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4 **Benthic foodweb structure in a large shallow lake studied by stable isotope analysis**

5

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15

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17 **Abstract:** The benthic foodweb structure of Lake Võrtsjärv, a large (270 km<sup>2</sup>), shallow, and  
18 turbid Estonian lake, was evaluated based on C and N stable-isotope signatures ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ).  
19 Variation in  $\delta^{13}\text{C}$  between sampling sites was not related to site proximity to the littoral zone or  
20 the more vegetated southern part of the lake, but rather appeared to be influenced by in-situ site  
21 peculiarities.  $\delta^{13}\text{C}$  was stable temporally and between functional feeding groups, a result  
22 implying that the whole benthic food web of the lake relies largely on the same C source  
23 admixture, essentially particulate organic matter (POM). Thus, the foodweb composition of Lake  
24 Võrtsjärv is remarkably homogeneous given the lake's large surface area. Apparent trophic-level  
25  $\delta^{15}\text{N}$  fractionation between total collectors and total predators (mean 1.7‰) was lower than the  
26 value of 3.4‰ generally adopted in foodweb studies, but the higher value was valid for specific  
27 prey–predator links. The low  $\delta^{13}\text{C}$  signature of some chironomid samples indicated probable  
28 assimilation of methane-oxidizing bacteria (MOB) by these sediment-dwelling invertebrates.  
29 However, the lack of similar  $^{13}\text{C}$  depletion in benthic filterers (mussels) indicated that the MOB  
30 layer is essentially confined to the sediments and does not reach the water column, which  
31 probably constrains transfer of methane-derived C through the food web to fish in this lake. Our  
32 study demonstrates that the benthic food web of shallow turbid lakes like Võrtsjärv is simplified  
33 and is mostly sustained by phytoplanktonic C sources.

34 **Key words:** carbon and nitrogen stable isotopes, benthic food webs, Lake Võrtsjärv,  
35 macroinvertebrates, carbon source, methane-oxidizing bacteria, trophic levels

36

37           Benthic food webs have been underresearched compared to pelagic food webs, but they  
38 are of great importance for energy flux and organic-matter cycling in lakes (Vadeboncoeur et al.  
39 2002). In clear-water oligotrophic lakes, primary production is usually dominated by benthic  
40 periphyton (Ask et al. 2009). Conversely, in eutrophic, turbid lakes, most of the primary  
41 production is by phytoplankton because the euphotic zone does not reach the bottom of the lake.  
42 Thus, light restricts periphyton production (Vadeboncoeur et al. 2003). Nevertheless, benthic  
43 secondary production often is extremely important in these lakes. Phytoplankton assemblages in  
44 eutrophic lakes frequently comprise poorly grazed colonial or toxic cyanobacteria (Scheffer et al.  
45 1997). Instead of being consumed by zooplankton, this phytoplankton production is assimilated  
46 into the microbial loop by bacterioplankton (Agasild and Nöges 2005, Zingel et al. 2007) or  
47 provides food for benthic detritivores after it sediments to the bottom of the lake. In shallow  
48 lakes, the main benthic consumers are bacteria and macroinvertebrates like chironomid larvae  
49 and bivalve mollusks (Wetzel 2001). Thus, in shallow, well-mixed lakes, benthic food chains  
50 appear to play an important role in the cycling of organic matter (OM) produced by pelagic  
51 phytoplankton (Weidel et al. 2008).

52           Shallow, eutrophic Lake Võrtsjärv (Estonia) exemplifies the coupling between pelagic  
53 primary production and benthic secondary production. The Võrtsjärv benthic zone can be  
54 divided into 2 main areas: a sheltered, macrophyte-rich zone from the southern end of the lake  
55 (Väike-Emajõgi river mouth) to the broadening of the lake basin south of Tondisaar islet; and a  
56 much larger, mostly unvegetated area that occupies the rest of the lake (Feldmann and Mäemets  
57 2004). In the main basin, wind-induced resuspension of sediments causes high turbidity in the  
58 water column (Secchi depth < 1 m) and restricts the euphotic zone to a depth of  $\leq 2$  m (Reinart  
59 and Herlevi 1999). Low irradiance in the water column selects for shade-tolerant phytoplankton

60 species, and benthic primary production is severely light limited (Nõges et al. 2004b). In  
61 Vörtsjärv, most of the primary producer biomass is constituted by planktonic cyanobacteria  
62 (Nõges and Nõges 2012). Diatoms are present, but they are mostly planktonic (Heinsalu et al.  
63 2008), so over most of the lake bottom, epipelagic and epipsammic primary production is assumed  
64 to be very low. Thus, benthic food webs in Vörtsjärv are probably heterotrophic, sustained by C  
65 from the microbial loop, sedimenting phytoplankton, and particulate organic matter (POM)  
66 originating from the littoral and inflow rivers (Nõges et al. 2004a). A study of Vörtsjärv fish  
67 communities revealed that, in 2009–2011, benthivorous fish composed ~50% of the species and  
68 70% of the fish total biomass (Järvalt et al. 2011). These findings demonstrate the importance of  
69 the benthic food web in transferring OM from phytoplankton to fish in this lake. However,  
70 pelagic and littoral components of Vörtsjärv have received extensive attention, but detailed  
71 knowledge of the benthic foodweb structure is lacking (Kangur et al. 2004).

