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8

9 **Trophic state changes can affect the importance of methane-derived carbon in aquatic**
10 **food webs**

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25 **Key words:** Chironomidae; *Daphnia*; Methane; Eutrophication; Lakes; Stable Carbon
26 Isotopes

27 **Abstract**

28 Methane-derived carbon, incorporated by methane oxidizing bacteria, has been identified as a
29 significant source of carbon in food webs of many lakes. By measuring the stable carbon
30 isotopic composition ($\delta^{13}\text{C}$ values) of particulate organic matter, Chironomidae, and *Daphnia*
31 spp. and their resting eggs (ephippia) we show that methane-derived carbon presently plays a
32 relevant role in the food web of hypertrophic Lake De Waay, the Netherlands. Sediment
33 geochemistry, diatom analyses and $\delta^{13}\text{C}$ measurements of chironomid and *Daphnia* remains
34 in the lake sediments indicate that oligotrophication and re-eutrophication of the lake during
35 the 20th century had a strong impact on in-lake oxygen availability. This, in turn, influenced
36 the relevance of methane-derived carbon in the diet of aquatic invertebrates. Our results show
37 that, contrary to expectations, methane-derived relative to photosynthetically produced
38 organic carbon became more relevant for at least some invertebrates during periods with
39 higher nutrient availability for algal growth, indicating a proportionally higher utilization of
40 methane-derived carbon in the lake's food web during peak eutrophication phases.
41 Contributions of methane-derived carbon to the diet of the investigated invertebrates are
42 estimated to have ranged from 0-11 % during the phase with the lowest nutrient availability to
43 13-20 % during the peak eutrophication phase.

44

45 **Introduction**

46 Eutrophication of inland waters as a consequence of human impact has a detrimental effect on
47 different aspects of the water quality of lakes, rivers and streams [1]. For example, the process
48 can change the chemical properties of the water, leading to oxygen depletion [2] and
49 accumulation of nutrients in the anoxic hypolimnion [3]. Lake ecosystems with low oxygen
50 concentrations and high nutrient loading are characterized by higher output of the important
51 greenhouse gas methane (CH_4) than oxygen-rich lakes and lakes with lower nutrient

52 availability [4,5], particularly via gas bubbles (ebullition) and release of CH₄ stored in the
53 anoxic hypolimnion during lake overturning [6]. CH₄ formed in lakes can be oxidized by
54 methane-oxidizing bacteria (MOB), predominantly in oxygenated sections of the lake basin
55 [7]. Biogenic CH₄ in freshwater systems is characterized by distinctly low ratios between the
56 stable carbon isotopes ¹³C and ¹²C (expressed as δ¹³C values; -80 to -50 ‰) [8,9] and MOB
57 are known to discriminate against the heavier ¹³C when metabolizing CH₄ resulting in even
58 lower δ¹³C values of MOB biomass [10]. These very low values do not occur in aquatic and
59 terrestrial photosynthetic primary producers (-35 to -10 ‰ [11–15]). The very low observed
60 δ¹³C values of e.g. larvae of non-biting midges (Chironomidae) of the tribe Chironomini
61 [16,17] and planktonic water fleas of the genus *Daphnia* (Cladocera) [18] in some lakes are
62 therefore considered a clear indication of MOB, or organisms feeding on MOB, forming a
63 relevant part of the diet of these organisms.

64 Planktonic filterers such as *Daphnia* can graze MOB from the water column during
65 stratification, a process which can effectively reduce MOB biomass and lead to increased
66 epilimnetic CH₄ concentrations at least in some shallow boreal lakes [19]. Furthermore,
67 *Daphnia* has been shown to rely strongly on MOB-derived carbon during autumn overturning,
68 when the CH₄ stored in deep anoxic water layers of stratified lakes comes into contact with
69 oxygen [20]. Benthic invertebrates that can incorporate CH₄-derived carbon, such as
70 chironomid larvae of the tribe Chironomini, either feed on MOB in the sediments (deposit
71 feeders) or MOB associated with suspended organic particles (filter feeders). Some
72 Chironomini larvae have been shown to actively maintain an oxic-anoxic interface
73 within their tubes, providing a habitat for MOB which they feed on [21]. δ¹³C values of
74 chironomids and *Daphnia* closely reflect those of their food source (differences of 0-1 ‰) and
75 of their fossilizing chitinous structures (reported offsets of 0-1 ‰) [22–25]. Chitinous remains
76 deposited and buried in the lake sediments retain their original isotopic composition [23].
77 Hence, analysis of δ¹³C values of “fossil” chironomid and *Daphnia* remains can provide

80 insights on their past food sources and into whether CH₄-derived carbon formed a major
81 component of their diet [23,26–31]. Available studies indicate that chitinous remains with low
82 $\delta^{13}\text{C}$ values are deposited in lakes with high surface and deep water CH₄ concentrations and
83 diffusive CH₄ emissions [28,29].

