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

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

1. Introduction

Eutrophication of inland waters as a consequence of human impact has a detrimental effect on different aspects of the water quality of lakes, rivers and streams [1]. For example, the process can change the chemical properties of the water, leading to oxygen depletion [2] and accumulation of nutrients in the anoxic hypolimnion [3]. Lake ecosystems with low oxygen concentrations and high nutrient loading are characterized by higher output of the important greenhouse gas methane (CH_4) than oxygen-rich lakes and lakes with lower nutrient availability [4,5], particularly via gas bubbles (ebullition) and release of CH_4 stored in the anoxic hypolimnion during lake overturning [6]. CH_4 formed in lakes can be oxidized by methane-oxidizing bacteria (MOB), predominantly in oxygenated sections of the lake basin [7]. Biogenic CH_4 in freshwater systems is characterized by distinctly low ratios between the stable carbon isotopes ^{13}C and ^{12}C (expressed as $\delta^{13}C$ values; −80 to −50‰) [8,9] and MOB are known to discriminate against the heavier ^{13}C when metabolizing CH_4, resulting in even lower $\delta^{13}C$ values of MOB biomass [10]. These very low values do not occur in aquatic and terrestrial photosynthetic primary producers (−35 to −10‰ [11–15]). The very low observed $\delta^{13}C$ values of, for example, larvae of non-biting midges (Chironomidae) of the tribe Chironomini [16,17] and planktonic water fleas of the genus *Daphnia* (Cladocera) [18] in some lakes are therefore considered a clear indication of MOB, or organisms feeding on MOB, forming a relevant part of the diet of these organisms.

Planktonic filterers such as *Daphnia* can graze MOB from the water column during stratification, a process which can effectively reduce MOB biomass and lead to increased epilimnetic CH_4 concentrations at least in some shallow boreal lakes [19]. Furthermore, *Daphnia* has been shown to rely strongly on MOB-derived carbon during autumn overturning, when the CH_4 stored in deep anoxic water layers of stratified lakes comes into contact with oxygen [20]. Benthic invertebrates that can incorporate CH_4-derived carbon, such as chironomid larvae of the tribe Chironomini, either feed on MOB in the sediments (deposit feeders) or MOB associated with suspended organic particles (filter feeders). Some Chironomini larvae have been shown to actively maintain an oxic–anoxic interface within their tubes, providing a habitat for MOB which they feed on [21]. $\delta^{13}C$ values of chironomids and *Daphnia* closely reflect those of their food source (differences of 0–1‰) and of their fossilizing chitinous structures (reported offsets of 0–1‰) [22–25]. Chitinous remains deposited and buried in the lake sediments retain their original isotopic composition [23]. Hence, analysis of $\delta^{13}C$ values of 'fossil' chironomid and *Daphnia* remains can provide insights on their past food sources and into whether CH_4-derived carbon formed a major component of their diet [23,26–31]. Available studies indicate that chitinous remains with low $\delta^{13}C$ values are deposited in lakes with high surface and deep water CH_4 concentrations and diffusive CH_4 emissions [28,29].

It is unclear whether an increase in nutrient loading, in addition to a higher CH_4 output, also results in a higher contribution of CH_4-derived carbon to the lake food webs. Higher photosynthetic primary productivity associated with higher nutrient loading can increase the availability of algal organic matter. As a consequence, a higher proportion of algae in the diet of invertebrate groups that may also incorporate CH_4-derived carbon could be expected. However, higher algal productivity can also lead to decreased oxygen concentrations in lake sediments and deep water layers, and associated increases in CH_4 production and CH_4 availability in lake ecosystems. This can favour the growth of MOB and their temporal and spatial availability within lakes. Therefore, how the relevance of CH_4-derived carbon in the food web of lakes changes under influence of (past or future) changes in nutrient concentrations and productivity remains poorly constrained, particularly on decadal time scales which are not covered by instrumental measurements of CH_4 concentrations and $\delta^{13}C$ values in aquatic ecosystems.

