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Zooplankton allochthony is spatially heterogeneous in a boreal lake

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SUMMARY

1. The proportion of consumer biomass from terrestrial origin (i.e. allochthony) has been shown to vary greatly among lakes and also seasonally, but has been assumed to be spatially homogeneous within a lake. Given that the distribution of different organic carbon (C) sources tends to be spatially patchy in most lakes, this assumption may not be warranted.

2. We tested this hypothesis using a spatially intensive sampling designed to capture the in-lake heterogeneity in terrestrial inputs, phytoplankton, benthic algae and a dominant aquatic macrophyte (*Brasenia schreberi*: Cabombaceae) in a medium-sized boreal lake, and used a dual-isotope Bayesian mixing approach ($\delta^{13}C$, $\delta^{2}H$) to establish the degree of allochthony of the dominant copepod *Leptodiaptomus minutus* (Diaptomididae) across these sites. Samples were collected in spring when tributaries had high flow rates and aquatic primary producers (phytoplankton, macrophytes) had rapid growth rates, and in mid-summer when tributary flows were at the lowest.

3. There was substantial spatial variability in the stable-isotope composition of the copepod and consequently in its levels of allochthony in both seasons. Allochthony in *L. minutus* varied from 34 to 50% in spring and from 45 to 65% in summer, and this range was linked to the spatial variability in the main sources of organic C (terrestrial inputs via tributaries, *B. schreberi* and phytoplankton). Allochthony in *L. minutus* was lowest in areas dominated by macrophytes, and further influenced by the distribution of tributary-derived terrestrial C across the lake. Macrophyte and phytoplankton carbon contributed, respectively, up to 28 and 38% during growing season (spring) to the diet of the *L. minutus*, while benthic algae contribution was negligible.

4. Our results clearly show that the reliance of zooplankton on terrestrial C may be spatially heterogeneous even in a relatively small lake and, in particular, that macrophytes, whose distribution is typically patchier than that of phytoplankton, may play a major role in shaping the spatial patterns of zooplankton allochthony in lakes.

Keywords: Copepoda, macrophytes, mixing model, stable isotopes, terrestrial carbon

Introduction

One of the major interactions between terrestrial and aquatic ecosystems is mediated by the movement of terrestrial organic carbon to lakes and rivers (Polis, Anderson & Holt, 1997; Solomon *et al.*, 2015). At least some of this terrestrial carbon eventually enters aquatic food webs and is selectively allocated to different functions by the aquatic organisms. For example, recent studies have shown that lake bacteria tend to respire algal-derived C, whereas terrestrial carbon is preferentially allocated to biosynthesis (Guillemette, Leigh Mccallister & Del Giorgio, 2016). Terrestrial C assimilation leads to variable but often significant proportion of aquatic consumer biomass of terrestrial origin which we refer to as allochthony. The magnitude, variability and regulation of allochthony in freshwaters have received an increasing interest over the past decade, especially in

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zooplankton (Rautio, Mariash & Forsstrom, 2011; Wilkinson *et al*., 2013a; Berggren, Bergström & Karlsson, 2015). It is now clear that there is a large degree of variability in zooplankton allochthony among lakes (Berggren *et al*., 2014), from negligible (Pace *et al*., 2007; Francis *et al*., 2011) in large, clear water lakes, to >60% in smaller humic systems (Karlsson *et al*., 2012; Kelly *et al*., 2014). Allochthony has been shown to vary as a function of lake productivity (Karlsson *et al*., 2012), season (Rautio *et al*., 2011; Berggren *et al*., 2015), catchment type or size (Babler, Pilati & Vanni, 2011; Tanentzap *et al*., 2014) and lake size (Wilkinson *et al*., 2013a). This high variability in consumer allochthony results from the combination of the terrestrial influence in food sources, the availability of aquatic primary producers and the feeding strategy (Berggren *et al*., 2014). The main groups of freshwater zooplankton have major functional differences in terms of diet and food preference, and in the retention rates of carbon relative to food source availability and quality (Koussoroplis, Kainz & Striebel, 2013), which together generate a large degree of variability in allochthony between major zooplankton even within a given lake (Matthews & Mazumder, 2003; Brett, 2014). While some degree of spatial variability, for example, between profundal and littoral communities (Syväranta, Hämäläinen & Jones, 2006) has been addressed, the spatial variability in the relative importance of terrestrial C to lake consumers remains a major uncertainty in our understanding of lake food webs.

As the variability in allochthony has been shown to be related to organic matter sources among lakes (Wilkinson *et al*., 2013a), we can reasonably think that within-lake heterogeneity in the various C sources may lead to intra-lake variability in allochthony. For example, although some soil carbon enters the lake by runoff along the land–water interface, most of the terrestrial organic matter (OM) arriving to lakes is loaded through the tributaries (Polis *et al*., 1997). This generates a spatial pattern in the availability of terrestrial C, which has been shown to influence consumer diets (Doi, 2009). The spatial patterns of allochthony in aquatic consumers, particularly zooplankton, may be further influenced by the distribution of autochthonous carbon sources, i.e. aquatic primary producers (Taipale *et al*., 2013). Phytoplankton, benthic algae and aquatic macrophytes all contribute to the autochthonous carbon pool, the latter two playing a larger role in shallow depths where light and substrata are not limiting photosynthesis and settlement (Auderset-Joye *et al*., 2006; Cazzanelli *et al*., 2012). Currents from tributaries further influence the patterns of macrophyte settlement keeping their biomass low in fast flowing areas (Chambers *et al*., 1991). Macrophytes and phytoplankton in turn compete for light and nutrients (Scheffer *et al*., 1993; Vanderstukken *et al*., 2014) in addition to allelopathic interactions (Erhard & Gross, 2006), such that littoral zones with extensive macrophyte development are less likely to have high phytoplankton concentrations. These processes structure the spatial distribution of the different autochthonous organic carbon sources within aquatic ecosystems (Lapierre & Frenette, 2009).

The trophic link between terrestrial carbon sources and consumers is mediated by the consumption of dissolved (t-DOC) and particulate terrestrial organic carbon (t-POC) (Wilkinson *et al*., 2013a; Berggren *et al*., 2014). Most of the terrestrial OM arriving to lakes is in the form of t-DOC, which itself cannot be taken up by zooplankton and other metazoan consumers. The t-DOC pool can nevertheless be consumed by bacteria and has been shown to support a substantial fraction of the production at the base of the microbial food web in many lakes (Karlsson *et al*., 2012). Zooplankton acquire allochthonous organic carbon either through the consumption of bacteria or bacterial grazers, or by directly feeding on allochthonous particles (Cole *et al*., 2006). Although there is still considerable debate as to the importance of these two pathways (Pace *et al*., 2004; Cole *et al*., 2006), the current evidence would suggest that the latter is generally minor for zooplankton (Jansson *et al*., 2007; Berggren *et al*., 2010; Mehner *et al*., 2015), although it may be significant for benthic macroinvertebrates (Gerlach *et al*., 2014). The autochthonous signature of zooplankton, on the other hand, is acquired through direct feeding on phytoplankton cells, or POC that contains either live or detrital algal C. Benthic algae and associated microbial material and detritus are not available as POC for pelagic suspension feeder zooplankton (Paffenhöfer, Strickler & Alcaraz, 1982), although some cladocerans may feed directly on benthic mats (Cazzanelli *et al*., 2012; Mariash *et al*., 2014). To our knowledge, there is also no evidence for a direct consumption of macrophyte-derived POC, even when macrophytes contribute to the POC pool (Marinho *et al*., 2010; Cole & Solomon, 2012). However, macrophytes have been shown to release large amounts of DOC to the environment when they are growing (Alber & Valiela, 1994; Demarty & Prairie, 2009), and are decomposed (Maie *et al*., 2006). This DOC represents between 1 and 43% of the total DOC in shallow boreal lakes (Demarty, 2009). It is thus likely that the transfer of macrophyte-derived C to zooplankton is also mediated by bacteria, which have been shown to use this OM very efficiently (Findlay

et al., 1986; Mann & Wetzel, 1996; Wetzel & Sondergaard, 1998).