84 It is unclear whether an increase in nutrient loading, in addition to a higher CH₄
85 output, also results in a higher contribution of CH₄-derived carbon to the lake food webs.
86 Higher photosynthetic primary productivity associated with higher nutrient loading can
87 increase the availability of algal organic matter. As a consequence, a higher proportion of
88 algae in the diet of invertebrate groups that may also incorporate CH₄-derived carbon could be
89 expected. However, higher algal productivity can also lead to decreased oxygen
90 concentrations in lake sediments and deep water layers, and associated increases in CH₄
91 production and CH₄ availability in lake ecosystems. This can favour the growth of MOB and
92 their temporal and spatial availability within lakes. Therefore, how the relevance of CH₄-
93 derived carbon in the food web of lakes changes under influence of (past or future) changes in
94 nutrient concentrations and productivity remains poorly constrained, particularly on decadal
95 time scales which are not covered by instrumental measurements of CH₄ concentrations and
96 $\delta^{13}\text{C}$ values in aquatic ecosystems.

97 The $\delta^{13}\text{C}$ values of chitinous remains of aquatic invertebrates were previously mainly
98 studied in oligo- to mesotrophic, often remote lakes (e.g. [27,29,30,32]). Here, we present a
99 study of the $\delta^{13}\text{C}$ values of fossil Chironomini head capsules and *Daphnia* resting eggs
100 (ephippia) from recent (20th century) sediments from a small and presently hypertrophic
101 dimictic lake in the Netherlands (Figure 1). The study lake, Lake De Waay, underwent a
102 transition from eutrophic (TP ~100 $\mu\text{g l}^{-1}$) to more mesotrophic conditions (TP ~40 $\mu\text{g l}^{-1}$) and
103 then again to hypertrophic conditions (TP >100 $\mu\text{g l}^{-1}$) during the past ~100 years [33,34].
104 The impact of 20th century temperature changes on lake ecosystems in the Netherlands is

103 likely to have been relatively minor compared to direct anthropogenic environmental
104 disturbances. Therefore, this study provides an opportunity to investigate the effects of both
105 oligotrophication and (re-)eutrophication and the associated changes in oxygenation regime
106 on the contribution of CH₄-derived carbon to the aquatic food web, and indirectly on in-lake
107 dissolved CH₄ availability, under relatively stable climatic conditions. We compare fossil
108 invertebrate $\delta^{13}\text{C}$ values with a diatom-inferred reconstruction of total phosphorus
109 concentrations (DI-TP) in the lake water and the Fe : Mn ratio of the sediments, which is
110 expected to increase with decreasing oxygen availability at the sediment-water interface [35].
111 If CH₄-derived carbon became more relevant for Lake De Waay's foodweb under conditions
112 with higher nutrient availability we expect to see a positive relationship between nutrient
113 availability (DI-TP) and anoxia (Fe : Mn ratio), which in turn are expected to be negatively
114 related to $\delta^{13}\text{C}$ values of the examined invertebrate groups that can incorporate CH₄-derived
115 carbon. Conversely, if higher availability of algal material in the eu- to hypertrophic phases
116 led to a lower relevance of CH₄-derived carbon for the investigated aquatic invertebrates, we
117 expect to see positive relationships between the $\delta^{13}\text{C}$ values of the studied invertebrate groups
118 and DI-TP and Fe : Mn in Lake De Waay.

119

120 **Methods**

121 *Current conditions in the lake*

122 To assess the current range of $\delta^{13}\text{C}$ values of chironomids, *Daphnia*, floating *Daphnia*
123 ephippia, and suspended particulate organic matter (POM) in the water column of Lake De
124 Waay, field sampling was conducted on September 3 and November 30 2009, and March 1
125 and June 1 2010. Living organisms were collected using plankton nets, kicknets and
126 inspection of submerged wood and rope. In the lab, organisms were sorted and transferred to

127 tin cups and water was filtered for $\delta^{13}\text{C}$ analysis (see supplementary material for details on
128 sampling and processing).