The $\delta^{13}C$ values of chitinous remains of aquatic invertebrates were previously mainly studied in oligo- to mesotrophic, often remote lakes [27,29,30,32]. Here, we present a study of the $\delta^{13}C$ values of fossil Chironomini head capsules and *Daphnia* resting eggs (ephippia) from recent

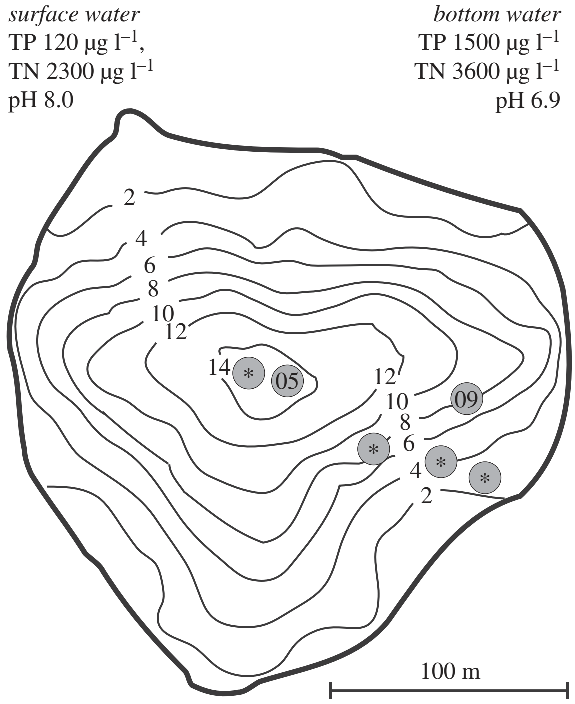

Figure 1. Bathymetric map of Lake De Waay. The circles indicate the coring locations (see the electronic supplementary material): (09) core WAY09 presented in this study, (05) core WAY05 investigated by Kirilova *et al*. [33] from which we used the XRF data (see below). Asterisks (*) indicate locations of the surface sediment samples at 2.5, 5, 8 and 14.5 m depth. Lake water nutrient concentrations and pH were measured in August 2011. TP, total phosphorus; TN, total nitrogen.

(twentieth century) sediments from a small and presently hypertrophic dimictic lake in The Netherlands (figure 1). The study lake, Lake De Waay, underwent a transition from eutrophic (total phosphorus (TP) $\sim 100\ \mu g\ l^{-1}$) to more mesotrophic conditions (TP $\sim 40\ \mu g\ l^{-1}$) and then again to hypertrophic conditions (TP $> 100\ \mu g\ l^{-1}$) during the past approximately 100 years [33,34]. The impact of twentieth-century temperature changes on lake ecosystems in The Netherlands is likely to have been relatively minor compared with direct anthropogenic environmental disturbances. Therefore, this study provides an opportunity to investigate the effects of both oligotrophication and (re-)eutrophication and the associated changes in oxygenation regime on the contribution of CH_4-derived carbon to the aquatic food web, and indirectly on in-lake dissolved CH_4 availability, under relatively stable climatic conditions. We compare fossil invertebrate $\delta^{13}C$ values with a diatom-inferred reconstruction of total phosphorus concentrations (DI-TP) in the lake water and the Fe : Mn ratio of the sediments, which is expected to increase with decreasing oxygen availability at the sediment–water interface [35]. If CH_4-derived carbon became more relevant for Lake De Waay's food web under conditions with higher nutrient availability, we expect to see a positive relationship between nutrient availability (DI-TP) and anoxia (Fe : Mn ratio), which in turn are expected to be negatively related to $\delta^{13}C$ values of the examined invertebrate groups that can incorporate CH_4-derived carbon. Conversely, if higher availability of algal material in the eutrophic to hypertrophic phases led to a lower relevance of CH_4-derived carbon for the investigated aquatic invertebrates, we expect to see positive relationships between the $\delta^{13}C$ values of the studied invertebrate groups and DI-TP and Fe : Mn in Lake De Waay.