72 Benthic primary production is very low in Vörtsjärv because the compensation depth is  
73 well above the bottom of the lake, so benthic invertebrate consumers are mostly sustained by a  
74 limited number of sources: POM derived from sedimented phytoplankton and detrital material  
75 originating from macrophyte decay (Nõges et al. 2004a). Thus, markers of consumer food source  
76 and trophic level, like C and N stable isotopes (SI) signatures, respectively ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ;  
77 Peterson and Fry 1987), are expected to reflect these patterns. In general,  $\delta^{13}\text{C}$  varies little with  
78 trophic level and constitutes a good tracer for organic-matter source (Peterson and Fry 1987,  
79 Cremona et al. 2009). On the other hand,  $\delta^{15}\text{N}$  increases 1 to 4‰ between food source and  
80 consumer (Post 2002, Cremona et al. 2010) and is a good indicator of the trophic level of an  
81 organism. Therefore, we used C and N SI analysis to assess the structure of Lake Vörtsjärv  
82 benthic food webs. Our working hypotheses were that: 1)  $\delta^{13}\text{C}$  signatures of benthic consumer

83 taxa at the same site would show little variation because of their similar dependence on  
84 homogeneous C sources; and 2)  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures would show a shift between sites along  
85 a south–north, on-shore/off-shore axis as the contribution of sedimented phytoplankton to the  
86 total OM increases relative to the littoral or riverine contributions.

87

## 88 **METHODS**

### 89 **Study site**

90 Lake Võrtsjärv (surface area at mean water level ca 270 km<sup>2</sup>) is a eutrophic, polymictic  
91 lake in southern Estonia. The lake is shallow (mean depth = 2.8 m) and, because of poor outflow  
92 conditions and unregulated flow, experiences important water-level fluctuations (Nõges et al.  
93 2003) that affect its surface area and volume (Järvet 2004). The lake has well buffered water (pH  
94 = 8.1) with medium hardness (335  $\mu\text{S}/\text{cm}$ ; Tuvikene et al. 2004). Two 4<sup>th</sup>-order rivers (Õhne and  
95 Väike Emajõgi) are the largest inflows to the lake. The pH in their lower reaches (8.0 and 8.1,  
96 respectively) is similar to that in the lake (Järvekülg 2001). Sapropel (lake mud) forms about  $\frac{2}{3}$   
97 of the upper part of the bottom sediments, dominating particularly in the southern part of the lake  
98 (Raukas 2004). The distribution of aquatic vegetation is uneven. The southern tip of the lake is  
99 heavily vegetated, but vegetation becomes gradually sparser as the lake widens and is more wind  
100 exposed. The northern part of the lake is essentially vegetation free, except along the shores,  
101 because of unfavorable conditions (Fig. 1). Tondisaar islet can be regarded as a boundary point,  
102 south of which conditions are better for macrophyte colonization (Feldmann and Mäemets 2004).

103

### 104 **Sample collection**

105 Foodweb component samples were collected during the ice-free periods of 2008–2010.

106 Eight lake (L1–8) and 3 inflow river (S1–3) sites (Table S1) were selected for invertebrate  
107 sampling (Estonian State Environmental Monitoring 2011). The lake sites stretched along a  
108 south–north gradient from the southernmost part of the lake to north of Tondisaar islet (Fig. 1).  
109 The river sites comprised downstream reaches of the 2 main inflow rivers Väike Emajõgi and  
110 Õhne. The main substratum in the rivers and in the lake is fine sand and silt. Sites S1–3 and L1–  
111 5 were covered with macrophytes, with the 2 dominant species being emergent *Phragmites*  
112 *australis* (Cav.) Trin. ex Steud. and submerged *Myriophyllum spicatum* L. 1753. Sites L6 and 7  
113 did not have noticeable macrophyte presence, whereas site L8 had very low macrophyte density.  
114 Flow at the river sites was very low or absent (0–0.5 m/s; Estonian Institute of Hydrology and  
115 Meteorology). Invertebrate samples were collected with a 0.5-mm-mesh hand net with different  
116 handle lengths (2–6 m) and by Borutski and Zabolotski grabs. In the laboratory, taxa were  
117 identified to the lowest practical taxonomical unit, which was often genus for Chironomidae  
118 (*Chironomus* in offshore areas, *Stictochironomus* in nearshore areas). The following taxonomic  
119 keys were used for identifying invertebrates: Killeen et al. (2004) for mussels, Timm (2009) for  
120 annelids, and Nilsson (1996, 1997) for insects. Individual taxa were isolated with steel forceps  
121 under a microscope. Chironomid larvae and unionid mussels formed most of the material.

122 Sediment samples were collected only at L1 and L6 with a Willner sediment corer. In the  
123 laboratory the 25-cm-long sediment core was sectioned into 2-cm (top layer), 3-cm, and three 5-  
124 cm-thick slices, which were freeze-dried for the later analyses. POM from rivers and Võrtsjärv  
125 was sampled once a month by sieving a depth-integrated water sample through a 100- $\mu$ m net and  
126 then collecting material onto precombusted (550°C, 3 h) GF/F filters. The 2 dominant  
127 macrophytes (*P. australis* and *M. spicatum*) were collected manually 3 times during the  
128 vegetated period (May, August, and October) in 2008 from L1.

129

130 **SI analyses**

131 Because of the high inorganic C content in Vörtsjärv lake water,  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of  
132 sediment, *M. spicatum* (not *P. australis* because this species has aerial leaves), and POM filters  
133 were measured in separate analyses. These samples were all acidified prior to the  $\delta^{13}\text{C}$  analyses.  
134 The freeze-dried sediment was treated with 12% HCl to remove inorganic C. Approximately 3  
135 mg of dried, acidified (only for  $\delta^{13}\text{C}$ ), and pure material was used for  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  analyses.  
136 For sediments, average stable N and C signatures of the sliced 25-cm sediment core were used.  
137 For macrophytes, the whole aboveground plant (leaves and stems) was used for SI analyses.  
138 POM filters and macrophytes were maintained for 24 h in HCl vapors and then dried to constant  
139 mass at 60°C. The collected material was dried, pulverized, and stored as a ground powder.