129 On September 3 2009, nine sediment cores were taken using a gravity corer
130 (UWITEC, Austria): five at 14.5 m water depth and four at 5 m water depth. The top 2 cm of
131 sediment were sampled in the field. Sediment was treated with 10% KOH for 2 hours at room
132 temperature and sieved (200 μm). From the sieve residue approximately 50 subfossil *Daphnia*
133 ephippia and head capsules of chironomids of the tribe Chironomini were cleaned with a
134 forceps and placed in pre-weighed tin cups for isotope analysis, resulting in one sample for
135 $\delta^{13}\text{C}$ analysis of *Daphnia* ephippia from 14.5 m and two from 5 m water depth, and two
136 samples of Chironomini head capsules from 5 m water depth.

137 On August 9 2011, water samples were collected to characterize CH_4 concentration in
138 the lake water during late summer stratification, and for analysis of $\delta^{13}\text{C}$ values of CH_4 as a
139 basis for isotope mixing models (see [36] and the supplementary material for full details). In
140 addition, we retrieved more surface sediments (0-2 cm) from 2.5, 8, and 14.5 m depth as
141 described above which were sent to the Netherlands Institute of Ecology (NIOO) for
142 quantitative polymerase chain reaction (qPCR) analysis to assess the presence and abundance
143 of methanogens and MOB (see supplementary material for details on the methods).

144

145 *Down-core study*

146 On November 30 2009, a 68 cm long core (WAY09) was taken at 8 m water depth (just
147 beneath the summer thermocline) using a gravity corer, and sampled on site at 2 cm intervals.
148 17 samples were prepared for gamma spectrometric determination of ^{137}Cs (see
149 supplementary material). A subsample from the same sampling depths (~12 mg dry weight)
150 was treated with 2.5 % HCl to remove carbonates [35], then freeze dried and subsequently

151 loaded into tin cups for bulk sediment $\delta^{13}\text{C}$ analysis. Further subsamples were used to
152 reconstruct diatom-inferred total phosphorus concentrations (DI-TP) following Kirilova et al.
153 [33,34] (see supplementary material).

154 Sediment cores taken by Kirilova et al. [34] from Lake De Waay (WAY05) were
155 previously analyzed using a XRF core scanner (Avaatech, the Netherlands). Here we use the
156 ratio between Fe and Mn as an indication of past changes in the oxygen regime of the lake.
157 Higher values in this ratio are indicative for lower oxygen availability [37,38]. Cores WAY09
158 (this study) and WAY05 were correlated by comparing their ^{137}Cs profiles (see supplementary
159 material). Invertebrate remains were sorted from 17 samples and analysed for their $\delta^{13}\text{C}$
160 values, following the same procedure as for subfossil remains in the surface sediment samples
161 (see above and supplementary material). Relationships and lags between records were
162 quantified by cross-correlation analysis, after linear detrending of the time series (see
163 supplementary material).

164 To assess how much carbon in the diet of Chironomini and *Daphnia* could derive from
165 CH_4 , and how strongly this contribution may have varied in the past, we applied a two-source
166 mixing model [39] to the modern and down-core invertebrate $\delta^{13}\text{C}$ data. As end-members,
167 $\delta^{13}\text{C}$ values of POM (average) and a CH_4 sample from the sediment pore space were chosen,
168 the latter modified by -6 ‰ to account for fractionation by MOB [10] (see supplementary
169 material for further details). For the mixing model we assumed no change in baseline $\delta^{13}\text{C}$ of
170 CH_4 and POM over time because $\delta^{13}\text{C}$ values of bulk sedimentary organic matter remained
171 very stable within our record (-30.5 ± 0.5 ‰).