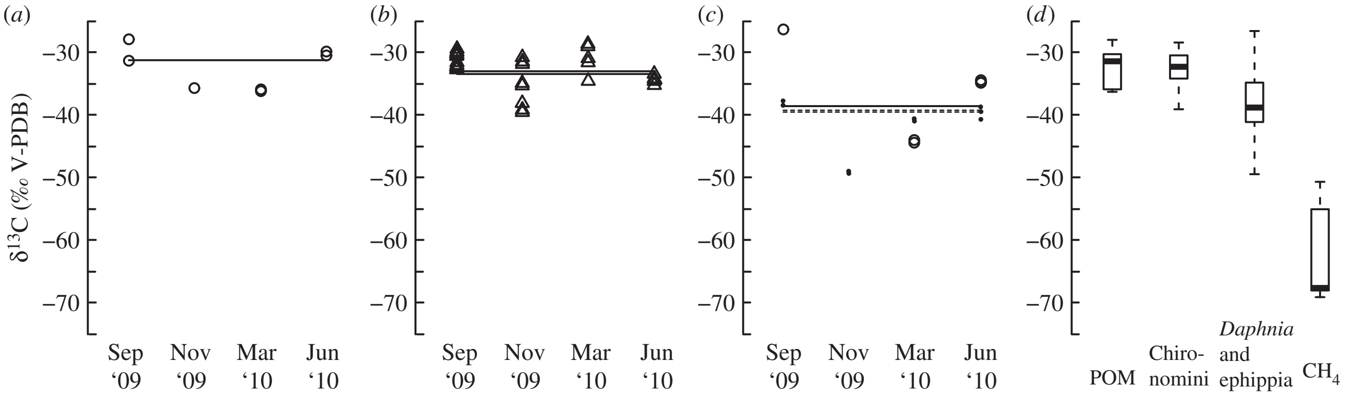

Figure 2. $\delta^{13}C$ values of (*a*) POM, (*b*) Chironomini body tissue, (*c*) *Daphnia* body tissue (open circles) and *Daphnia* ephippia (dots) sampled on 3 September and 30 November 2009 and 1 March and 1 June 2010. The lines indicate the $\delta^{13}C$ values of bulk sediment organic matter from the top sample of the core (*a*), $\delta^{13}C$ values of Chironomini head capsules from surface sediment at 5 m water depth (two replicates) (*b*), and $\delta^{13}C$ values of *Daphnia* ephippia from surface sediments at 5 m water depth (two replicates, dashed lines) and 15 m water depth (solid line) (*c*). (*d*) Boxplot showing the range of $\delta^{13}C$ values from the field survey, as well as the $\delta^{13}C$ of CH_4 sampled in the sediment and in oxic and anoxic water. Whiskers of the boxplots encompass data points no more than 1.5 times the interquartile range from the box. V-PDB, Vienna Pee Dee Belemnite.

2. Material and methods

(a) Current conditions in the lake

To assess the current range of $\delta^{13}C$ values of chironomids, *Daphnia*, floating *Daphnia* ephippia and suspended particulate organic matter (POM) in the water column of Lake De Waay, field sampling was conducted on 3 September and 30 November 2009, and 1 March and 1 June 2010. Living organisms were collected using plankton nets, kicknets and inspection of submerged wood and rope. In the laboratory, organisms were sorted and transferred to tin cups and water was filtered for $\delta^{13}C$ analysis (see the electronic supplementary material for details on sampling and processing).