140 An average of 10 individuals from each invertebrate sample was used for SI analyses.  
141 The shells of snails and mussels do not reflect the SI signature of their diets (McConnaughey et  
142 al. 1997), so they were removed manually with forceps and only the soft tissue was used. Prior to  
143 analysis, invertebrate samples were dried to constant mass at 60°C. Failure to allow organisms  
144 clear their gut content could bias SI analyses done on whole individuals (Junger and Planas  
145 1994), but the need to clear gut content has been questioned (Kaehler and Pakhomov 2001,  
146 Jardine et al. 2005, Cremona et al. 2009). Therefore, we tested the extent to which gut clearance  
147 would influence SI signatures of Lake Vörtsjärv Chironomidae because they made up the  
148 overwhelming majority of the macroinvertebrate samples. Three groups of animals were kept  
149 alive for 12 h before drying to permit clearance of their gut contents. The SI values for these  
150 groups were compared with those of control groups dried immediately without gut clearance.  
151 Comparison made with a nonparametric Mann–Whitney *U* test revealed no significant difference



152 between the treated and untreated groups ( $p > 0.05$ ,  $n = 10\text{--}24$ ; Table S2). Therefore, subsequent  
153 samples were processed without gut clearance.

154 All SI analyses were done at the University of Jyväskylä (Finland) using a Flash EA 1112  
155 elemental analyzer connected to a DELTAplus Advantage IRMS (Thermo Finnigan, Bremen,  
156 Germany). SI ratios are expressed as  $\delta$  values ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ) in ‰ referenced to the international  
157 standards for C (PeeDee Belemnite) and N (atmospheric  $\text{N}_2$ ). Internal laboratory standards were  
158 homogenized potato leaves for POM, macrophytes, and sediment and white muscle tissue of pike  
159 for invertebrates. Lipids have more negative  $\delta^{13}\text{C}$  values relative to other major biochemical  
160 compounds, so the lipid-corrected value for C was used for statistical analyses (Logan et al.  
161 2008). Both corrected and uncorrected data are presented in Table S3.

162

### 163 **Data treatment**

164 Benthic taxa were classified into 3 functional feeding groups (FFGs) according to their  
165 feeding mode (Kangur et al. 2004): collectors, filterers and predators. The collector group  
166 comprises organisms feeding on bottom detritus like POM and settled phytoplankton. Filterers  
167 are taxa that obtain their food from the water column. In this study, they were particle feeders  
168 like mussels. Predators feed on other live benthic invertebrates. Samples were not separated  
169 according to year because most (75%) of our samples were collected in 2010 and because the  
170 year effect on isotopic signature is weak (Cremona et al. 2010). Several taxa were not found  
171 either during all time periods or at all the sites, so a general linear model (GLM) with factorial  
172 test effect was used for data analysis. Adjusted values (i.e., Least Square Means, LSM) of  $\delta^{13}\text{C}$   
173 and  $\delta^{15}\text{N}$  were used as the response variable. LSMs are predicted values from the model across  
174 the level of categorical effects where the other model variables are controlled by setting them to

175 neutral values (SAS Institute 1991; Uryu et al. 2001). For a model with 3 categorical variables,  
176 when comparisons are made within 1 variable, the weights of the other 2 variables are  
177 neutralized. Categorical variables were time (month), space (site), and trophic level (functional  
178 feeding group). Tukey's Honestly Significant Difference (HSD) tests were done on the model  
179 outputs (i.e., adjusted values of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) to test monthly, site, and functional group  
180 differences. All analyses were done with JMP (version 10; SAS Institute Inc., Cary, North  
181 Carolina).

182

## 183 **RESULTS**

### 184 **$\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of invertebrate taxa and OM sources**

185 Of the 200 invertebrate samples analyzed, 143 were collectors, 41 were filterers, and 16  
186 were predators. The lipid-corrected  $\delta^{13}\text{C}$  signatures of the samples varied from  $-57.7 \pm 1.8\text{‰}$  in  
187 unidentified *Chironomus* larvae (Diptera) to  $-30.2 \pm 1\text{‰}$  in *Hydroporus* (Coleoptera). The  
188 largest taxonomic group, *Chironomus plumosus* (Linnaeus), which accounted for nearly  $\frac{1}{2}$  of the  
189 analyzed samples, had a mean  $\delta^{13}\text{C}$  signature of  $-37.1 \pm 0.4\text{‰}$  (Fig. 2). The 2<sup>nd</sup> largest taxon  
190 sample, *Unio tumidus* Philipsson, had a slightly higher signature ( $-35.6 \pm 0.5\text{‰}$ ). Thus, despite  
191 the apparent amplitude and taxonomic richness observed, most sample  $\delta^{13}\text{C}$  values clustered  
192 within the range  $-36$  to  $-38\text{‰}$ , indicating a low number of food sources or low variability of the  
193 food-source signatures.  $\delta^{15}\text{N}$  signature ranged from  $0.6\text{‰}$  in unidentified *Chironomus* to  $12.3\text{‰}$   
194 in Zygoptera (Odonata). Though this amplitude is less than that of  $\delta^{13}\text{C}$ , the main taxa exhibited  
195 greater differences in  $\delta^{15}\text{N}$  than in  $\delta^{13}\text{C}$ . The  $\delta^{15}\text{N}$  of the 2 most numerous taxa, *Chironomus*  
196 *plumosus* and *Unio tumidus*, were  $7.4 \pm 0.1\text{‰}$  and  $10.8 \pm 0.3\text{‰}$ , respectively. Predatory and  
197 filterer taxa exhibited generally higher  $\delta^{15}\text{N}$  signatures than collectors.