Regardless of its origin, the biologically labile portion of the DOC pool is usually very small and is taken up very rapidly by heterotrophic bacteria upon release to the water (Rosenstock & Simon, 2001; Berggren *et al.*, 2010). Consequently, although the bulk DOC from different sources will move with the water and eventually mix throughout a lake, the labile portion associated to the different DOC sources will most likely be consumed and exhausted locally in the vicinity of the source. Similarly, it is likely that particles originating from the various C sources will sink in the surrounding area of the source and fuel local benthic metabolism. If these various C sources are characterised by different chemical and isotopic properties, then these properties will be transferred to the microbial food web that is utilising this C locally, and to zooplankton feeding on this food web, in turn potentially generating variability in zooplankton isotopic composition. To our knowledge, there have been no studies to date assessing this potential spatial patchiness in zooplankton allochthony, and the underlying assumption of most previous studies has been that allochthony should be uniform within a given lake.

In this paper we have explicitly tested this assumption by carrying out a high-resolution study to quantify the spatial variability in zooplankton allochthony within Lake Simoncouche (Canada), a shallow (2.2 m average depth), medium-sized (0.83 km^2) boreal lake that receives large terrestrially derived OM inputs from several separate tributaries, but which also has extensive macrophyte development distributed in clumps, and significant algal production in its pelagic and benthic regions. This lake is therefore characterised by a strong spatial heterogeneity in the potential C sources, and we explicitly attempted to link this spatial variability to zooplankton allochthony. In order to do this we first estimated the contributions of different putative carbon sources to Lake Simoncouche by measuring the incoming terrestrial fluxes of DOC and POC, photosynthetic carbon production of phytoplankton and benthic algae, and the DOC release rates of the dominant aquatic macrophyte *Brasenia schreberi* (Cabombaceae). We then used DOC aromaticity and biolability as well as ^{13}C isotopic composition of POC to assess how the above mentioned sources contribute to creating spatial heterogeneity across the lake in the putative zooplankton resource pool. Furthermore, we determined the $\delta^{13}C$ and $\delta^{2}H$ of the various sources and of zooplankton biomass to estimate zooplankton allochthony across 10 sites within the lake, which covered five habitats dominated by distinct C sources: (i) tributaries with high terrestrial inputs, (ii) vicinity of *B. schreberi* beds, (iii) tributary flowing through macrophyte beds to account for terrestrial–macrophyte interactions, (iv) pelagic zones dominated by phytoplankton and (v) near shore control sites far from tributaries or macrophyte clumps to calculate zooplankton allochthony in sites that were not clearly dominated by any of the sources above. Additionally, benthic algae were considered to potentially influence zooplankton allochthony at all sites within Lake Simoncouche given that the mean depth is 2.2 m. Our study was carried out in two seasons to increase the variability range in the relative contribution of different putative zooplankton resources: during the spring, when phytoplankton bloomed, the macrophytes were starting to grow and tributary discharge was in its annual maximum, and in mid-summer, clear water phase, when macrophytes were abundant but growing slowly and discharge from the tributaries was low. We hypothesised that allochthony in zooplankton is spatially structured across the lake driven by the spatial distribution of carbon sources, with the highest allochthony potentially within the plumes of the tributaries. Furthermore, in order to limit the confounding effects of inter-species differences in diet and food preference, we focused our study on one copepod species *Leptodiaptomus minutus* (Calanoida: Diaptomididae), the dominant zooplankton throughout the year in this lake, which is also widespread across the boreal landscape (Carter *et al.*, 1980).

Methods

Study lake and sampling

Lake Simoncouche (48.23°N, 71.25°W, mean depth 2.2 m, maximum depth 8 m, surface area 0.8 km^2, see bathymetric map in Figure S1) is a mesotrophic boreal lake that is surrounded by a dense boreal forest, with its drainage basin dominated by *Abies balsamea* (Pinaceae), *Picea mariana* (Pinaceae) and *Betula papyrifera* (Betulaceae) populations (Montoro Girona *et al.*, 2016). Mean total dissolved phosphorous and nitrogen concentrations are, respectively, 8.2 ± 3.1 μg P L^{-1} and 0.3 ± 0.3 mg N L^{-1}, Secchi depth is 3.3 ± 0.3 m, conductivity 115 ± 86 μS cm^{-1}, pH 6.9 ± 0.6, DOC 5.3 ± 0.8 mg C L^{-1} and POC 1.1 ± 0.4 mg L^{-1}. The lake is divided into three basins (Fig. 1), with the third basin characterised by an extensive macrophyte bed that can cover more than 25% of total lake surface area, largely dominated by *B. schreberi*, with

isolated plants of *Nuphar* sp. (Nymphaeaceae) and *Potamogeton* sp. (Potamogetonaceae). The macrophyte community was also composed to a lesser extent of the submerged *Myriophyllum sibiricum* (Haloragaceae) and the emergent *Typha angustifolia* (Typhaceae). In this study, we focused on the dominant macrophyte *B. schreberi* which has floating leaves. The main tributary represents 70% of the incoming water to the lake and enters in the third basin. The two remaining basins have some isolated areas of macrophytes and the lake is supplied by 6 permanent and 10 intermittent tributaries. From the main tributary in the south to the main outlet in the north, the water crosses the three basins from basin 3 to basin 1. The mean lake water residence time is 50 days, although each of the three major basins likely differs in their own average water residence time, due to their own particular morphometry (see lake morphometry, Figure S1). The residence time is also seasonally variable (Vachon & Del Giorgio, 2014) amounting to only 30 days in spring but extending to as much as 76 days in winter (D. Vachon, pers. comm.). *Leptodiaptomus minutus* dominates the zooplankton community, representing up to 93% of the total zooplankton biomass in the lake, is found active all year long and is the only species found everywhere in the lake (G. Grosbois, unpubl. data). *Cyclops scutifer* (Cyclopidae), *Mesocyclops edax* (Cyclopidae), *Tropocyclops prasinus* (Cyclopidae), *Aglaodiaptomus spatulocrenatus* (Diaptomidae), *Daphnia* spp. (Daphniidae), *Bosmina* spp. (Bosminidae), *Diaphanosoma* spp. (Sididae) and *Holopedium* sp. (Holopedidae) constitute the rest of