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

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

(b) Down-core study

On 30 November 2009, a 68 cm long core (WAY09) was taken at 8 m water depth (just beneath the summer thermocline) using a gravity corer, and sampled on site at 2 cm intervals. Seventeen samples were prepared for gamma spectrometric determination of ^{137}Cs (see the electronic supplementary material). A subsample from the same sampling depths (approx. 12 mg dry weight) was treated with 2.5% HCl to remove carbonates [35], then freeze dried and subsequently loaded into tin cups for bulk sediment $\delta^{13}C$ analysis. Further subsamples were used to reconstruct diatom-inferred total phosphorus concentrations (DI-TP) following Kirilova *et al*. [33,34] (see the electronic supplementary material).

Sediment cores taken by Kirilova *et al*. [33] from Lake De Waay (WAY05) were previously analysed using a XRF core scanner (Avaatech, The Netherlands). Here, we use the ratio between Fe and Mn as an indication of past changes in the oxygen regime of the lake. Higher values in this ratio are indicative for lower oxygen availability [37,38]. Cores WAY09 (this study) and WAY05 were correlated by comparing their ^{137}Cs profiles (see the electronic supplementary material). Invertebrate remains were sorted from 17 samples and analysed for their $\delta^{13}C$ values, following the same procedure as for subfossil remains in the surface sediment samples (see above and the electronic supplementary material). Relationships and lags between records were quantified by cross-correlation analysis, after linear detrending of the time series (see the electronic supplementary material).

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

3. Results

(a) Current conditions in the lake

POM $\delta^{13}C$ values (both 0–60 and 0–250 μm fractions were analysed) were around −36‰ in late autumn and late winter, and −30‰ in late spring and late summer (figure 2*a*). The $\delta^{13}C$ value of bulk sedimentary organic matter in the top sediment layer at the coring site was −31.3‰ and the atomic C : N ratio was 13.1, suggesting a predominantly lacustrine origin of organic material with some terrestrial contributions [35]. Chironomids of the tribe Chironomini (figure 2*b*) had an average $\delta^{13}C$ value of −32.5‰ ($n = 31$, standard deviation (s.d.) ± 1.95‰), which

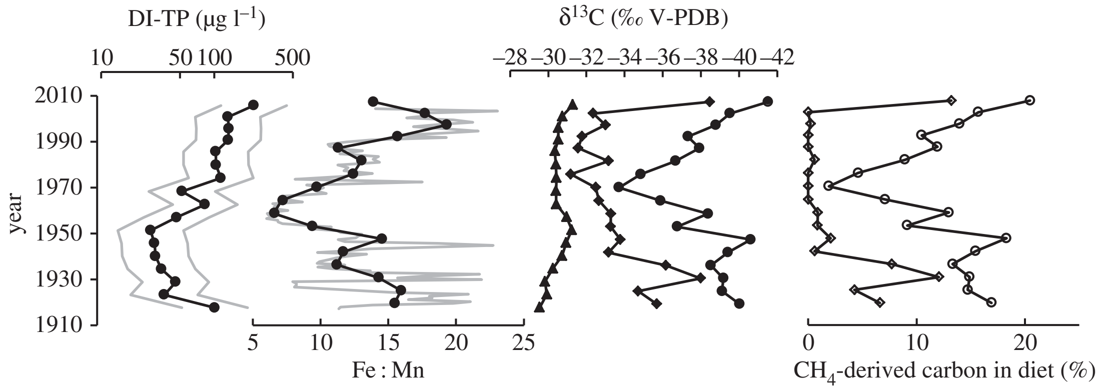

Figure 3. Diatom-inferred TP (DI-TP) for the De Waay sediment record, grey lines indicate estimated standard errors of prediction (note the log scale); Fe : Mn of the sediments (grey line: high resolution measurements (near-yearly resolution), black symbols: average values for intervals represented by fossil samples); $\delta^{13}C$ values of bulk sediment organic matter (closed triangles), Chironomini head capsules (closed diamonds) and *Daphnia* ephippia (closed circles) (note inverted scale); and the estimated contribution of CH_4-derived carbon in the diet of Chironomini (open diamonds) and *Daphnia* (open circles) based on a two-source mixing model. Fe : Mn ratios are from core WAY05 taken by Kirilova *et al*. [33] in the lake centre, and DI-TP and $\delta^{13}C$ data from core WAY09 obtained from 8 m water depth (this study). V-PDB, Vienna Pee Dee Belemnite.