198 C signatures of sediments were  $-30\text{‰}$  for the southernmost site (L1) with no difference  
199 between depth of sampling (Table S4) and  $-33\text{‰}$  in the northernmost site (L6). Sediment  
200 samples from L1 exhibited a slight depletion ( $\sim 1.15\text{‰}$ ) of the heavier isotope for  $\delta^{15}\text{N}$  between  
201 the surface and 25 cm. River and lake POM SI signatures were nearly identical, which is  
202 indicative of similar environmental conditions between phytoplankton from tributaries and  
203 Vörtsjärv (Fig. 2). According to the signatures of foodweb components, the main food sources  
204 for invertebrate consumers appeared to be POM, a little sediment OM, and another, much more  
205  $^{13}\text{C}$ -depleted source that was not sampled. Macrophyte SI values indicated that vascular plants do  
206 not contribute substantially to the invertebrate dietary OM.

207

#### 208 **Influence of categorical variables on SI signatures**

209 The GLM explained 60% and 69% of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  variation, respectively (Table 1). For  
210 both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , sampling site appeared as the most important variable, followed by month  
211 and FFG. No significant differences were observed between the center of the lake and the south  
212 shore or between on-shore and off-shore areas (Fig. 3A–D). Significant differences in isotopic  
213 signatures were observed among invertebrate samples from a few different sites (Fig. 4A, Table  
214 2). These differences can be explained by the more negative  $\delta^{13}\text{C}$  signature of *C. plumosus* and  
215 unidentified *Chironomus* at these sites. Thus, the spatial variations in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in Vörtsjärv  
216 appeared to correspond to local in-site peculiarities. River site (S1–3) isotopic signatures did not  
217 differ from those of most lake sites (L1, 4, 5, 7, 8).  $\delta^{13}\text{C}$  signature tended to decrease from the  
218 eastern shore (L8) to the center (L6), but the differences were not statistically significant. For  
219 example, benthos collected at sites L2 and L3 had a significantly lower  $\delta^{13}\text{C}$  signature than  
220 benthos collected from other sites, but samples collected from the most vegetated sites (L1–5)

221 did not differ significantly from samples collected at less-vegetated sites (L6–8).  $\delta^{13}\text{C}$  signature  
222 varied little between months. September was the only month that differed from the others (Fig.  
223 4B, Table 3). Thus, spatial homogeneity of  $\delta^{13}\text{C}$  paralleled temporal stability of the same  
224 signature, a result implying reliance on the same C source by benthic consumers during most of  
225 the growing season.

226 Output values from the GLM revealed that FFG did not affect  $\delta^{13}\text{C}$  ( $p = 0.49$ ), and LSM  
227 (adjusted) values were within the range of standard error (Fig. 4C, Table 4). LSM  $\delta^{13}\text{C}$  values  
228 were  $-36.5 \pm 0.9\text{‰}$  for collectors,  $-37.2 \pm 1.26\text{‰}$  for filterers, and  $-34.8 \pm 1.95\text{‰}$  for predators.  
229 This result implies that the Vörtsjärv benthic food web is sustained primarily by 1 C source or by  
230 a fixed proportion of sources. On the other hand, FFG did affect  $\delta^{15}\text{N}$  ( $p < 0.0001$ ) for. There  
231 was a significant difference between collector and filterer LSM  $\delta^{15}\text{N}$  values, but not between  
232 predators and the 2 other feeding functional groups (Fig. 2). LSM  $\delta^{15}\text{N}$  values were  $7.11 \pm$   
233  $0.36\text{‰}$  for collectors,  $10.28 \pm 0.49\text{‰}$  for filterers and  $8.81 \pm 0.76\text{‰}$  for predators. The average  
234  $\delta^{15}\text{N}$  enrichment between the collector group and the next trophic level represented by the  
235 invertebrate predator group was therefore  $1.7\text{‰}$ .

236

## 237 **DISCUSSION**

238 Our 1<sup>st</sup> hypothesis was supported because no difference was detected among  $\delta^{13}\text{C}$   
239 signatures of consumers within sampling sites. Samples collected from in-shore sites and rivers  
240 generally had  $\delta^{13}\text{C}$  values between  $-30\text{‰}$  and  $-40\text{‰}$ , which would correspond to a primary  
241 uptake of POM ( $\sim -32\text{‰}$ ). In a long-term study of a shallow Danish lake, benthic  
242 macroinvertebrates had relatively lower  $\delta^{13}\text{C}$  signatures and taxonomic richness during years  
243 when macrophyte cover was scarce and turbidity high (Boll et al. 2012). Lower consumer  $\delta^{13}\text{C}$

244 values were argued to be caused by consistently higher CO<sub>2</sub> concentration in the water column  
245 (and thus, more <sup>13</sup>C discrimination in primary producers) during turbid-water years. However, in  
246 Vörtsjärv, POM probably is supplemented by another, more negative source, e.g., methane-  
247 oxidizing bacteria (MOB; <-60‰; Grey et al. 2004, Jones and Grey 2011, Yasuno et al. 2012).  
248 Some samples had even lower δ<sup>13</sup>C signatures (<-40‰) that strongly suggested assimilation of  
249 C from MOB. δ<sup>15</sup>N values of benthic consumers was relatively high, so uptake of terrestrially  
250 derived OM (δ<sup>13</sup>C ≈ -28‰, δ<sup>15</sup>N ≈ 0‰) is very unlikely (Peterson and Fry 1987, Cremona et al.  
251 2009), as it is for submerged macrophytes.