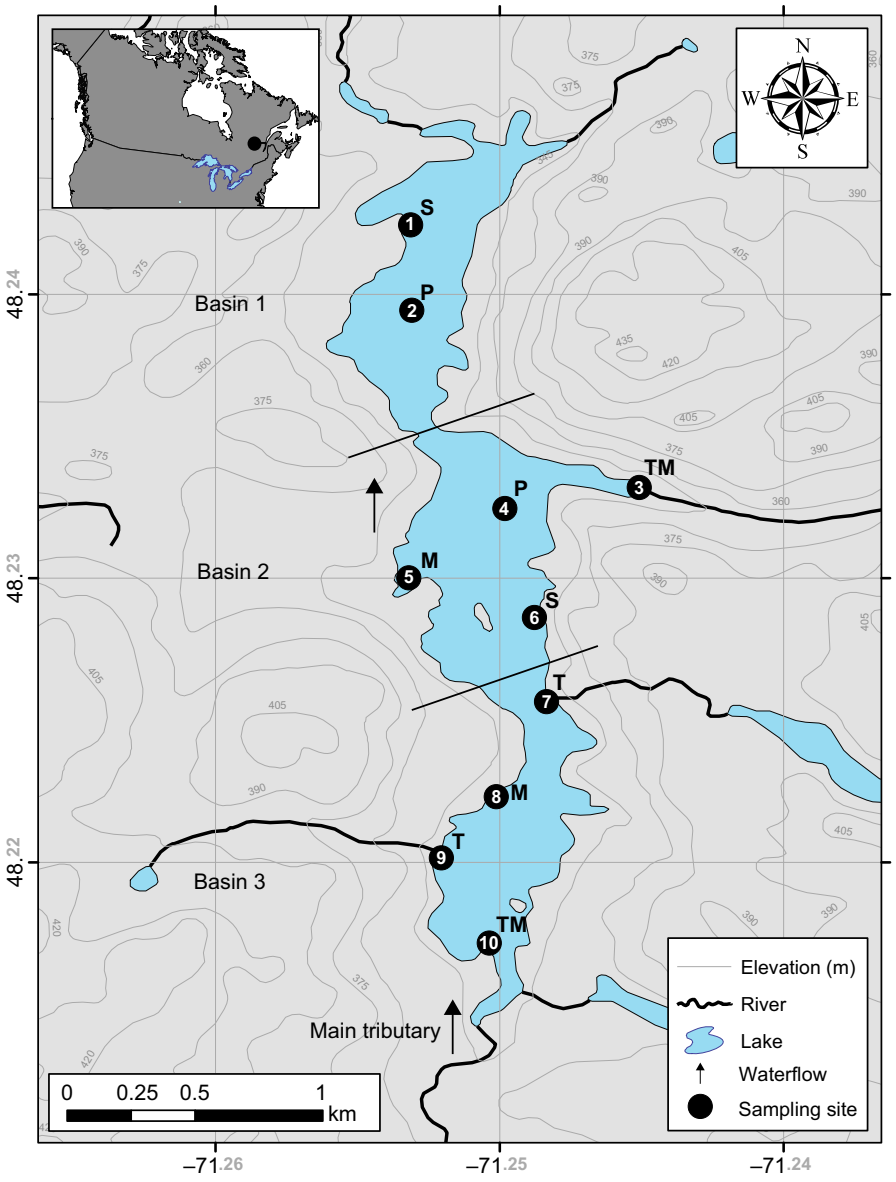

Fig. 1 Location of Lake Simoncouche (48.23°N, 71.25°W) in boreal Quebec, Canada. Numbered black dots show the sampling sites in different habitats: tributary (T), macrophytes (M), tributary + macrophytes (TM), pelagic (P) and shore (S). Water flow direction is from basin 3 to basin 1.

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zooplankton community in Lake Simoncouche. The lake was sampled twice in 2013; 2 weeks after the ice-out (20 and 21 May) when phytoplankton typically produce a spring bloom, macrophytes are in full development, and tributary discharge is high, and again in mid-summer during the clear water phase (5 and 6 August) when phytoplankton are less abundant, macrophytes are widespread but tributary discharge is low.

Characterisation of resource heterogeneity

To characterise the relative contributions of terrestrial carbon inputs, and phytoplankton, benthic algae and macrophyte production to the lake's putative resource pool for zooplankton, we estimated the inputs of t-DOC and t-POC from tributaries, as well as algal production rates and the DOC release from macrophytes. Inputs of t-DOC and t-POC from tributaries were calculated as a function of water discharge, measured with a flow meter (2030R; General Oceanics Inc., Miami), and DOC and POC concentrations at the tributary mouths, assuming terrestrial OM dominates the incoming carbon pools (Caraco & Cole, 2004). Water for DOC was filtered through combusted GF/D filters and stored in the dark at 4 °C for subsequent DOC analyses as in Lapierre & Del Giorgio (2014). This type of filter allows the passage of a small portion of the bacterial community, and was chosen because it is the one also used to prepare water for the DOC degradation assays (see below). Previous work in our group has shown that there are no measurable differences in DOC concentration relative to the use of the more conventional 0.45 μm pore size filters (P. A. del Giorgio, pers. comm.). Concentrations of POC were estimated only for the size fraction that represents *L. minutus* food source. This was done by passing 20 L of 50 μm sieved lake water (to remove animals) through a 20-μm sieve. Keeping only the >20 μm fraction allowed collecting the potential food that the selective adult copepods are directly feeding on (Wilson, 1973), while very small particles possibly ingested by nauplii, copepodites or rotifers were discarded. Here, this fraction represented about 50% of total POC. The POC samples were kept at −20 °C until freeze-drying.

Average macrophyte cover was estimated from aerial photographs taken over several years (1983–2007) and MapInfo professional software v.11.5 (S. Lévesque, unpubl. data). Although the submerged macrophytes cannot be detected using aerial photographs, our field observations showed that the distribution of the latter overlapped almost perfectly with that of *B. schreberi*. Macrophyte DOC release rates were derived on the basis of macrophyte cover following Demarty & Prairie (2009) who measured DOC release rates from similar macrophyte beds also in comparable lakes in Quebec. We used the average rates reported in that study (4.57 mg C m^{-2} h^{-1}) and multiplied these by the total surface covered by macrophytes, and by an average number of daylight hours to estimate the potential whole-lake DOC production of *B. schreberi*.

Gross primary production of phytoplankton was calculated with diurnal variations in hourly measurements of dissolved oxygen concentration (O_2) in surface water as in Vachon & Del Giorgio (2014). In short, GPP represented by the net O_2 production corrected for respiration rates was calculated from net ecosystem metabolism (NEM). Daily NEM is defined with the hourly changes of O_2 concentrations over time corrected for gas exchange with the atmosphere and integrated over a period from midnight to 23:00 hours, whereas night-time changes, NEM_{night}, represents respiration. Assuming that daily and night-time respiration are similar, GPP is calculated from the difference between integrated night and daily O_2 concentrations ($GPP = NEM + NEM_{night}$). Photosynthetic rates of benthic algae were measured *in situ* following the ^{14}C-bicarbonate protocol as in Rautio & Vincent (2006). Benthic algae (0.5 cm diameter) suspended in GF/F prefiltered lake water in replicated 20 mL vials, were spiked with ^{14}C-bicarbonate (specific activity: 80 μCi mL^{-1}) and exposed to eight different light intensities (100, 75, 30, 10, 4, 2, 1 and 0% of total solar radiation) at the water surface of the lake shore to obtain P-I curves. After 1 h incubation the samples were filtered on GF/F and kept at −20 °C until radioactivity was measured with a scintillation counter (TriCarb 2910TR PerkinElmer, Waltham). PAR intensity was measured at the surface and in the water column during the incubations using a PAR-meter connected to a LiCor Li1000 Data logger, Lincoln. These measurements were used to obtain the vertical light profile, from which the diffuse vertical attenuation coefficient was calculated for the whole lake estimations of phytoplankton and benthic algal productions.

A more detailed spatial characterisation of carbon resources to zooplankton was based on POC (>20 μm) and DOC concentrations, ^{13}C carbon isotopic composition of POC (hereafter $PO^{13}C$), DOC aromaticity and biolability as well as to chlorophyll-*a* concentration (Chl-*a*). These were measured at 10 sites in spring (May) and summer (August) 2013 that were characterised either with macrophyte beds (M), tributaries (T), both

macrophyte and tributary influence (TM), pelagic sites (P) and control sites on shore (S) without tributary or macrophytes. Each of these five habitat types was replicated twice resulting in 10 sampling sites. The four tributaries selected for this study contributed >95% of the water input to the lake (D. Vachon, pers. comm.). The specific UV absorbance ($SUVA_{254}$) was used as an index of DOC aromaticity and the relative proportion of allochthonous (terrestrial) versus autochthonous (algal) carbon sources (Weishaar *et al.*, 2003). It was measured as a DOC normalised absorbance at the wavelength 254 nm using a Cary 100 UV-Vis spectrophotometer (Agilent, Santa Clara). DOC biolability was measured as in Guillemette & Del Giorgio (2011). Briefly, water samples (0.5 L) were filtrated through GF/D filter to remove organisms larger than 2.7 μm, but to retain the bacteria community. Water was incubated in glass bottles at room temperature in the dark during 14 days. Aliquots were taken every 2 days, measured for DOC, and lability was estimated from the linear regression of DOC concentration versus time. Chlorophyll-*a* concentration was measured for each sampling site by filtering 500 mL on GF/F and extracting it on ethanol and measuring by fluorescence following Yentsch & Menzel, (1963).