172

173 **Results**

174 *Current conditions in the lake*

175 POM $\delta^{13}\text{C}$ values (both 0-60 and 0-250 μm fractions were analysed) were around -36 ‰ in
176 late fall and late winter, and -30 ‰ in late spring and late summer (Figure 2A). The $\delta^{13}\text{C}$
177 value of bulk sedimentary organic matter in the top sediment layer at the coring site was -31.3
178 ‰ and the atomic C : N ratio was 13.1, suggesting a predominantly lacustrine origin of
179 organic material with some terrestrial contributions [35]. Chironomids of the tribe
180 Chironomini (Figure 2B) had an average $\delta^{13}\text{C}$ value of -32.5 ‰ (n = 31, standard deviation
181 (SD) \pm 1.95 ‰), which agrees well with values of Chironomini head capsules from the
182 surface sediment samples (-33.0 and -33.4 ‰). Only three individual chironomid larvae had
183 lower $\delta^{13}\text{C}$ values than the POM, all belonging to *Glyptotendipes barbipes*-type and collected
184 in late fall (-37.8, -39.0 and -39.0 ‰). *Daphnia* $\delta^{13}\text{C}$ values were highly variable (average -
185 36.6 ± 6.8 ‰, n = 6; Figure 2C). In late winter they were much lower than the POM (-44.2
186 and -44.3 ‰) as opposed to late summer (-26.5 ‰) and late spring (-35.0, -34.6 and -34.8 ‰).
187 Insufficient *Daphnia* were collected in late fall for a measurement. Floating *Daphnia* ephippia
188 $\delta^{13}\text{C}$ values were lower (average -41.7 ± 4.8 ‰, n = 9; Figure 2C) than POM throughout the
189 year and the values were in agreement with those found in ephippia from surface sediments (-
190 38.6, -39.3 and -39.6 ‰). Most notably, in late fall the floating ephippia reached $\delta^{13}\text{C}$ values
191 as low as -49.4 ‰.

192 $\delta^{13}\text{C}$ values of six CH_4 samples from anoxic waters and sediments ranged from -69.0
193 to -67.5 ‰, whereas values for two samples from oxygen-rich waters were -51.3 and -50.6 ‰
194 (Figure 2D). CH_4 concentrations in the surface and bottom waters were 1.1 and 479 μM ,
195 respectively. QPCR analyses revealed that gene copy numbers of methanotrophic bacteria as
196 well as of methanogenic archaea in sediment samples increased with water depth
197 (supplementary Table 1). At 2.5 m water depth the numbers of methanotrophs in the
198 sediments were below detection limit ($\sim 10^3$ gene copies gram sediment⁻¹). However, a nested
199 PCR approach indicated that MOB were present but below detection of qPCR assays. Type Ia
200 MOB dominated the methanotrophic community while type II MOB could not be detected.

201

202 *Down-core study*

203 Trophic history and oxygen availability

204 Fossil diatom assemblages (presented in the supplementary material) indicate clear shifts in
205 TP in Lake De Waay in the past ca. 100 years. The DI-TP values for Lake De Waay suggest
206 hypertrophic conditions ($>100 \mu\text{g TP l}^{-1}$, Figure 3) in the lower section of the core. This is
207 followed by a phase until ca. 1955 with a drop in DI-TP to around $35 \mu\text{g l}^{-1}$ which is related to
208 hydrological changes in the lake's catchment as a result of surface water management [34].
209 DI-TP increases again gradually between ca. 1955 and 1975 to $100 \mu\text{g l}^{-1}$ as the lake
210 underwent a distinct re-eutrophication. From thereon, the lake reverted back to hypertrophic
211 conditions as DI-TP exceeds $100 \mu\text{g l}^{-1}$, which is confirmed by water column TP
212 measurements in 2011 (Figure 1; [36]).

213 The oligotrophication during the early 20th century is associated with a distinct
214 lowering of the Fe : Mn ratio from 15 to 7 in core WAY05 (Figure 3). This suggests an
215 increase in oxygen availability at the sediment-water interface in the centre of the lake
216 [37,38]. The last part of the 20th century is then characterized by increasing Fe : Mn to values
217 around 15 indicating more anoxic conditions at the sediment-water interface. Both trends in
218 Fe : Mn follow the oligo- and eutrophication of the lake as inferred by diatoms. Cross-
219 correlation analysis revealed maximum correlations between DI-TP and Fe : Mn, if the
220 records are shifted by 1-2 sample steps (ca. 5 - 10 years; correlation coefficients 0.55 and
221 0.56, respectively), suggesting that the response of lake oxygenation lags ca. 10 years behind
222 the changes in trophic state (supplementary Figure 3).