252 Contrary to our 2<sup>nd</sup> hypothesis, no lake-wide gradient was observed in Vörtsjärv  
253 macroinvertebrate SI signatures. Only organisms collected at the southern sites L2 and L3 had  
254 significantly lower δ<sup>13</sup>C signatures than organisms collected at other sites. The main cause of this  
255 difference lies in the greater abundance of strongly <sup>13</sup>C-depleted Chironomidae (*C. plumosus* and  
256 unidentified *Chironomus*) relative to other taxa at these 2 sites. Whether on a south–north or  
257 offshore–onshore axis, the observed variations in δ<sup>13</sup>C and δ<sup>15</sup>N did not appear to be linked to  
258 any diet shift on a large scale, but rather seemed to correspond to some local site or community  
259 composition differences (greater abundance of Chironomidae). Therefore, and despite its large  
260 surface area, the benthic zone of Vörtsjärv is a very homogeneous ecosystem. Most of the  
261 benthic organisms evidently feed on a limited number of C sources, and the food web essentially  
262 comprises 2 trophic compartments, i.e., benthic collectors and filterers, and predators (Table S5).  
263 This monotonous foodweb structure, dominated by omnivorous chironomids and mussels, is  
264 typical of shallow turbid lakes with unvegetated bottoms (Hargeby et al. 1994) or of the “turbid  
265 state” experienced by clearer lakes (Boll et al. 2012). In the profundal (i.e., below compensation  
266 point) zone of lakes the low diversity of feeding modes among invertebrates corresponds to a

267 low diversity of available food sources (Jonasson 1972). However, contrary to what is observed  
268 in clearer lakes, the contribution of macrozooplankton (by the sinking of dead individuals or  
269 fecal pellets to the benthic zone) to the invertebrate diet is negligible in Vörtsjärv because of the  
270 low abundance of these organisms in the water column (Zingel et al. 2007). More generally, the  
271 importance of benthic consumers in shallow lakes is consistent with the results of previous  
272 studies in which an inverse relationship between lake mean depth and zoobenthos contribution to  
273 whole-lake secondary productivity was described (Jeppesen et al. 1997, Vadeboncoeur et al.  
274 2002). Based on the depth–benthos relationship compiled by Vadeboncoeur et al. (2002; fig. 7 in  
275 their paper), Vörtsjärv, with a mean depth of only 2.8 m, should lie at one extreme end of the  
276 continuum with the contribution of zoobenthos to lake secondary production as high as 90%.

277         The mean  $\delta^{15}\text{N}$  enrichment observed in Vörtsjärv between the collector and predator  
278 trophic levels (1.7‰) was lower than the typical trophic fractionation value of ~3.4‰ that has  
279 been reported by many previous investigators (e.g., Minagawa and Wada 1984, Vander Zanden  
280 et al. 1999, Post 2002). Several factors could contribute to this discrepancy. First, an apparent  
281 trophic enrichment <3.4‰ could indicate consumer omnivory, i.e., feeding at >1 trophic level  
282 (Zah et al. 2001). Aquatic invertebrates are often opportunistic in their feeding behavior, and  
283 taxa like chironomids that have primitive chewing mouth parts can graze on particles and cells of  
284 a wide size range (Tall et al. 2006). Low trophic enrichment can also be caused by invertebrate-  
285 specific assimilation rates that differ from those of vertebrates (Webb et al. 1998, McCutchan et  
286 al. 2003). The overall fractionation value we obtained is very close to values of 1.8‰ reported  
287 by Anderson and Cabana (2005) and 1.6‰ by Cremona et al. (2010) for Québec benthic  
288 invertebrates. However, our collector isotope values were mainly for Chironomidae, and  
289 especially *Chironomus plumosus* because these make up most of the benthic biomass in

290 Vörtsjärv. Kangur et al. (2004) estimated that Chironomidae represent 84% of bottom animal  
291 abundance in the lake with *C. plumosus* alone constituting 73%. The invertebrate predators we  
292 analyzed are not capable of consuming the bivalve mollusks that constitute the only other  
293 significant component of the benthic biomass (Kangur et al. 2004), so chironomid larvae are  
294 likely to be the principle prey for the invertebrate predators. We obtained a mean  $\delta^{15}\text{N}$  value of  
295 7.4‰ for *C. plumosus* and a 3.4‰ trophic enrichment above this value would give a  $\delta^{15}\text{N}$  value  
296 of 10.8‰, which is actually slightly lower than most of the predator  $\delta^{15}\text{N}$  values we obtained.  
297 Furthermore, the average  $\delta^{15}\text{N}$  of *C. plumosus* is exactly 3.22‰ enriched relative to the upper  
298 layer of sediments that constitute its natural food input. Thus, N trophic fractionation among  
299 benthic invertebrates in Vörtsjärv is consistent with widely adopted values when considered in  
300 the context of probable prey–predator interactions rather than as community-wide averages.

301       Among the 3 FFGs categorized in our study, the filterers exhibited the highest adjusted  
302  $\delta^{15}\text{N}$  signature. In Lake Vörtsjärv, the filterer FFG essentially comprises freshwater mussels, and  
303 some physiological and ecological characteristics specific to these taxa might explain their high  
304  $\delta^{15}\text{N}$  signatures. Mussels are larger bodied and longer lived organisms than chironomids. They  
305 have a very efficient rate of retention for particles  $>5\ \mu\text{m}$ , which is the size of the large  
306 flagellates and ciliates (Jørgensen et al. 1984, Thorp and Casper 2002) that constitute a high  
307 proportion of the zooplankton biomass in Vörtsjärv (Zingel and Nõges 2010). The  $\delta^{15}\text{N}$  signature  
308 of some Vörtsjärv zooplankton is  $\sim 8.5\text{‰}$  (H. Agasild, Estonian University of Life Sciences,  
309 unpublished data),  $\sim 1.5$  to  $3.0\text{‰}$  lower than that of the mussels and at the same level as that of  
310 the chironomids, which suggests that mussels in Vörtsjärv might feed extensively on the  
311 abundant protozoan zooplankton.