Stable-isotope analyses

Terrestrial leaves, macrophytes, benthic algae, phytoplankton, POC and adult *L. minutus* copepods were analysed for stable isotopes. Once collected, the samples were freeze-dried in the laboratory, grinded and homogenised before encapsulation. The terrestrial signature for $\delta^{13}C$ and $\delta^{2}H$ was obtained either from dead litter collected near each site or live leaves from the main surrounding tree species ($n = 28$). Macrophytes were sampled in sites where they were present ($n = 12$) and analysed for the two isotopes. Most samples were from *B. schreberi*, although samples taken from *Nuphar* sp. ($n = 3$), which was also abundant at site 3, were isotopically indistinguishable from those of *B. schreberi* ($t = 1.47$, $P = 0.19$) and allowed the use of *B. schreberi* as a generic floating leaf macrophyte indicator. Benthic algae for $\delta^{13}C$ were collected scraping the surface of Nalgene bottles installed in the lake for several months allowing colonisation. Due to the lack of material on Nalgene bottles and because it was not possible to physically separate the benthic algal cells from the bulk mat material, the benthic algae stable-isotope signature $\delta^{2}H$ was estimated using the Bayesian mixing model with eqn 1 developed by Wilkinson *et al.* (2013a) using $\delta^{2}H$ of 0.2-μm filtered H_2O from each site in the lake and a fractionation distribution ε_H (144.5 ± 14.7‰) taken from Berggren *et al.* (2014). The POC (>20 μm) was collected as described above and was analysed for $\delta^{13}C$. Zooplankton were sampled from the whole water column with a 50-μm mesh net. The organisms were placed in 500 mL plastic containers with lake water and kept in a cooler until sorting live under a binocular. About 200 adult individuals of *L. minutus* were sorted for each replicate (3) from every site. Samples were then freeze-dried, ground to powder and encapsulated in tin ($\delta^{13}C$ and $\delta^{15}N$) or silver cups ($\delta^{2}H$). The zooplankton $\delta^{15}N$ signature was used to determine zooplankton trophic level, which was then used in the two isotope mixing model. In order to remove storage lipids that might reflect long-term storage diet, lipid extractions were carried out on zooplankton samples (Syväranta & Rautio, 2010). Lipids were removed from zooplankton using 1-mL wash of chloroform/methanol (2:1 v/v) (Bligh & Dyer, 1959). Samples were slowly shaken overnight and rinsed three times to remove all the lipids. Lipid-free zooplankton samples were dried in the oven (+60 °C) overnight.

Samples were analysed for $\delta^{13}C$ and $\delta^{15}N$ using a FlashEA 1112 elemental analyser (Thermo Fisher Scientific Corporation, Waltham) coupled to a Thermo Finnigan DELTA plus Advantage mass spectrometer in the University of Jyväskylä (Jyväskylä, Finland). Deuterium analyses ($\delta^{2}H$) were carried out at Colorado Plateau Stable-Isotope Laboratory in Northern Arizona University. Lake water and solid material $\delta^{2}H$ were measured according to Doucett *et al.* (2007), using a 1400 C TC/EA coupled to a Thermo-Electron Delta Plus XL mass spectrometer, Bremen.

To estimate the phytoplankton isotopic composition, $\delta^{13}C$ analyses were carried out on specific algal fatty acids that were recovered from bulk seston samples collected on GF/F filters (Pace *et al.*, 2007; Berggren *et al.*, 2014). We focused on 18:3ω3, 18:4ω3, 20:5ω3 and 22:6ω3 that are produced by algae (Mcleod & Wing, 2009; Barberá *et al.*, 2011). Fatty acids (FA) were extracted as in Mariash *et al.* (2011) using a modified extraction method from Bligh & Dyer (1959). Extracted FA methyl esters were obtained using a methylation procedure and evaporated to dryness. Samples were then shipped to Memorial University of Newfoundland for $\delta^{13}C$ analysis using a gas chromatograph interfaced with an IRMS via a combustion interface. We assumed a lipid fractionation of 3.8‰, and all FA $\delta^{13}C$ values were adjusted accordingly (Berggren *et al.*, 2014). We analysed 19 samples from years 2011 to 2013. They showed relatively low seasonal variability (−34.4 to −45.7‰) in algal FA $\delta^{13}C$, which were always

clearly separated from other FA. As for benthic algae, phytoplankton δ^2H signature were estimated using a fractionation distribution ($\varepsilon_H = 162.8 \pm 26.1‰$) from Berggren *et al.* (2014) and eqn 1:

$$\delta^2H \text{ Phytoplankton or Benthic algae(i)} = \delta^2H H_2O(i) - \varepsilon_H \quad (1)$$

Isotope mixing model

Four potential sources of C were considered to possibly contribute for *L. minutus* diet: terrestrial, phytoplankton, macrophytes (*B. schreberi*) and benthic algae. With two isotopes as tracers (^{13}C and ^{2}H), the mixing model is underdetermined and unique solutions are impossible. We consequently followed a two-step procedure, as recommended by Fry (2013). We initially run a Bayesian SIAR model that took into account the benthic, phytoplankton, terrestrial and macrophyte contributions, to determine which of these four sources likely contributed the least towards the diet of *L. minutus*. SIAR can be run with more sources ($n + 1$) than isotopes (n), and although this greatly increases output uncertainty (Parnell *et al.*, 2010) and number of possible feasible solutions for % source contributions (Fry, 2013), it does allow to establish a robust ranking of sources. This procedure allowed us to discard one of the sources (benthic algae), and we were then able to apply a three source, dual isotope ($\delta^{13}C$ and δ^2H) Bayesian mixing model, adapted from Wilkinson *et al.* (2014), to more robustly determine the relative contribution of the remaining three major C sources. In this Bayesian mixing model, uncertainties associated to not only source end-members and consumers but also for the isotopic fractionation between the sources and consumers (carbon fractionation for $\delta^{13}C$ and dietary water contribution for δ^2H) were accounted for. We added a correction to the model for potential trophic carbon fractionation, assuming a per trophic level enrichment (Δ_C) of $0.4 \pm 1.3‰$ (Post, 2002) adjusted to trophic level (τ). The trophic level of *L. minutus* was estimated on the basis of $\delta^{15}N$ using eqn 2, assuming that the $\delta^{15}N_{Daphnia}$ (Table S2) represent a food-web baseline, and $\delta^{15}N$ of *L. minutus* for each site as:

$$\tau = (\delta^{15}N_{L.\ minutus} - \delta^{15}N_{Daphnia})/\Delta_N + 1 \quad (2)$$

where Δ_N is the per-trophic-level stable nitrogen isotope fractionation of $3.4 \pm 1.0‰$ (Post, 2002). Trophic enrichment was then calculated accounting for the trophic level using eqn 3 for each site as:

$$\delta^{13}C \text{ trophic enrichment } (L.\ minutus) = \Delta_C \times \tau \quad (3)$$

where Δ_C is the per-trophic-level stable carbon isotope fractionation of $0.4 \pm 1.3‰$ (Post, 2002). The overall distribution of trophic $\delta^{13}C$ enrichment ± SD was then calculated running eqns 2 and 3 in sequence with 50 000 Monte Carlo iterations with random values of Δ_C and Δ_N generated from their assumed mean and SD.

The enrichment in δ^2H across trophic levels is not caused by trophic fractionation *per se* but rather to dietary water. Following Wilkinson *et al.* (2013a), we assumed that dietary water (ω) contributed 0.07 ± 0.10 per trophic level. The total contribution of water in the organism ($\omega_{tot;}$ eqn 4) was calculated as:

$$\omega_{tot} = 1 - (1 - \omega)^{\tau} \quad (4)$$

where τ is the trophic level. Dietary water enrichment was then calculated with eqn 5 for each sample as:

$$\delta^2H \text{ enrichment} = \delta^2H_{L.\ minutus} - (\delta^2H_{L.\ minutus} - \omega_{tot} \times \delta^2H_{water})/(1 - \omega_{tot}) \quad (5)$$

The overall distribution of δ^2H enrichment ± SD was then calculated with 50 000 Monte Carlo simulations, running eqns 2, 4 and 5 in sequence for each sample. The spatial distribution of allochthony in the lake was visualised in R (R development Core Team, 2011) with kriging interpolation with packages 'gstat' and 'maptools', which extrapolate unknown values of zooplankton allochthony for the entire lake surface area from the mixing model outputs (medians) calculated at known locations. The high spatial resolution of this study further allowed us to derive robust average estimates of allochthony for spring and summer for the entire lake with a high number of replicates ($n = 30$), i.e. accounting for all stable-isotope variation in the lake.

Statistical analysis

ANOVAs and Wilcoxon signed-rank tests were performed using the statistical computing environment of R to analyse within lake differences in DOC, POC, Chl-*a* concentrations and biolability. Pair-wise comparisons were performed using a *post hoc* test (Tukey's HSD). Pearson correlations were performed using SigmaPlot v.12.3 to test correlations between DOC biolability and Chl-*a*. Food sources, $PO^{13}C$ and zooplankton isotopic composition ($\delta^{13}C$ and δ^2H) were normalised subtracting means and dividing by standard deviation when tested using PermANOVA run with PRIMER v 6.1.11 & PERMANOVA+ v1.0.1 (Anderson, Gorley & Clarke, 2008).