223

224 Down-core $\delta^{13}\text{C}$

225 Bulk organic matter in the sediments had an average $\delta^{13}\text{C}$ value of -30.5 ‰, with little change
226 (SD \pm 0.5 ‰) throughout core WAY09 (Figure 4). Before ca. 1940 and after ca. 2000 head
227 capsules of the Chironomini had low $\delta^{13}\text{C}$ values (-35 ‰ and lower). Clearly higher values
228 are recorded between ca. 1940 and 2000 (-33.8 to -31.2 ‰). *Daphnia ephippia* $\delta^{13}\text{C}$ values
229 rise gradually from -40.0 to -33.7 ‰ between ca. 1920 and ca. 1970, after which a strong
230 opposite trend is apparent, with *Daphnia* $\delta^{13}\text{C}$ reaching values as low as -41.5 ‰ just below
231 the sediment surface. Cross correlation indicates strong negative correlations between Fe : Mn
232 and invertebrate $\delta^{13}\text{C}$ values at lags of 0 - 2 sample steps (ca. 0-10 years; correlation
233 coefficients -0.52 to -0.80) for *Daphnia* and 1-2 sample steps (ca. 5 - 10 years; correlation
234 coefficients -0.58 to -0.70) for Chironomini (supplementary Figure 3). The strongest negative
235 relationships were found for both invertebrate groups for a lag of 2 sample steps (ca. 10
236 years).

237

238 **Discussion**

239 *Current conditions in the lake*

240 Stable carbon isotope analysis of the different organisms and sedimentary remains in Lake De
241 Waay provided evidence for both photosynthetically produced and CH_4 -derived carbon
242 contributing to the aquatic food web. POM $\delta^{13}\text{C}$ values in Lake De Waay (-36 to -30 ‰) were
243 in the range of POM collected in small, eutrophic and/or high DIC lakes and characteristic for
244 algal biomass (-39 to -18 ‰ [13,40,41]). $\delta^{13}\text{C}$ values of *Daphnia* in late winter (-44.3 ‰) and
245 floating *Daphnia ephippia* in late fall (-49.4 ‰) in Lake De Waay were clearly lower than
246 reported for photoautotrophic biomass in small eutrophic lakes, and distinctly lower than the
247 $\delta^{13}\text{C}$ values of water column POM we observed. Low $\delta^{13}\text{C}$ values of zooplankton, and
248 *Daphnia* in particular, have been linked to the uptake of CH_4 -derived carbon [18,42]. The
249 pronounced difference in $\delta^{13}\text{C}$ values (\sim -19 ‰) between CH_4 sampled in the sediment and in

250 the oxic surface waters is an indication of MOB activity within the lake, as preferential uptake
251 of $^{12}\text{CH}_4$ by MOB [10] leads to higher $\delta^{13}\text{C}$ values of the CH_4 pool. This is supported by the
252 qPCR analysis that indicated presence of DNA of MOB type I in the surface sediments. The
253 low $\delta^{13}\text{C}$ values we found in *Daphnia* and their ephippia confirm that these organisms
254 incorporate MOB-derived carbon in Lake De Waay.

255 Temperature, starvation and lipid content can influence invertebrate $\delta^{13}\text{C}$ values, but
256 these effects are typically small (± 0 to 2 ‰ [11,25,43,44]) compared to the shifts we
257 observed, indicating that seasonal variations in $\delta^{13}\text{C}$ values of *Daphnia* in De Waay mainly
258 reflect changing availability and $\delta^{13}\text{C}$ values of available food sources. Based on the two-
259 source mixing model, we estimate a contribution of CH_4 -derived carbon to the diet of
260 *Daphnia* ranging from 0 % (in late spring and summer) to 27 % (based on body tissue) and 39
261 % (based on ephippia) in late fall and winter. This is in agreement with findings by Taipale et
262 al. [20], who found the strongest contribution of CH_4 -derived carbon to the diet of *Daphnia* in
263 a polyhumic boreal lake in Finland in fall. Similarly, Harrod and Grey [45] and Morlock et al.
264 [46] reported Cladoceran $\delta^{13}\text{C}$ values 10-20 ‰ lower in fall and winter than in summer in
265 eutrophic lakes in Germany and Switzerland, respectively. These results indicate that these
266 invertebrates can utilise a CH_4 -derived carbon source when the preferred food sources are less
267 readily available.

268 The Chironomini larvae were sampled in the littoral zone, whereas MOB-feeding
269 chironomids are mostly found in sediment exposed to low oxygen concentrations [47–49].
270 The sampling location may explain why the majority of the living Chironomini we sampled
271 did not exhibit as low $\delta^{13}\text{C}$ values as in some studies (e.g. [47]), even though CH_4 and MOB
272 appear to play a major role in the lake food web. Nevertheless, several individuals had $\delta^{13}\text{C}$
273 values distinctly lower than observed for POM and the other Chironomini larvae, suggesting
274 that CH_4 -derived carbon may have contributed to their diet even in littoral habitats (12 to 15

275 % based on the mixing model). Agasild et al. [49] also reported at least 40 % CH₄-derived
276 carbon in the diet of *Chironomus plumosus* found in the littoral, macrophyte-covered zone of
277 a shallow lake.