312       The  $\delta^{13}\text{C}$  signature of benthic consumers was more negative than the POM, sediment, and

313 macrophyte signatures despite a slight positive  $\delta^{13}\text{C}$  fractionation (+0.4‰) from source to  
314 consumer (Post 2002). This lower benthic consumer signature can be accounted for only by the  
315 assimilation of a more negative OM source not included in our sampling and analysis of putative  
316 food sources. Some samples, mostly chironomids, exhibited an even lower  $\delta^{13}\text{C}$  signature, in  
317 some cases approaching  $-60\text{‰}$ . This depleted  $\delta^{13}\text{C}$  signature of chironomids relative to other  
318 taxa is most plausibly accounted for by a contribution to their diet of MOB found in sediments  
319 (Grey et al. 2004, Jones and Grey 2011). For example, *Chironomus plumosus* is a taxon  
320 characteristic of eutrophic and dystrophic lakes that experience  $\text{O}_2$  depletion in the sediments  
321 (Jones and Grey 2011). These hypoxic conditions are likely to favor the activity of MOB and  
322 their subsequent assimilation by bottom-feeding invertebrates. The  $\delta^{13}\text{C}$  values obtained for *C.*  
323 *plumosus* in Vörtsjärv are not nearly as low as those reported from some other lakes by Jones  
324 and Grey (2011), but nevertheless, they suggest some contribution of methane-derived C to the  
325 chironomid biomass in this lake. Some other bottom/sediment feeding taxa collected in  
326 Vörtsjärv, like the Oligochaeta, also exhibited a strongly depleted  $\delta^{13}\text{C}$  signature (see Hershey et  
327 al. 2006 for similar findings in Oligochaeta). On the other hand *Stictochironomus* sp., which  
328 inhabits the vegetated littoral zone, exhibited a heavier C signature than *C. plumosus*, indicating  
329 a stronger reliance on POM and terrestrial detritus in its diet.

330         In some lakes, methane-derived C can be transferred through the food web to make a  
331 substantial contribution to fish biomass (Ravinet et al. 2010, Sanseverino et al. 2012). However,  
332 our findings suggest only limited potential transfer of methane-derived C to other parts of the  
333 food webs of Vörtsjärv. The lake is polymictic and its water column is well oxygenated  
334 throughout the year, so the MOB layer is certainly constrained in the deeper subsurface  
335 sediments (see fig. 4 by Jones and Grey 2011). This situation also means that zooplankton are



336 unlikely to graze MOB and transfer methane-derived C to the upper parts of the food webs.  
337 Hence, mobile top consumers in the lake like benthivorous fishes that rely heavily on chironomid  
338 larvae are likely to contain some methane-derived C in their biomass, but only to a small extent,  
339 and this situation is likely to be most evident for fish from the southern basin of the lake where  
340 the chironomids with particularly low  $\delta^{13}\text{C}$  values were collected (Agasild et al., in press). Our  
341 results suggest that MOB should be taken into account as potential energy mobilizers in addition  
342 to phytoplankton and detritus in foodweb studies in Lake Vörtsjärvi, but their role in C transfer to  
343 top consumers is probably a small one.

344         In summary, our results support previous research that benthic foodweb structure of  
345 turbid shallow lakes is remarkably homogenous and dominated by a few abundant taxa, mainly  
346 chironomid larvae and bivalve mollusks, with somewhat different feeding strategies but  
347 sustained by few OM sources. Vörtsjärvi benthic foodweb structure is similar to that of other  
348 productive turbid lakes of the hemiboreal region, i.e., lakes that are generally more light- than  
349 nutrient-limited, but we suggest that it stands at the very end of the benthic/pelagic continuum  
350 regarding the importance of benthos in secondary production. A turbid state, whether temporary  
351 or permanent, induces strong coupling between primary and secondary production in the pelagic  
352 and benthic zones, with primary production essentially carried out by phytoplankton and  
353 secondary production processed by benthic consumers. Three major OM sources (macrophytes,  
354 terrestrial detritus and benthic algae) that are used by consumers of nonturbid, not light-limited  
355 lakes are excluded in the simplified food webs of these lakes.

356

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- 522

523 **FIGURE CAPTIONS**

524 Fig. 1. Location of sampling sites on the main river inflows (S1–3, left) and in Vörtsjärv (L1-L8,  
525 right). Dashed line represents lower limit of macrophyte distribution.

526 Fig. 2. Mean ( $\pm 1$  SE) C and N stable isotope signature biplot of foodweb components sampled in  
527 Vörtsjärv.

528 Fig. 3. Adjusted values of sampling site mean  $\delta^{13}\text{C}$  (A, B) and  $\delta^{15}\text{N}$  (C, D) of benthic  
529 macroinvertebrates calculated by the test-effect model plotted against distance from the  
530 southern end of the lake (A, C) and distance from nearest shore (B, D). Only data from  
531 the in-lake sites (L1–8) are shown in the plots.

532 Fig. 4. Mean ( $\pm 1$  SE) Least Square Means (LSM) adjusted values of isotopic signatures of  
533 macroinvertebrates among sampling sites (see Fig. 1 for locations) (A), months (B),  
534 functional feeding groups (C). The adjusted values were calculated by the test effect  
535 model.

536

537 Table 1. Analysis of variance for the test effect model of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of benthic  
 538 macroinvertebrates with the variables site (L1–8), month (June, July, August, September,  
 539 October, November), and functional feeding group (FFG; collector, filterer, predator). For  $\delta^{13}\text{C}$ ,  
 540 the analysis was done on lipid-corrected values. \* indicates significant effect.