PermANOVA analysis was used as a multivariate analysis ($\delta^{13}C$ and $\delta^{2}H$) due to the nature of the stable-isotope data which did not meet the conditions of Gaussian distribution and homoscedasticity.

Results

Contribution of autochthonous and allochthonous sources to lake resource pool

Terrestrial carbon, phytoplankton, benthic algae and macrophytes all made an important contribution to the Lake Simoncouche carbon pool (Table 1). The average spring discharge of the main tributary (site 10) was 689 L s^{-1} while the discharge in the other three tributaries (sites 3, 7 and 9) ranged from 67 to 187 L s^{-1}. Main tributary discharge was about sevenfold lower and the only measurable discharge in summer (97 L s^{-1}). Following the discharge rates, the spring t-DOC input was highest from the main tributary (316 kg day^{-1}) while the smaller tributaries contributed 36–94 kg day^{-1}. Summer t-DOC input from the main tributary was almost sixfold lower (53 kg day^{-1}). Spring input of t-POC was much lower than the t-DOC with the highest inputs coming from the main tributary (9 kg day^{-1}) compared to the other tributaries (2–6 kg day^{-1}). Summer t-POC input from the main tributary was about twofold lower (4 kg day^{-1}). Mean spring primary production was 330 kg C day^{-1} for the entire lake and decreased in summer to 263 kg C day^{-1}. Benthic production measurements reached 791 kg C day^{-1} in summer accounting for 75% of algal primary production. Macrophytes (*B. schreberi*) were distributed by clump and were totally submersed in spring while leaves reached the surface in summer. Mean cover estimates of macrophytes showed that basins 1, 2 and 3 had macrophyte coverage of 0.54, 2.59 and 11.41 ha, respectively, and contained 79% of the total lake macrophyte biomass. Applying the macrophyte DOC releasing rates from Demarty & Prairie (2009), basins 1, 2 and 3 received 0.4, 1.9 and 8.6 kg C day^{-1}, totalling 10.9 kg C day^{-1} DOC generated by macrophytes in the lake as a whole (Table 1).

Table 1 Contribution of terrestrial, phytoplankton, macrophyte and benthic carbon to Lake Simoncouche in spring and summer. Terrestrial contribution is expressed as t-DOC and t-POC input by the tributaries, phytoplankton and benthic carbon as rate of primary production, and macrophyte carbon contribution as a DOC release from macrophyte beds. NA, not available; DOC, dissolved organic carbon; POC, particulate organic carbon.

Source	Spring	Summer
t-DOC (kg C day^{-1})	445.8	52.9
t-POC (kg C day^{-1})	17.7	4.4
Phytoplankton (kg C day^{-1})	329.7	262.9
Macrophytes (kg C day^{-1})	NA	10.9
Benthic algae (kg C day^{-1})	NA	790.9

Spatial heterogeneity in the putative zooplankton resource pool

The carbon isotopic composition of POC in the 10 different sampling sites (−27.2 ± 1.3‰, Table 2) showed similar although slightly less negative values than the measured signature for terrestrial carbon suggesting an overwhelming contribution from terrestrial material with some contribution from an isotopically less depleted source (macrophytes: −24.4‰, benthic algae: −23.0‰). Macrophyte contribution to the resource pool was further evidenced by the spatial distribution of DOC concentrations, which was significantly higher in sites with macrophytes (7.1 mg C L^{-1}, $F_{1,16} = 3.40$, $P = 0.01$) than without macrophytes (5.8 mg C L^{-1}) in spring. DOC summer concentrations were less heterogeneous and no spatial distribution was observed ($F_{9,10} = 1.655$, $P = 0.22$). The quality of DOC was spatially variable, aromaticity ($SUVA_{254}$) in spring ranged from 2.3 to 4.6, whereas summer values had smaller range (2.7–3.2). DOC biolability, which reflects the potential DOC consumption by bacterial communities, varied among the habitats in spring ($F_{4,6} = 6.95$, $P = 0.02$) with highest values in the pelagic (Table 2), and was correlated with Chl-*a* ($R = 0.80$, $P = 0.02$). Summer DOC biolability showed a different pattern but still varied across the lake ($F_{9,10} = 9.00$, $P = 0.001$). Spring concentrations of Chl-*a* were spatially heterogeneous and significantly different between sites ($F_{9,18} = 9.29$, $P < 0.001$). This spatial distribution of Chl-*a* followed a gradient with lower concentrations in southern basins 2 (2.3 ± 0.9) and 3 (2.7 ± 1.8) and higher concentration at the opposite of the main tributary in northern basin 1 (3.9 ± 0.7). Summer Chl-*a* concentrations were also spatially distributed and significantly different between sites ($F_{9,20} = 5.99$, $P < 0.001$). Site 10 near the main tributary had the lowest concentration (0.9 ± 0.4) when compared to the other sites (mean 2.36 ± 0.8).

The average $\delta^{13}C$ isotopic compositions of phytoplankton (−40 ± 3‰), terrestrial matter (−28 ± 2‰), macrophyte *B. schreberi* (−24 ± 1‰) and benthic algae (−23 ± 1‰) were significantly different ($F_{3,53} = 158.36$, $P = 0.001$) from each other (all data pooled). The mean $\delta^{2}H$ isotopic composition also differed significantly ($F_{3,85} = 221.9$, $P = 0.001$) between the sources

Table 2 Dissolved and particulate organic carbon characteristics of the sampled habitats in spring and summer, including dissolved organic carbon (DOC), specific UV absorbance (SUVA), rate of DOC degradation (biolability), particulate organic carbon (POC), chlorophyll-*a* (Chl-*a*) and ^{13}C of POC ($PO^{13}C$). Sites as indicated in Fig. 1. Values are means ± SD. NA, not available.

		Dissolved organic carbon			Particulate organic carbon		
Habitat	Site	DOC (mg L^{-1})	SUVA (L mg C^{-1} m^{-1})	Biolability (μgC L^{-1} day^{-1})	POC (mg L^{-1})	Chl-*a* (μg L^{-1})	$PO^{13}C$ (‰)
Spring							
Tributary (T)	7, 9	5.7 ± 0.2	2.5 ± 0.2	18 ± 12	0.4 ± 0.3	3.5 ± 2.2	−26.9 ± 1.2
Macrophytes (M)	5, 8	6.0 ± 0.2	2.6 ± 0.2	26 ± 1	0.4 ± 0.1	1.8 ± 1.1	−27.5 ± 1.0
Tributary + macrophytes (TM)	3, 10	8.2 ± 2.4	3.6 ± 0.1	53 ± 15	0.6 ± 0.4	3.0 ± 0.8	−25.9 ± 1.3
Pelagic (P)	2, 4	6.0 ± 0.8	4.6 ± NA*	84 ± 22	0.3 ± 0.4	3.4 ± 1.1	−27.2 ± 1.4
Shore (S)	1, 6	5.7 ± 0.2	3.0 ± NA	25 ± NA	0.3 ± 0.2	2.3 ± 1.3	−27.2 ± 1.8
Summer							
Tributary (T)	7, 9	6.6 ± 0.3	2.9 ± 0.3	30 ± 10	0.8 ± 0.2	3.0 ± 0.5	−27.7 ± 1.2
Macrophytes (M)	5, 8	6.3 ± 0.3	2.8 ± 0.2	31 ± 17	0.3 ± 0.1	2.1 ± 0.7	−27.5 ± 0.3
Tributary + macrophytes (TM)	3, 10	6.5 ± 0.2	3.0 ± 0.0	48 ± 21	0.6 ± 0.3	1.9 ± 1.2	−26.9 ± 0.8
Pelagic (P)	2, 4	6.4 ± 0.1	2.7 ± 0.0	44 ± 9	0.5 ± 0.2	2.4 ± 0.4	−28.2 ± 1.4
Shore (S)	1, 6	6.5 ± 0.1	2.8 ± 0.1	60 ± 17	0.7 ± 0.5	1.7 ± 1.0	−27.9 ± 0.9

*Measured only in site 2 in basin 1.

(phytoplankton −240 ± 7‰, terrestrial OM −154 ± 11‰, macrophytes *B. schreberi* −149 ± 37‰ and benthic algae −224 ± 8‰; Fig. 2, Table S1).