278 Chironomini head capsules and *Daphnia* ephippia in the uppermost analysed sample
279 in core WAY09 were also characterized by low $\delta^{13}\text{C}$ values (-38.5 ‰ for Chironomini and -
280 41.5 ‰ for *Daphnia* ephippia), values which are again well below the average of modern
281 water column POM and the sedimentary organic matter in the surface sediments. This
282 suggests that the imprint of CH₄-derived carbon on the $\delta^{13}\text{C}$ values of the organisms is
283 registered in the fossil record, even though this record integrates seasonal and spatial
284 variability in *Daphnia* and chironomid $\delta^{13}\text{C}$ values.

285

286 *Carbon sources during changing nutrient levels*

287 The DI-TP reconstruction confirms, with higher temporal resolution, the conclusions
288 by Kirilova et al. [33,34] that the lake went from eutrophic conditions at the beginning of the
289 20th century to more mesotrophic conditions between ca. 1925 and 1955, followed by a trend
290 to the current, hypertrophic conditions (Figure 3). The Fe : Mn record indicates lower
291 availability of oxygen at the sediment-water interface during the eutrophic and hypertrophic
292 phases (Figure 3), conditions that promote methanogenesis [50], although the variations in
293 oxygen availability take place more gradually and lag those in nutrient concentrations by ca.
294 5-10 years. This lag may reflect the time needed to accumulate organic rich, oxygen-
295 demanding sediments during eutrophication and the lingering oxygen demand of such
296 sediments after oligotrophication [51]. The oldest and the most recent sediments, representing
297 the highest nutrient levels and lowest oxygen availability, featured $\delta^{13}\text{C}$ values in chitinous
298 remains of *Daphnia* and Chironomini that resemble the low values we found during the field
299 survey (Figure 2; Figure 3). As discussed above, this suggests a contribution of CH₄-derived

300 carbon to the diet of *Daphnia* (up to 20 % based on the mixing model, Figure 3) and
301 Chironomini (up to 12 %). In intermediate sections of the record, the analyzed invertebrate
302 remains had distinctly higher $\delta^{13}\text{C}$ values, which may indicate a lower (or even a lack of)
303 contribution of CH_4 -derived carbon to the diets of *Daphnia* (less than 10 %) and Chironomini
304 (less than 2 %) during this period. Variations in $\delta^{13}\text{C}$ values of *Daphnia* were more gradual
305 than variations in Chironomini $\delta^{13}\text{C}$ values. However, the maxima in both curves closely
306 followed the observed minimum in Fe : Mn values, with the strongest negative relationships
307 between the records observed for a small lag of ca. 5 - 10 years (Supplementary Figure 3). This
308 suggests that variations in invertebrate $\delta^{13}\text{C}$ values were related to changes in oxygen
309 availability in the hypolimnion resulting from changes in lake productivity.

310 There are alternative explanations for changes in invertebrate $\delta^{13}\text{C}$ values in lake
311 sediment records. However, these cannot explain the full range of invertebrate $\delta^{13}\text{C}$ values
312 observed for lake De Waay. $\delta^{13}\text{C}$ values of autochthonous photoautotrophic primary
313 production may vary in lakes, due to changing ^{12}C -preference of algae during carbon uptake,
314 and/or shifts in baseline $\delta^{13}\text{C}$ values of DIC. Lower algal growth rates under lower nutrient
315 availability lead to higher discrimination against ^{13}C during photosynthesis and therefore
316 more ^{13}C -depleted algal biomass (e.g. [52]). Therefore, this mechanism would have caused
317 lower $\delta^{13}\text{C}$ values of algal biomass and correspondingly lower *Daphnia* $\delta^{13}\text{C}$ values during
318 the mesotrophic conditions reconstructed for the lake ca. 1925-1955. This implies that a major
319 increase in baseline $\delta^{13}\text{C}$ values of DIC would have been necessary to explain the increase in
320 *Daphnia* $\delta^{13}\text{C}$ values, even exceeding the 8 ‰ shift observed in *Daphnia ephippia*.
321 Considering the present DIC $\delta^{13}\text{C}$ values of -9.1 ‰ [28] this would only be possible if DIC
322 reached unrealistically high $\delta^{13}\text{C}$ values of ~0 ‰, which exceeded the range of DIC $\delta^{13}\text{C}$ values
323 reported in a wide range of lakes (-31.1 to -2.1 ‰, [53]).

