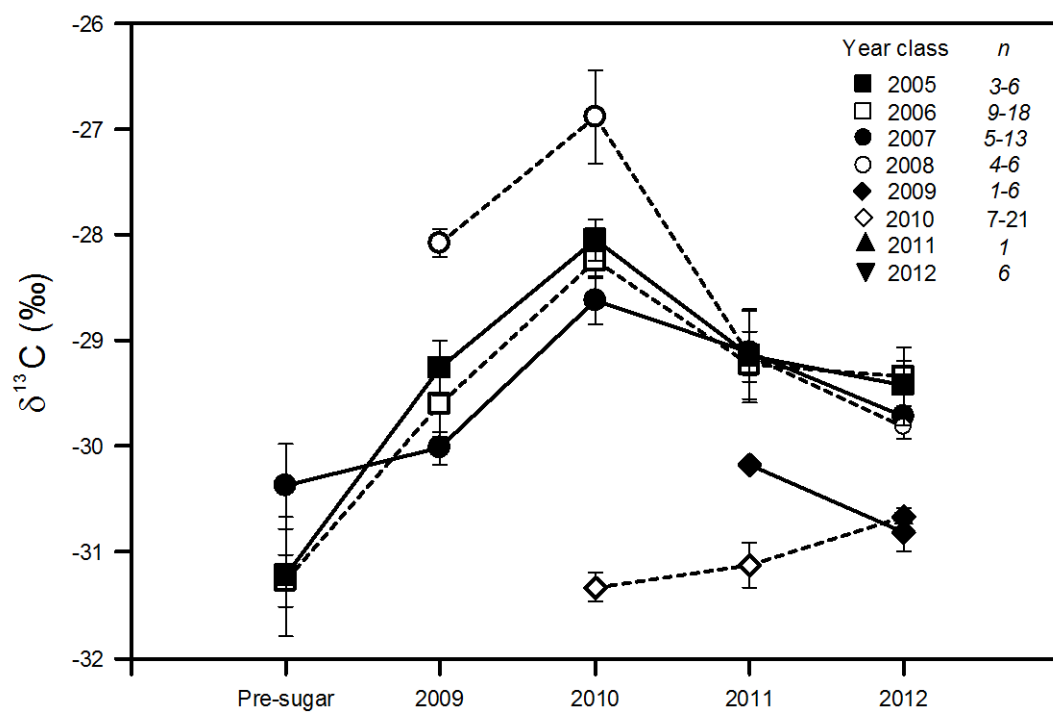
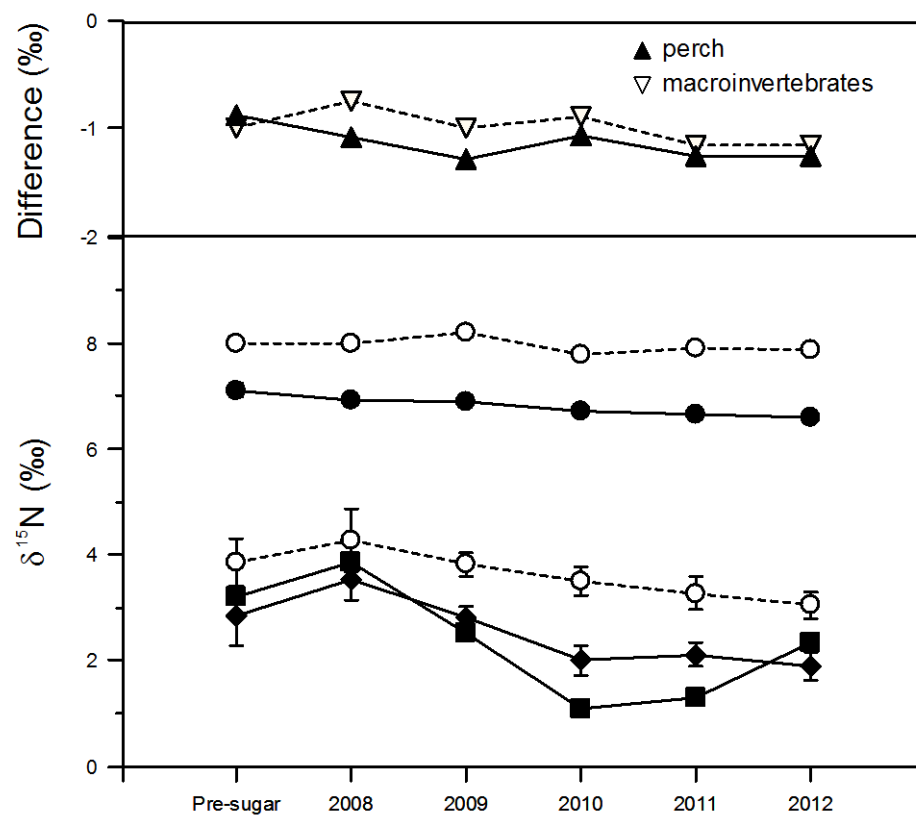


Fig. 6



Supplementary Information 1 Details of perch analysed from the manipulated lake Alinen-Mustajärvi (AM) and the reference lake Valkea-Kotinen (VK). Total number of fish analysed for each year is presented as numbers of males (M), females (F) and immature (I) individuals.

Lake	Year	Sampling months	Sex	Age (years)	Weight (g)	Length (mm)
				Mean (\pm SE)	Mean (\pm SE)	Mean (\pm SE)
AM	2007	May 2008	16M/17F/1I	3.9 (0.45)	20.6 (3.3)	117 (7.4)
	2008	Jul/Oct	13M/26F/0I	3.8 (0.44)	27.6 (3.0)	133 (5.1)
	2009	May/Jul/Oct	21M/41F/0I	4.6 (0.38)	27.2 (2.3)	135 (4.0)
	2010	May/Jul/Oct	25M/38F/0I	4.3 (0.33)	29.6 (2.6)	136 (3.9)
	2011	May/Jun/Aug	24M/35F/5I	4.1 (0.30)	24.6 (1.9)	129 (4.0)
	2012	May/Jul/Sept	16M/40F/4I	3.8 (0.31)	24.0 (2.0)	124 (4.1)
VK	2007	Sept	2M/19F/0I	4.1 (0.47)	35.0 (8.9)	132 (10.0)
	2008	May/Jul/Oct	6M/31F/1I	5.5 (0.47)	112.9 (15.1)	188 (11.5)
	2009	May/Jul/Oct	21M/41F/2I	3.8 (0.29)	35.8 (5.5)	131 (6.3)
	2010	May/Jul/Oct	24M/38F/1I	3.9 (0.28)	43.4 (7.8)	137 (6.8)
	2011	May/Jul/Sept	19M/35F/5I	3.6 (0.33)	28.9 (3.7)	127 (5.7)
	2012	May/Jul/Sept	32M/26F/5I	3.8 (0.33)	36.6 (5.4)	131 (6.0)