Spatial distribution of allochthony in L. minutus

The $\delta^{13}C$ variability in *L. minutus* among samples showed a range between minimum and maximum value of 3.2‰ in spring (n = 28) and 3.0‰ in summer (n = 30). The spatial range of *L. minutus* δ^2H values was 15.3‰ in spring (n = 26) and 25.7‰ in summer (n = 30) (Table S2). There were no significant differences in $\delta^{13}C$ and δ^2H values of *L. minutus* in the sites associated with tributaries in spring (TM and T sites, Fig. 1) and all other sites ($F_{1,24}$ = 0.31, P = 0.72). However, *L. minutus* in sites with macrophyte beds had significant differences in $\delta^{13}C$ and δ^2H values compared to those in non-macrophyte sites ($F_{1,24}$ = 4.76, P = 0.02). In addition, *L. minutus* collected in sites that had a combination of macrophytes and tributaries (TM) showed $\delta^{13}C$ and δ^2H values that were significantly different from all other sites ($F_{1,24}$ = 12.67, P = 0.001). Zooplankton had less depleted $\delta^{13}C$ signatures in sites with both macrophytes and tributary. In summer, no differences were found between the stable-isotope signatures of *L. minutus* sampled at sites with and without tributary influences ($F_{1,26}$ = 2.56, P = 0.09), but there were significant differences between sites with and without macrophytes ($F_{1,26}$ = 3.21, P = 0.048).

The SIAR outputs showed that the zooplankton diet was expected to be mostly made of phytoplankton, terrestrial and macrophyte originating carbon sources while the benthic organic material was predicted to contribute only 7.2 ± 5‰ (mean ± SD of posterior probability distribution) in spring and 8.2 ± 7‰ in summer to zooplankton diet for all sites (Fig. 3a). Given this low apparent contribution of benthic algae, these were excluded from further analyses, which were based on the more robust Bayesian mass balance model on two isotopes and three food sources (terrestrial, phytoplankton and macrophyte *B. schreberi*). All the isotopic data for *L. minutus* fit well within the source end-member polygons (Fig. 2), although in spring the terrestrial and macrophyte end-members were somewhat aligned with the zooplankton (Fig. 2a). Consequently, the model did not effectively discriminate the terrestrial and macrophyte contributions in the pooled spring data, as is reflected in the large probability ranges around the mean estimates (Fig. 3b). The terrestrial contribution or overall allochthony in spring had a median of 30% but included a high range of the 95% highest probability densities (0–71%), whereas for the summer data, the model output was clearer with a median allochthony of 63% (41–74%) to the *L. minutus* diet. Phytoplankton were the dominant C source in spring (median 42% with a probability distribution of 28–54%, and had a similar but lower contribution in summer (median 34% with a probability distribution of 25–42%). Macrophytes appeared to be a significant source in spring, with a median of 28%, but the probability range was large (0–51%), although in summer their contribution to the

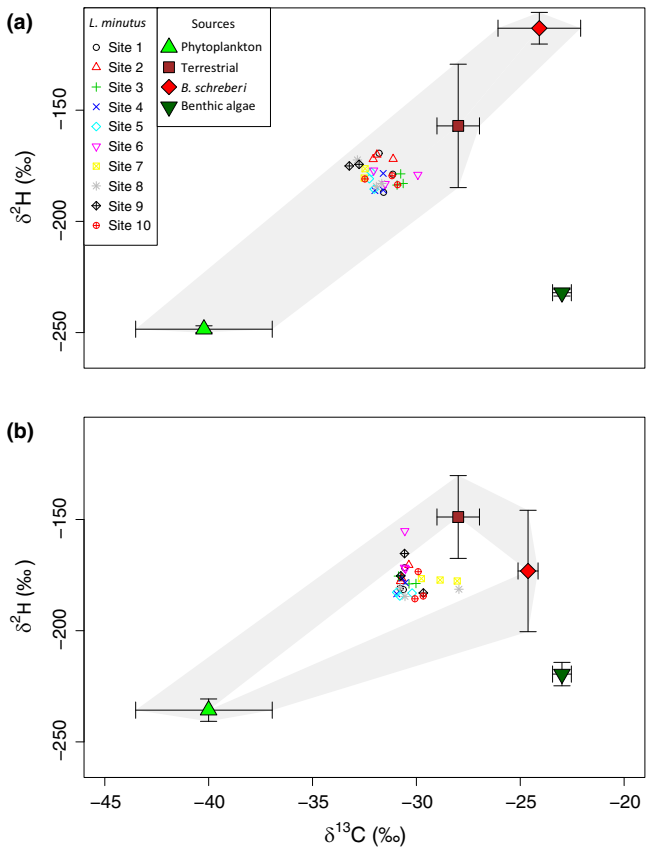

Fig. 2 The distribution of $\delta^{13}C$ and $\delta^{2}H$ signatures of *Leptodiaptomus minutus* (corrected for dietary water and carbon fractionation) inside a polygon of the potential food sources + SD: terrestrial, macrophyte (*Brasenia schreberi*) and phytoplankton. *Leptodiaptomus minutus* stable-isotope signatures are represented according to sampling sites. (a) Spring (May 2013) and (b) summer (August 2013). The isotopic composition of benthic algae that was not included in the Bayesian model is also represented in the figure.

zooplankton diet was small (Fig. 3b). At a spatial scale, zooplankton collected from the sites that had no or few macrophytes expressed very low (median 1%) macrophyte-derived C in their tissues. In sites dominated by macrophytes, zooplankton had a much higher putative macrophyte contribution of 29% (0–50%; Fig. 4). Spatial distribution of allochthony, on the contrary, was not as confined to sites that were in the vicinity of tributaries. In spring when the tributary discharge was at its maximum and water residence time was short (30 days), the incoming terrestrial carbon was assimilated mainly in basin 1, across the lake from the main tributary, where it was reflected as high allochthony values in zooplankton. In summer during the low flow rates, most terrestrial carbon was assimilated close to the main tributary and reflected in zooplankton tissues in the southern part of the lake in basin 3 (Fig. 5).

Discussion

As expected, we observed a large spatial heterogeneity in the various C sources, both between seasons and spatially across Lake Simoncouche. The pelagic, macrophyte and tributary-dominated sites were characterised by different quantity and quality of organic carbon, as evidenced by varying DOC concentrations and biolability among sites. Our results clearly show that this carbon source heterogeneity was reflected in the zooplankton stable isotopic composition. The $\delta^{13}C$ variability in *L. minutus* among samples showed a range between minimum and maximum value of 3.2‰ in spring and 3.0‰ in summer, close to the within-lake variability range of 2.7–3.1‰ reported for zooplankton in other lakes (Matthews & Mazumder, 2006; Syväranta *et al.*, 2006; Karlsson *et al.*, 2012). The spatial range of *L. minutus* $\delta^{2}H$ values was 15.3‰ in spring and 25.7‰ in summer. To our knowledge, such within-lake $\delta^{2}H$ variability has not been reported for zooplankton in the past, and the variability in zooplankton $\delta^{13}C$ has been either ignored, or attributed to change in community composition or life stages (Grey, Jones & Sleep, 2001). Here we show that the isotopic variability in *L. minutus* $\delta^{2}H$ and $\delta^{13}C$ composition was high and attributable to differential use of terrestrial, phytoplankton and macrophyte-based diets (and to a lesser extent, to benthic algae) among different sites. At a fine spatial scale, allochthony in *L. minutus* was most influenced by the presence of macrophytes, which tended to result in decreased proportion of terrestrial C incorporated near macrophyte beds. At a broader spatial scale, the rate of movement of tributary water across the lake and its associated C, which showed strong seasonal patterns, contributed to generating spatial heterogeneity and resulted in zooplankton assimilating different quantities of allochthonous carbon in different lake basins.