324 Heterotrophic respiration of DOC can also lead to ^{13}C -depletion of DIC available to
325 algae and consequently of organisms that feed on them, a process often reported for lakes
326 with high DOC concentrations [54]. Since Lake De Waay is presently characterized by
327 relatively low DOC concentrations (0.5 mmol l^{-1} ; [55]), a strong increase in heterotrophic
328 respiration of DOC during the second half of the 20th century is unlikely. Moreover, we would
329 expect that major variations in $\delta^{13}\text{C}$ values of of algal production in Lake De Waay would
330 have led to distinct variations in $\delta^{13}\text{C}$ values of bulk organic matter in the sediments of Lake
331 De Waay.

332 As indicated above, factors such as starvation, temperature and lipid composition have
333 only minor effects on $\delta^{13}\text{C}$ values of aquatic invertebrates ($\pm 0 - 2 \text{ ‰}$) [11,25,43,44]. These
334 factors therefore cannot (fully) explain the major changes in fossil invertebrate $\delta^{13}\text{C}$ values
335 observed in De Waay. Finally, changes in the timing of *Daphnia* ehippia production may
336 potentially have some effect on the $\delta^{13}\text{C}$ values of the fossil assemblage. However, given the
337 supporting evidence of changes in trophic state and oxygenation regime, as well as the similar
338 trends in Chironomini $\delta^{13}\text{C}$ values we consider it highly unlikely that this is the primary cause
339 for changes in ehippia $\delta^{13}\text{C}$ values in the sediments of Lake de Waay.

340

341 **Conclusions**

342 We have shown that in the currently hypertrophic Lake De Waay CH_4 -derived carbon plays a
343 relevant role in the pelagic food web and most likely also in parts of the benthic food web,
344 based on the very low $\delta^{13}\text{C}$ values of *Daphnia*, floating *Daphnia* ehippia and some
345 chironomids in comparison to POM values and sedimentary organic matter. This is clearest in
346 fall (Figure 2), when photosynthetic primary productivity as food source is declining, and
347 stored hypolimnetic CH_4 is mixed with oxygen-rich water layers, providing favourable
348 conditions for MOB. Our down-core study revealed that during the beginning of the 20th

349 century higher nutrient levels and relatively lower oxygen availability occurred, comparable
350 to the modern situation (Figure 3). Under these conditions $\delta^{13}\text{C}$ values of remains of *Daphnia*
351 and Chironomini were very low, and lower than may be expected from feeding on
352 photoautotrophic biomass only [11–15]. This suggests a significant contribution of CH_4 -
353 derived carbon to the lake's food web, comparable to the modern situation. In contrast, the
354 more mesotrophic phase between ca. 1925 and 1955, which was associated with higher
355 oxygen availability at the sediment-water interface, was associated with distinctly (up to 8 ‰)
356 higher $\delta^{13}\text{C}$ values in the investigated invertebrate remains (Figure 3).

357 We conclude that the eutrophication of the lake resulted in an increase in primary
358 productivity and an increase in strength and duration of hypoxic conditions, which allow for
359 both increased CH_4 production in the sediment and increased build-up of dissolved CH_4 in the
360 hypolimnion [4–6]. MOB can be expected to thrive under these conditions and can therefore
361 provide a more readily available food source for *Daphnia* in the water column and
362 Chironomini living in sediments near the oxycline. This implies that even though
363 eutrophication can lead to a higher availability of algal organic matter in lakes, some
364 invertebrate taxa may benefit from the higher availability of CH_4 -derived carbon as an
365 alternative food source, leading to an increased role of CH_4 -derived carbon for at least some
366 sections of the lake food webs, and that oligotrophication can have the opposite effect. It is
367 likely that the increased CH_4 -derived carbon utilisation is due to higher CH_4 production (in
368 addition to a longer build-up of hypolimnetic CH_4)[6], which implies that CH_4 emissions by
369 lakes are potentially higher after eutrophication events and that (re-)oligotrophication may
370 lower CH_4 emissions. This is also confirmed by experiments that revealed increased
371 methanogenesis in sediments after addition of both P and N [56]. Based on our record there
372 may be multiannual to decadal-scale lags between variations in nutrient concentrations and
373 changes in CH_4 -derived carbon entering lake food webs.

374

375 **Data**

376 The down-core stable isotope data as well as the DI-TP reconstruction can be found in the
377 supplementary material in comma-delimited text format.