agrees well with values of Chironomini head capsules from the surface sediment samples (−33.0 and −33.4‰). Only three individual chironomid larvae had lower $\delta^{13}C$ values than the POM, all belonging to *Glyptotendipes barbipes*-type and collected in late autumn (−37.8, −39.0 and −39.0‰). *Daphnia* $\delta^{13}C$ values were highly variable (average −36.6 ± 6.8‰, $n = 6$; figure 2*c*). In late winter, they were much lower than the POM (−44.2 and −44.3‰) as opposed to late summer (−26.5‰) and late spring (−35.0, −34.6 and −34.8‰). Insufficient *Daphnia* were collected in late autumn for a measurement. Floating *Daphnia* ephippia $\delta^{13}C$ values were lower (average −41.7 ± 4.8‰, $n = 9$; figure 2*c*) than POM throughout the year and the values were in agreement with those found in ephippia from surface sediments (−38.6, −39.3 and −39.6‰). Most notably, in late autumn, the floating ephippia reached $\delta^{13}C$ values as low as −49.4‰.

$\delta^{13}C$ values of six CH_4 samples from anoxic waters and sediments ranged from −69.0 to −67.5‰, whereas values for two samples from oxygen-rich waters were −51.3 and −50.6‰ (figure 2*d*). CH_4 concentrations in the surface and bottom waters were 1.1 and 479 µM, respectively. qPCR analyses revealed that gene copy numbers of methanotrophic bacteria as well as of methanogenic archaea in sediment samples increased with water depth (electronic supplementary material, table S1). At 2.5 m water depth, the numbers of methanotrophs in the sediments were below detection limit (approx. 10^3 gene copies gram sediment^{-1}). However, a nested PCR approach indicated that MOB were present but below detection of qPCR assays. Type Ia MOB dominated the methanotrophic community, while type II MOB could not be detected.

(b) Down-core study

(i) Trophic history and oxygen availability

Fossil diatom assemblages (presented in the electronic supplementary material) indicate clear shifts in TP in Lake De Waay in the past *ca* 100 years. The DI-TP values for Lake De Waay suggest hypertrophic conditions (greater than 100 µg TP l^{-1}, figure 3) in the lower section of the core. This is followed by a phase until *ca* 1955 with a drop in DI-TP to around 35 µg l^{-1} which is related to hydrological changes in the lake's catchment as a result of surface water management [33]. DI-TP increases again gradually between *ca* 1955 and 1975 to 100 µg l^{-1} as the lake underwent a distinct re-eutrophication. From thereon, the lake reverted back to hypertrophic conditions as DI-TP exceeds 100 µg l^{-1}, which is confirmed by water column TP measurements in 2011 (figure 1; [36]).

The oligotrophication during the early twentieth century is associated with a distinct lowering of the Fe : Mn ratio from 15 to 7 in core WAY05 (figure 3). This suggests an increase in oxygen availability at the sediment–water interface in the centre of the lake [37,38]. The last part of the twentieth century is then characterized by increasing Fe : Mn to values around 15, indicating more anoxic conditions at the sediment–water interface. Both trends in Fe : Mn follow the oligo- and eutrophication of the lake as inferred by diatoms. Cross-correlation analysis revealed maximum correlations between DI-TP and Fe : Mn, if the records are shifted by one to two sample steps (*ca* 5–10 years; correlation coefficients 0.55 and 0.56, respectively), suggesting that the response of lake oxygenation lags approximately 10 years behind the changes in trophic state (electronic supplementary material, figure S3).