Model and variables	Sum of squares	df	<i>F</i> ratio	<i>p</i>	<i>r</i> <sup>2</sup>
$\delta^{13}\text{C}$					0.60
Site	4605	10	21.39	<0.0001*	
Month	1720	5	15.98	<0.0001*	
FFG	22	2	0.51	0.6	
$\delta^{15}\text{N}$					0.69
Site	534	10	16.28	<0.0001*	
Month	155	5	9.46	<0.0001*	
FFG	117	2	17.85	<0.0001*	

541

542

543 Table 2. Results of Tukey's Honestly Significant Difference test ( $\alpha = 0.05$ ) on the Least Square  
 544 Means (LSM) of C and N isotopic signatures for site categorical variables. Sites with the same  
 545 letter are not significantly different.

Site	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
L1	A	A
L2	CD	CD
L3	D	D
L4	A	ABCD
L5	A	ABC
L6	BCD	BCD
L7	A	AB
L8	A	A
S1	ABCD	ABCD
S2	AB	ABCD
S3	ABC	ABCD

546

547 Table 3. Results of Tukey's Honestly Significant Difference test ( $\alpha = 0.05$ ) on the Least Square  
548 Means (LSM) of C and N isotopic signatures for month categorical variable. Months with the  
549 same letter are not significantly different.

Month	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
June	A	AB
July	A	ABC
August	A	ABC
September	B	A
October	A	C
November	A	BC

550

551

552 Table 4. Results of Tukey Honestly Significant Difference test ( $\alpha = 0.05$ ) on the Least Square  
 553 Means (LSM) of C and N isotopic signatures for functional feeding groups (FFGs). FFGs with  
 554 the same letter are not significantly different.

Functional group	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
Collector	A	B
Filterer	A	A
Predator	A	AB