Spatial heterogeneity of C resources

We have shown that carbon sources were not uniformly distributed across the lake. The majority of terrestrial DOC and POC entered Lake Simoncouche in spatially highly defined location by its main tributary. Carbon inputs (DOC + POC) from this main tributary were highest in spring (325 kg C day^{-1}), about threefold higher than those of the 2nd tributary (100 kg C day^{-1}) and ninefold higher than from the 3rd tributary (38 kg C day^{-1}). This tributary was the only active input of terrestrial C in the summer (57 kg C day^{-1}), clearly demonstrating that t-DOC and

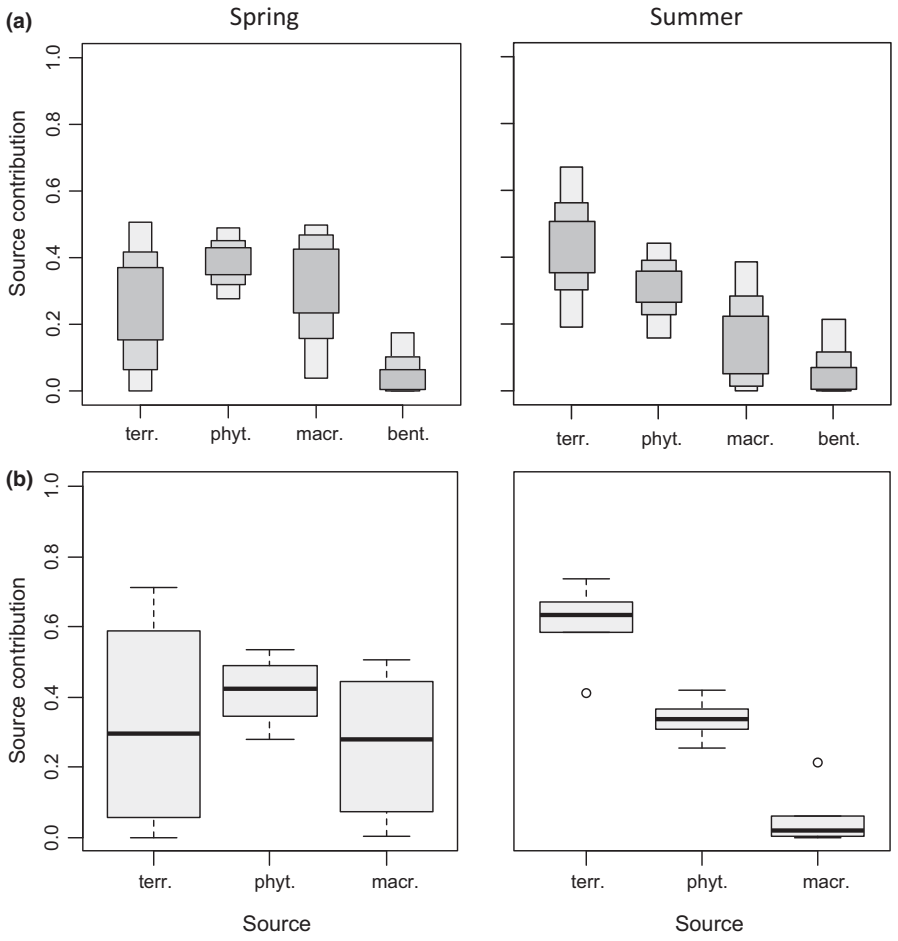

Fig. 3 (a) Spring and summer contributions of terrestrial organic matter, phytoplankton, macrophytes and benthic algae to *Leptodiaptomus minutus* tissues calculated with Bayesian SIAR model, and (b) fractional spring and summer contribution of phytoplankton, terrestrial organic matter and macrophytes to *L. minutus* tissues, based on Bayesian mixing model. Whisker plots show the distribution of 95% highest densities of contribution probabilities.

t-POC enter the lake unevenly. According to our measurements, basin 3 received around 78% of the tributary C while only 22% entered through basin 2 and very little in basin 1. These tributary C inputs appeared to then dominate the lake C pools: POC $\delta^{13}C$ measured in Lake Simoncouche (mean $-27.3‰$) were similar to values reported for terrestrial C3-plants ($-28.3‰$) indicating allochthonous particles were the dominant component of POC. Whereas we did not measure $\delta^{13}C$ of DOC in this study, previous studies have reported that the isotopic composition of DOC in Lake Simoncouche is also essentially identical to terrestrial C (Berggren *et al*., 2014). This would suggest that internal sources of DOC and POC are either smaller in magnitude, either yield C that is more labile and therefore is consumed more readily and does not build up in the bulk DOC (Wilkinson, Pace & Cole, 2013b). In the light of our results, the high biolability values associated with macrophyte beds and high seston Chl-*a* content point to the patchy production of labile internal sources, which however were masked with the overwhelming presence of terrestrial $\delta^{13}C$ in the seston C pool.

Lake Simoncouche, like most small and shallow boreal lakes, has a short water retention time (1–3 months), and materials brought in by tributaries must be quickly flushed through the lake. There were nevertheless spatial differences in some properties of POC and DOC within the lake. For example, as mentioned above, there were higher lability values in the pelagic (40–89 μg C^{-1} day^{-1}) compared to the inflowing waters (18–39 μg C^{-1} L^{-1}), suggesting that in spite of the high rate of flushing, there may still be local C signatures that reflect local C sources. In this regard, the presence of macrophytes increased the local DOC concentration by up to 20%, and this is probably linked to the release of highly biolabile DOC that bacteria may efficiently convert to new biomass (Findlay *et al*., 1986). The biolability was lower for the tributary DOC, but passage through macrophyte beds resulted in a 200% increase in biolabile DOC in spring. It is possible that the fresher and more biodegradable carbon from macrophytes (Stets & Cotner, 2008) acted as a primer for DOC bacteria degradation. Evidence of such priming effect has been increasingly shown in freshwater literature (Guenet *et al*., 2010;

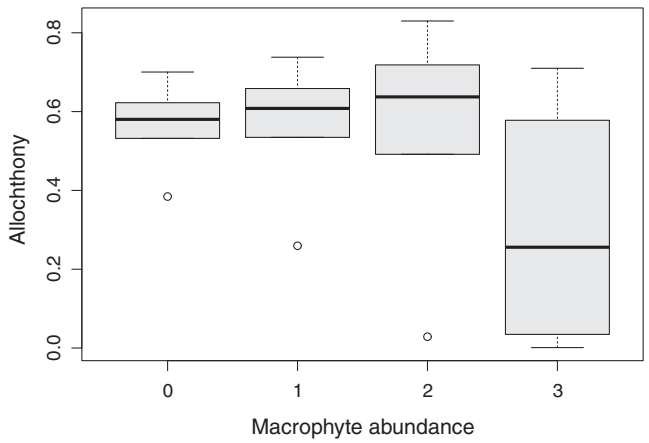

Fig. 4 *Leptodiaptomus minutus* allochthony in relation to the presence of macrophytes. 0 = sites without macrophytes, 1 = low abundance of macrophytes (basins 1 and 2), 2 = high abundance of macrophytes (basin 3) and 3 = macrophyte sites. Whisker plots show the distribution of 95% highest densities of contribution probabilities.

Danger *et al.*, 2013). Another source of biolabile DOC was revealed with a strong correlation between Chl-*a* and carbon biolability in spring ($R = 0.80$, $P = 0.02$) which suggest that the phytoplankton also contributed to a higher carbon consumption by bacteria via high quality DOC release, which is expected for phytoplankton in the exponential growth phase (Myklestad *et al.*, 1989; López-Sandoval *et al.*, 2013).