(ii) Down-core $\delta^{13}C$

Bulk organic matter in the sediments had an average $\delta^{13}C$ value of −30.5‰, with little change (s.d. ± 0.5‰) throughout core WAY09 (figure 3). Before *ca* 1940 and after *ca* 2000, head capsules of the Chironomini had low $\delta^{13}C$ values (−35‰ and lower). Clearly, higher values are recorded between *ca* 1940 and 2000 (−33.8 to −31.2‰). *Daphnia* ephippia $\delta^{13}C$ values rise gradually from −40.0 to −33.7‰ between approximately 1920 and approximately 1970, after which a strong opposite trend is apparent, with *Daphnia* $\delta^{13}C$ reaching values as low as −41.5‰ just below the sediment surface. Cross-correlation indicates strong negative correlations between Fe : Mn and invertebrate $\delta^{13}C$ values at lags of zero to two sample steps (approx. 0–10 years; correlation coefficients −0.52 to −0.80) for *Daphnia* and one to two sample steps (approx. 5–10 years; correlation coefficients −0.58 to −0.70) for Chironomini (electronic supplementary material, figure S3). The strongest negative relationships were found for both invertebrate groups for a lag of two sample steps (*ca* 10 years).

4. Discussion

(a) Current conditions in the lake

Stable carbon isotope analysis of the different organisms and sedimentary remains in Lake De Waay provided evidence for both photosynthetically produced and CH_4-derived carbon contributing to the aquatic food web. POM $\delta^{13}C$ values in Lake De Waay (−36 to −30‰) were in the range of POM collected in small, eutrophic and/or high dissolved inorganic carbon (DIC) lakes and characteristic for algal biomass (−39 to −18‰ [13,40,41]). $\delta^{13}C$ values of *Daphnia* in late winter (−44.3‰) and floating *Daphnia* ephippia in late autumn (−49.4‰) in Lake De Waay were clearly lower than reported for photoautotrophic biomass in small eutrophic lakes, and distinctly lower than the $\delta^{13}C$ values of water column POM we observed. Low $\delta^{13}C$ values of zooplankton, and *Daphnia* in particular, have been linked to the uptake of CH_4-derived carbon [18,42]. The pronounced difference in $\delta^{13}C$ values (approx. −19‰) between CH_4 sampled in the sediment and in the oxic surface waters is an indication of MOB activity within the lake, as preferential uptake of $^{12}CH_4$ by MOB [10] leads to higher $\delta^{13}C$ values of the CH_4 pool. This is supported by the qPCR analysis that indicated the presence of DNA of MOB type I in the surface sediments. The low $\delta^{13}C$ values we found in *Daphnia* and their ephippia confirm that these organisms incorporate MOB-derived carbon in Lake De Waay.

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

The Chironomini larvae were sampled in the littoral zone, whereas MOB-feeding chironomids are mostly found in sediment exposed to low oxygen concentrations [47–49]. The sampling location may explain why the majority of the living Chironomini we sampled did not exhibit as low $\delta^{13}C$ values as in some studies [47], even though CH_4 and MOB appear to play a major role in the lake food web. Nevertheless, several individuals had $\delta^{13}C$ values distinctly lower than observed for POM and the other Chironomini larvae, suggesting that CH_4-derived carbon may have contributed to their diet even in littoral habitats (12–15% based on the mixing model). Agasild *et al*. [49] also reported at least 40% CH_4-derived carbon in the diet of *Chironomus plumosus* found in the littoral, macrophyte-covered zone of a shallow lake.

Chironomini head capsules and *Daphnia* ephippia in the uppermost analysed sample in core WAY09 were also characterized by low $\delta^{13}C$ values (−38.5‰ for Chironomini and −41.5‰ for *Daphnia* ephippia), values which are again well below the average of modern water column POM and the sedimentary organic matter in the surface sediments. This suggests that the imprint of CH_4-derived carbon on the $\delta^{13}C$ values of the organisms is registered in the fossil record, even though this record integrates seasonal and spatial variability in *Daphnia* and chironomid $\delta^{13}C$ values.