378

379 **Competing interests**

380 We have no competing interests.

381

382 **Author's contributions**

383 The study was designed by JS, MvH and OH. The field work was carried out by JS and MvH.
384 Processing samples for isotope analysis was done by JS. PB performed the microbiological
385 analyses; EK performed the diatom analysis; AL was responsible for the dating of sediments
386 and ML for stable isotope analysis of the gas samples. All authors helped draft the MS and
387 gave final approval for publication.

388

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395

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558

559

560 **Figure 1:** Bathymetric map of Lake De Waay. The circles indicate the coring locations (see
561 supplementary material): (09) core WAY09 presented in this study, (05) core WAY05
562 investigated by Kirilova et al. (2010b) from which we used the XRF data (see below).
563 Asterisks (*) indicate locations of the surface sediment samples at 2.5, 5, 8 and 14.5 m depth.
564 Lake water nutrient concentrations and pH were measured in August 2011.

565

566 **Figure 2:** $\delta^{13}\text{C}$ values of (a) POM, (b) Chironomini body tissue, (c) *Daphnia* body tissue
567 (open circles) and *Daphnia* ehippia (dots) sampled on September 1 and December 1 2009
568 and March 1 and June 1 2010. The lines indicate the $\delta^{13}\text{C}$ values of bulk sediment organic
569 matter from the top sample of the core (a), $\delta^{13}\text{C}$ values of Chironomini head capsules from
570 surface sediment at 5 m water depth (two replicates) (b), and $\delta^{13}\text{C}$ values of *Daphnia* ehippia
571 from surface sediments at 5 m water depth (2 replicates, dashed lines) and 15 m water depth
572 (solid line) (c). (d) Boxplot showing the range of $\delta^{13}\text{C}$ values from the field survey, as well as
573 the $\delta^{13}\text{C}$ of CH_4 sampled in the sediment and in oxic and anoxic water. Whiskers of the
574 boxplots encompass data points no more than 1.5 times the interquartile range from the box.

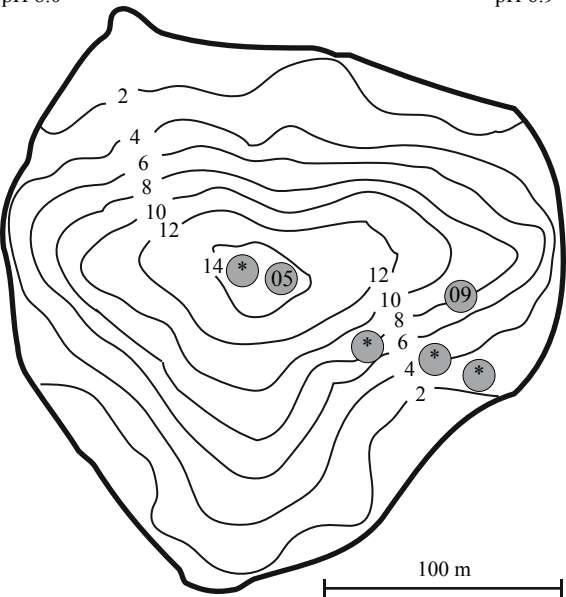
575

576 **Figure 3:** Diatom-inferred TP (DI-TP) for the De Waay sediment record, grey lines indicate
577 estimated standard errors of prediction (note the log scale); Fe : Mn of the sediments (grey
578 line: high resolution measurements (near-yearly resolution), black symbols: average values
579 for intervals represented by fossil samples); $\delta^{13}\text{C}$ values of bulk sediment organic matter
580 (closed triangles), Chironomini head capsules (closed diamonds) and *Daphnia* ehippia
581 (closed circles) (note inverted scale); and the estimated contribution of CH_4 -derived carbon in
582 the diet of Chironomini (open diamonds) and *Daphnia* (open circles) based on a two-source
583 mixing model. Fe : Mn ratios are from core WAY05 taken by Kirilova et al. [34] in the lake

584 centre, and DI-TP and $\delta^{13}\text{C}$ data from core WAY09 obtained from 8 m water depth (this
585 study).

Surface water
TP 120 $\mu\text{g l}^{-1}$,
TN 2300 $\mu\text{g l}^{-1}$
pH 8.0

Bottom water
TP 1500 $\mu\text{g l}^{-1}$
TN 3600 $\mu\text{g l}^{-1}$
pH 6.9



$\delta^{13}\text{C}$ (‰ V-PDB)