Spatial variability in putative allochthony

Combining the information of the distribution of the four putative resource pools and their isotopic signatures we were able to make estimates of zooplankton allochthony and its controlling factors across sites within the lake. On average, allochthony of the zooplankton in Lake Simoncouche was moderately high (medians: 34–65%) situated in the upper range of the reported allochthony in copepods (3–50%) for North American lakes (Wilkinson *et al.*, 2013a), reflecting the dominance of terrestrial organic material in the lake's resource pool. However, this allochthony was highly variable among different lake habitats and the two seasons considered. A whole-lake estimate based on incorporating all samples in the Bayesian mixing model showed a zooplankton allochthony of 30% in spring and 63% in summer. This lower spring allochthony

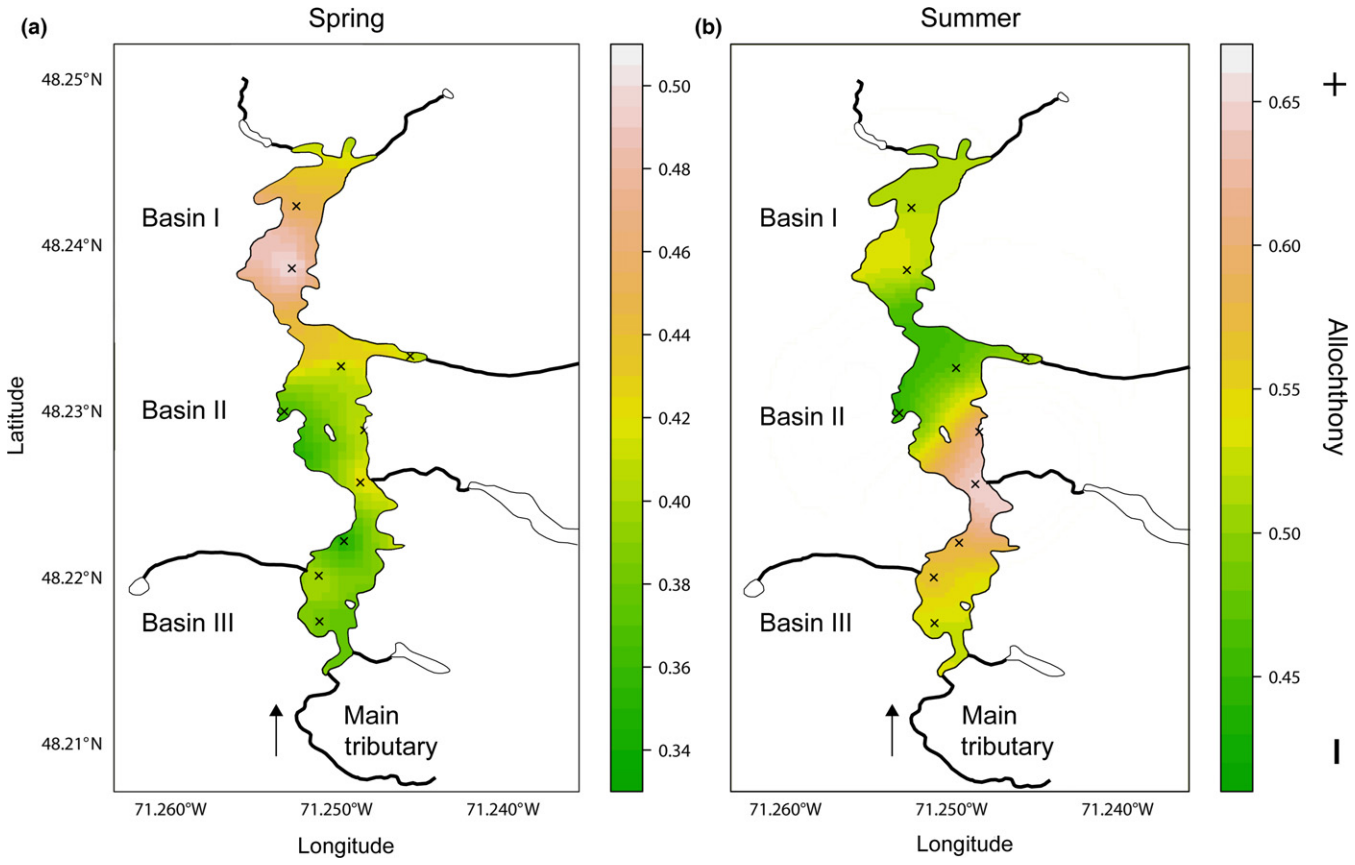

Fig. 5 Spatial distribution of *Leptodiaptomus minutus* allochthony calculated from median output of Bayesian mixing model and extrapolated by kriging in Lake Simoncouche for (a) spring and (b) summer. Notice the different scales.

agrees well with previous studies, which showed that calanoids switch to spring herbivory in boreal lakes (Berggren *et al*., 2015), in subarctic lakes (Rautio *et al*., 2011) or in marine environment (Gentsch *et al*., 2008). High zooplankton dependency on spring phytoplankton bloom (Grey *et al*., 2001) has been attributed to the higher quality of algal food sources and higher diet selectivity from zooplankton. Different physiological demands of spring zooplankton population may also play a role. *Leptodiaptomus minutus* demand for highly energetic compounds peaks in spring because individuals are reproducing (Schneider *et al*., 2016) and require algal-produced polyunsaturated fatty acids that are essential for reproduction (Muller-Navarra *et al*., 2000; Brett *et al*., 2009). The allochthony estimates increased in summer. This pattern is in accord with Lake Simoncouche switching from net autotrophy in early summer to net heterotrophy in August (Vachon & Del Giorgio, 2014), and supportive of the idea that consumers are more dependent on allochthonous carbon at times when local autochthonous production is reduced.

The spatial variability in allochthony was largely explained by the replacement of allochthonous carbon by macrophyte carbon. When macrophytes were absent or distant from the habitat, *L. minutus* showed high and well constrained allochthony (median 58%). Similarly high allochthony (median 61%) was observed for *L. minutus* in basins 1 and 2 that were located furthest from the main macrophyte bed. However, allochthony in zooplankton sampled next to the growing macrophytes had a median of only 26% (Fig. 4). The highly biodegradable carbon leaching out from macrophytes (Findlay *et al*., 1986) seemed to have reached *L. minutus*, most likely via the microbial loop, but in order to show an influence on the stable-isotope compositions of *L. minutus* the macrophytes needed not only to release large enough quantities of carbon but also to be located in the near vicinity of zooplankton. Such macrophyte influence has also been observed in different basins of a tropical urban lake (De Kluijver *et al*., 2015).

The spatial variability in *L. minutus* allochthony further indicated that the three lake basins were different in zooplankton assimilation of terrestrial C. Each basin was characterised by very different incoming water inflows and morphometry, with the main tributary in the shallow basin 3 dominating the total inflow. In spring, when the tributary discharge was at its maximum and water residence time was short (30 days), the incoming terrestrial C moved across the lake quickly and terrestrial C appeared to have been carried throughout basins 3 and 2, as was also indicated by the high SUVA values in basin 1, and assimilated mainly in basin 1. Thus, it is interesting to point out that basin 1, which is located furthest from the main tributary, was the basin with the highest degree of allochthony in spring (Fig. 5a). The assimilation of this terrestrial C into zooplankton requires a minimum processing time, because it needs uptake by bacteria and fungi and trophic transfer of the resulting biomass up the microbial food web (Wurzbacher, Bärlocher & Grossart, 2010). Our results clearly reflect this process, because at times of high tributary discharge and thus high flushing of the lake water, allochthonous carbon is not necessarily incorporated into zooplankton at the point of entry but rather further downstream, in our case, the distal points such as basin 1. In contrast, in periods of low tributary flow, the residence time of terrestrial C near the point of entry is longer, and this allows for a higher degree of allochthony in the local zooplankton, and we found the highest degree of allochthony (53–65%) in basin 3, close to the main tributary.

Collectively, these results suggest that it is not only the amount and quality of C loaded from land that will determine its influence on aquatic consumer allochthony, but also that the lake morphometry and residence time of this C within the system will play a major role, *sensu* Grey *et al.* (2001). Lake Simoncouche is a relatively small, shallow lake that has a short retention time and is thus constantly mixed and flushed, and it could be expected that larger lakes that have longer residence times, and greater spatial decoupling between pelagic, benthic and littoral communities could harbour an even higher degree of spatial variability in zooplankton allochthony. According to our findings, the degree of allochthony in zooplankton appears to be driven mostly by local increases in the availability of autochthonous C derived from phytoplankton and macrophytes. Although phytoplankton are distributed throughout lakes, macrophytes are extremely patchy, and this was here one of the main determinants of the spatial heterogeneity in zooplankton allochthony. Macrophytes are a major feature of the majority of lakes in the boreal biome and elsewhere, but have to our knowledge seldom been considered in food-web studies on zooplankton allochthony. It is clear that habitat heterogeneity and alternating states, *sensu* altering phytoplankton-dominated versus macrophyte-dominated states in shallow lakes (Janssen *et al*., 2014), will have to be taken into account in future studies of allochthony in lakes.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Bathymetric map of Lake Simoncouche.

Table S1. Raw stable-isotope signatures ($\delta^{13}C$, $\delta^{15}N$ and $\delta^{2}H$) of zooplankton (*Leptodiaptomus minutus*) and potential food sources in Simoncouche lake.

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