(b) Carbon sources during changing nutrient levels

The DI-TP reconstruction confirms, with higher temporal resolution, the conclusions by Kirilova *et al*. [33,34] that the lake went from eutrophic conditions at the beginning of the twentieth century to more mesotrophic conditions between *ca* 1925 and 1955, followed by a trend to the current, hypertrophic conditions (figure 3). The Fe : Mn record indicates lower availability of oxygen at the sediment–water interface during the eutrophic and hypertrophic phases (figure 3), conditions that promote methanogenesis [50], although the variations in oxygen availability take place more gradually and lag those in nutrient concentrations by approximately 5–10 years. This lag may reflect the time needed to accumulate organic rich, oxygen-demanding sediments during eutrophication and the lingering oxygen demand of such sediments after oligotrophication [51]. The oldest and the most recent sediments, representing the highest nutrient levels and lowest oxygen availability, featured $\delta^{13}C$ values in chitinous remains of *Daphnia* and Chironomini that resemble the low values we found during the field survey (figures 2 and 3). As discussed above, this suggests a contribution of CH_4-derived carbon to the diet of *Daphnia* (up to 20% based on the mixing model, figure 3) and Chironomini (up to 12%). In intermediate sections of the record, the analysed invertebrate remains had distinctly higher $\delta^{13}C$ values, which may indicate a lower (or even a lack of) contribution of CH_4-derived carbon to the diets of *Daphnia* (less than 10%) and Chironomini (less than 2%) during this period. Variations in $\delta^{13}C$ values of *Daphnia* were more gradual than variations in Chironomini $\delta^{13}C$ values. However, the maxima in both curves closely followed the observed minimum in Fe : Mn values, with the strongest negative relationships between the records observed for a small lag of approximately 5–10 years (electronic supplementary material, figure S3). This suggests that variations in invertebrate $\delta^{13}C$ values were related to changes in oxygen availability in the hypolimnion resulting from changes in lake productivity.

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

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

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

5. Conclusion

We have shown that in the currently hypertrophic Lake De Waay, CH_4-derived carbon plays a relevant role in the pelagic food web and most likely also in parts of the benthic food web, based on the very low $\delta^{13}C$ values of *Daphnia*, floating *Daphnia* ephippia and some chironomids in comparison to POM values and sedimentary organic matter. This is clearest in autumn (figure 2), when photosynthetic primary productivity as food source is declining, and stored hypolimnetic CH_4 is mixed with oxygen-rich water layers, providing favourable conditions for MOB. Our down-core study revealed that during the beginning of the twentieth century, higher nutrient levels and relatively lower oxygen availability occurred, comparable to the modern situation (figure 3). Under these conditions, $\delta^{13}C$ values of remains of *Daphnia* and Chironomini were very low, and lower than may be expected from feeding on photoautotrophic biomass only [11–15]. This suggests a significant contribution of CH_4-derived carbon to the lake's food web, comparable to the modern situation. By contrast, the more mesotrophic phase between *ca* 1925 and 1955, which was associated with higher oxygen availability at the sediment–water interface, was associated with distinctly (up to 8‰) higher $\delta^{13}C$ values in the investigated invertebrate remains (figure 3).

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

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

Authors' contributions. The study was designed by J.S., M.H. and O.H. The fieldwork was carried out by J.S. and M.H. Processing samples for isotope analysis was done by J.S. P.B. performed the microbiological analyses; E.P.K. performed the diatom analysis; A.F.L. was responsible for the dating of sediments and M.L. for stable isotope analysis of the gas samples. All authors helped draft the manuscript and gave final approval for publication.

Competing interests. We declare we have no competing interests.

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