555

Figure 1

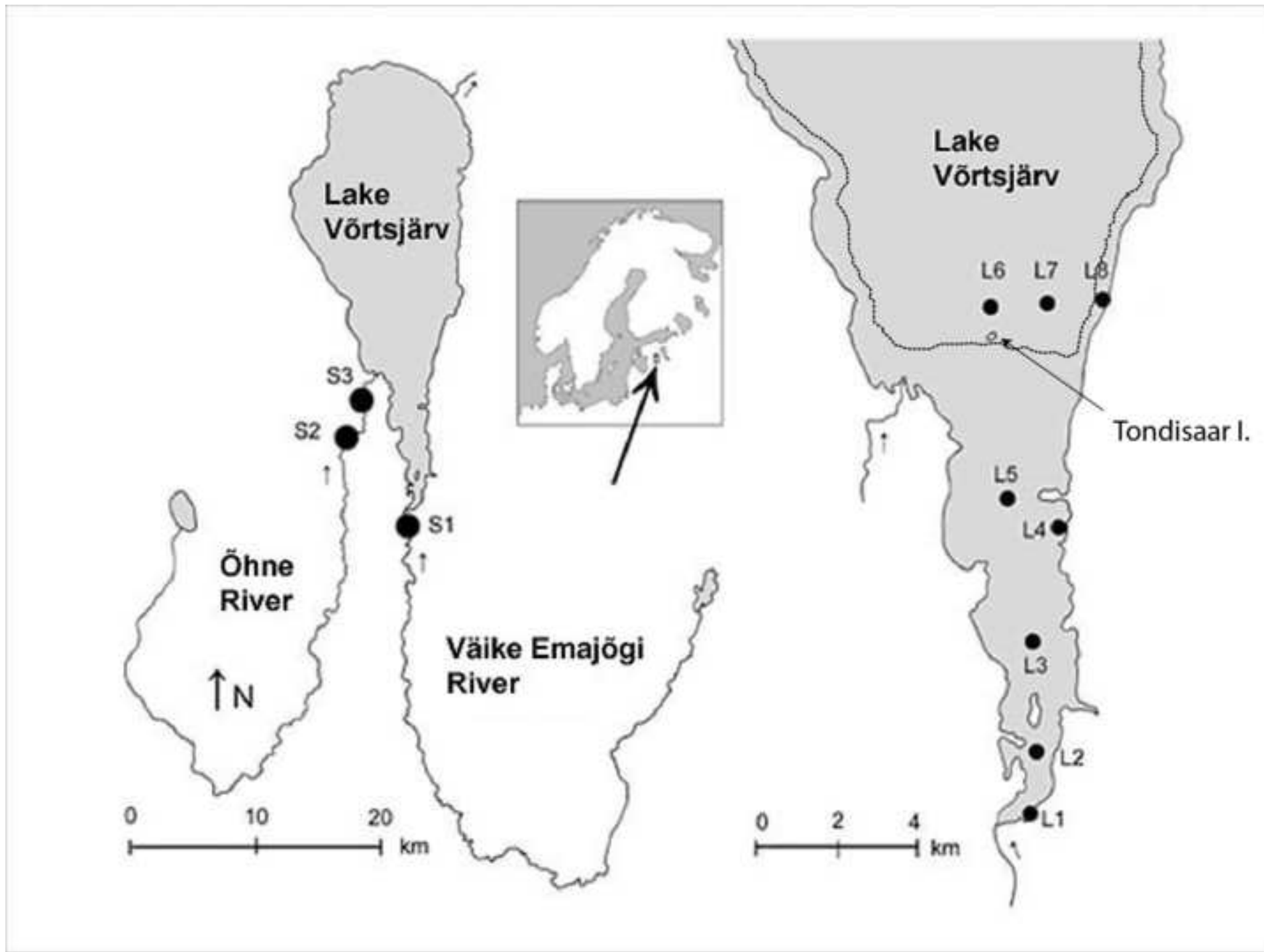


Figure 2

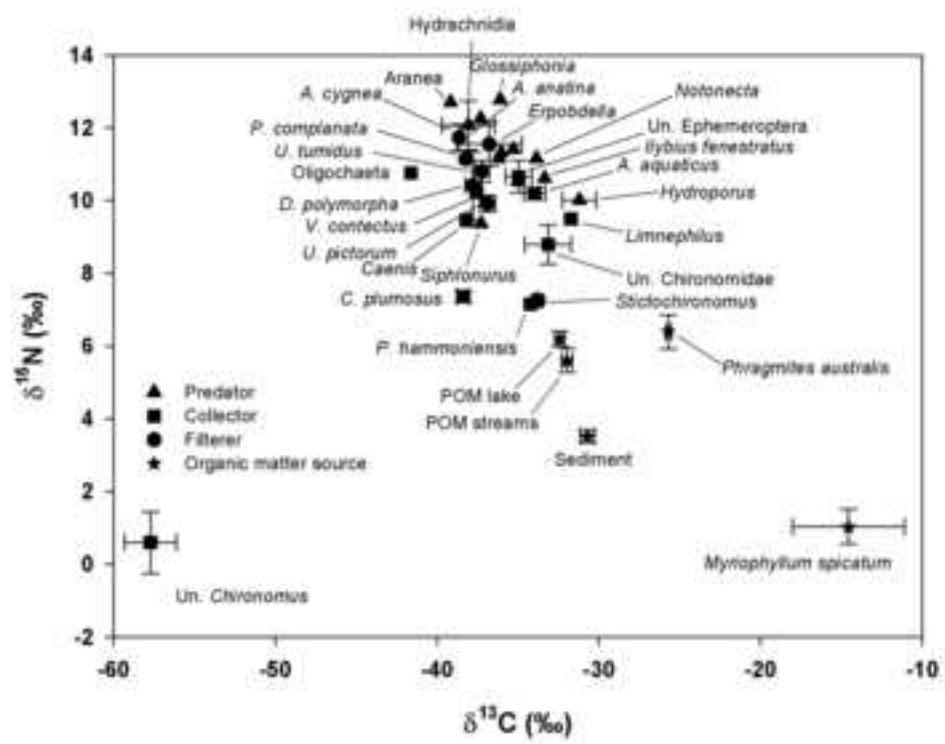




Figure 3

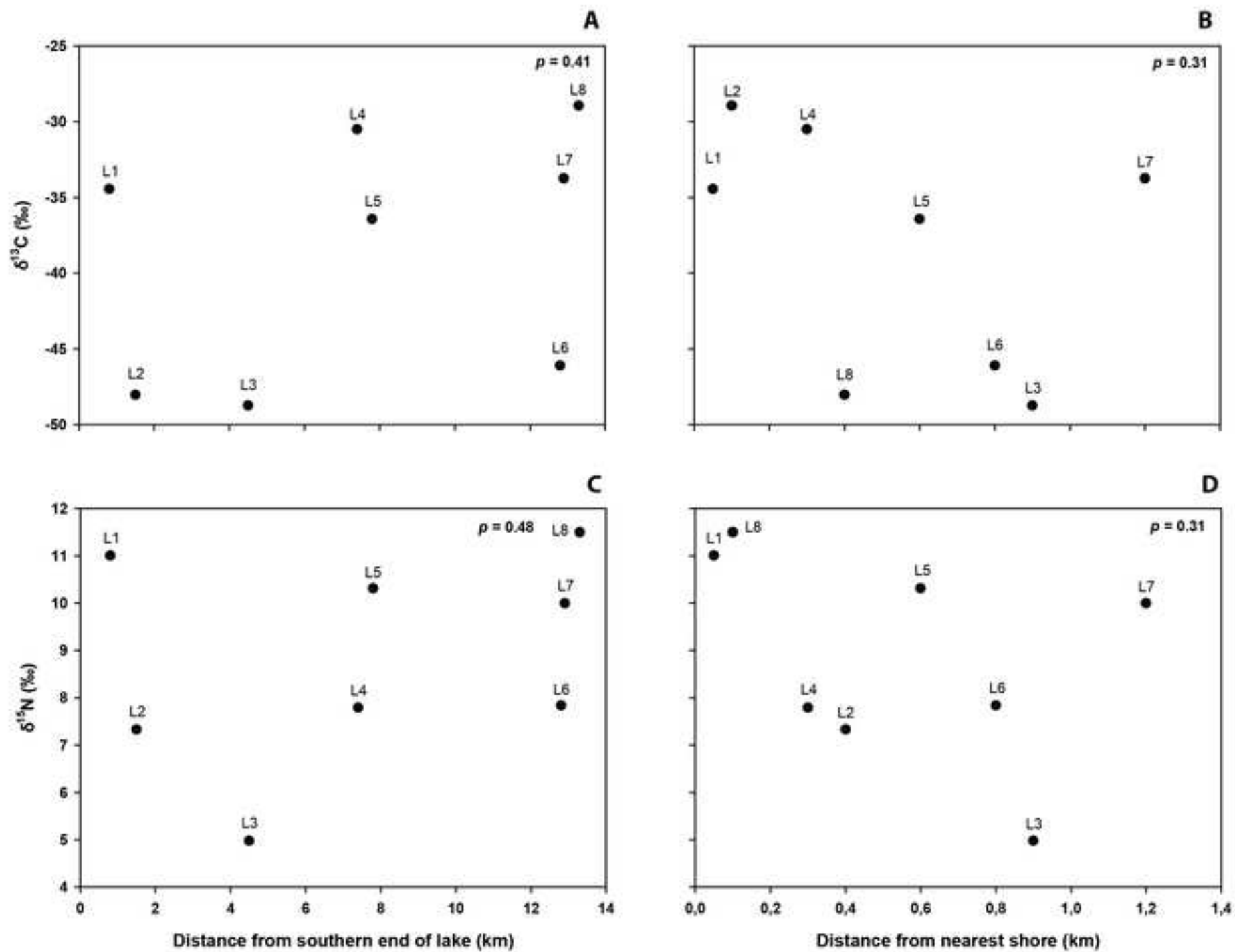


Figure 4

