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RESEARCH ARTICLE

Drivers and resilience of methane-derived carbon contribution to chironomid biomass in boreal lakes

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Abstract

General mechanisms underlying the pathways of methane (CH_4)-derived carbon in aquatic food webs are often associated with eutrophication-driven anoxia. Yet, the influence of changing nutrient availability on CH_4 cycling has been mainly investigated during the increasing phase (i.e. onset of anthropogenic eutrophication), thus leaving unclear whether nutrient reduction can lead to a simple reversion of the observed effects on CH_4 cycling. We combined stable isotopes of chironomid remains ($\delta^{13}C_{HC}$) and sedimentary ancient DNA of methanotrophic bacteria (MOB) to unravel the drivers of biogenic CH_4 contribution to chironomid biomass in boreal lakes. Using a spatial dataset, our study shows that $\delta^{13}C_{HC}$ values were more depleted in hypoxic lakes and were positively associated with methanotrophic bacteria belonging to the γ-proteobacteria class (MOB type I), therefore supporting the view of higher utilization of CH_4-derived carbon in anoxic environments. However, this space-for-time substitution approach failed to provide any reliable information on whether lake food webs follow the same pathway in forward and reverse directions. Using downcore reconstruction, our results show that despite a drastic mitigation-induced decrease in nutrient concentrations and strong evidence of biological recovery of algal and chironomid communities, chironomid biomass remained highly subsidized by methanotrophic bacteria throughout the study period. Results therefore suggest that mechanisms underlying the pathways of CH_4-derived carbon in aquatic food webs are likely not the same during perturbation and recovery trajectories and that complex feedback mechanisms can stabilize lakes in this CH_4-based food web state.

Keywords Lake food web · Chironomidae · Methane-oxidizing bacteria · Sedimentary ancient DNA · Carbon stable isotope · Paleolimnology

Introduction

Methanogenesis is the dominating degradation process in anoxic freshwaters (Mattson and Likens 1992), and the methane (CH_4) produced in anoxic sediments and/or water subsequently serves as an energy and carbon source for methanotrophic bacteria (or methane-oxidizing bacteria; MOB) in oxic environments (Sanseverino et al. 2012). Methanotrophic bacteria can therefore constitute a substantial food resource for aquatic consumers and may be transferred to higher trophic levels in the food web (Ravinet et al. 2010), thus representing an important link between anoxic and oxic environments in lakes. As energy and carbon flow through aquatic food webs are of fundamental importance for the function of lake ecosystems, unravelling the drivers of CH_4 cycling in lake food webs is key to our understanding of the response of lake ecosystems to global change.

General mechanisms underlying the pathways of CH_4-derived carbon in aquatic food webs are traditionally associated with anoxia (Jones et al. 2008), and research showed that the transfer of CH_4-derived carbon to aquatic consumers is favoured by warm temperature (Wooller et al. 2012; van Hardenbroek et al. 2013; Belle et al. 2017) and anthropogenic eutrophication (excess of nutrients inputs, Belle et al. 2014; Schilder et al. 2017). Yet, the influence of nutrient availability on CH_4 cycling has always been investigated during the increasing phase (Belle et al. 2014; Schilder et al. 2017), thus leaving it unclear whether nutrient reduction leads to a simple reversion of the observed effects on

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CH_4 cycling. However, the lack of long-term data beyond traditional monitoring windows (a few decades at best) makes it impossible, in most cases, to unravel to what extent nutrient reduction influences carbon processing within food webs and CH_4 cycling.

To overcome this issue, space-for-time substitutions have been increasingly applied in ecological studies to project responses of species distributions and trophic relationships in lakes (Belle et al. 2022), and this approach is commonly used to unravel drivers of CH_4 cycling in lake food webs (Jones et al. 2008). A space-for-time approach typically addresses how ecological processes are distributed across areas in landscape and relate to specific habitats/conditions and assumes that drivers of spatial gradients also drive temporal changes in the studied process. However, space-for-time substitution approaches are based on the assumption that the observed changes occur in both ways (e.g. both increasing and decreasing directions), therefore failing to capture more complex mechanisms of resilience, such as hysteresis referring to the phenomenon by which changes in ecological states follow different pathways in forward and reverse direction (Scheffer 1998). Numerous ecological feedback mechanisms can indeed stabilize lakes recovering from eutrophication (Scheffer 1998), and many studies have demonstrated the decoupling between lake water quality and benthic processes in lakes recovering from eutrophication (Little et al. 2000; Millet et al. 2010). Similar observations are therefore expected for CH_4 cycling.

Combined uses of chitinous remains and stable isotopes in paleolimnological studies have been developed to reconstruct long-term dynamics of past carbon flows through food webs (Heiri et al. 2012; van Hardenbroek et al. 2018; Belle et al. 2019), and this time-for-time approach provides a reliable alternative to the widely used space-for-time approach. Specifically, head capsules of Chironomidae larvae (Arthropoda; Diptera; Nematocera), non-biting midges with larvae growing in superficial lake sediments, are morphologically and chemically well preserved in sediments (Verbruggen et al. 2011a, b) and can therefore be utilized for carbon stable isotope analysis (expressed as $\delta^{13}C$). Several calibration studies showed a small and temperature-independent trophic fractionation (Belle et al. 2020) and a small offset between larvae and their chitinous remains (Heiri et al. 2012; Frossard et al. 2013), thus allowing to link $\delta^{13}C$ values of chironomid remains to their putative assimilated food resources. Biogenic CH_4 is typically known to exhibit very low $\delta^{13}C$ values (from − 110 to − 40‰; Rinta et al. 2015; Thottathil and Prairie 2021) largely exceeding the lowest $\delta^{13}C$ values of all other available resources (from − 35 to − 15‰ for aquatic and terrestrial primary producers: Jones et al. 1999; Vuorio et al. 2006). Therefore, chironomids relying on CH_4-rederived carbon typically show very low $\delta^{13}C$ values (Grey 2016). Furthermore, van Hardenbroek et al. (2010) found a positive relationship between $\delta^{13}C$ values of chironomid remains and CH_4 concentrations in boreal lakes, thus allowing us to reconstruct past changes in CH_4 availability for aquatic consumers. Chironomid larvae are also a keystone taxonomic group in lakes, forming an important trophic link between primary producers and top predators (Goedkoop and Johnson 1996), and the study of their chitinous remains will, therefore, help us to elucidate CH_4 cycling in lakes and the fate of biogenic CH_4 at the interface between anoxic and oxic environments. Furthermore, novel paleolimnological approaches based on sedimentary ancient DNA preserved in lake sediments were also successfully applied to the methanotrophic bacterial community (Belle and Parent 2017). Combined analysis of sedimentary ancient DNA of CH_4-oxidizing bacteria and stable isotope of chitinous remains (see also Belle et al. 2015) has the potential to produce novel insights into the mechanisms underlying lake CH_4 cycling responses to eutrophication and mitigation actions.

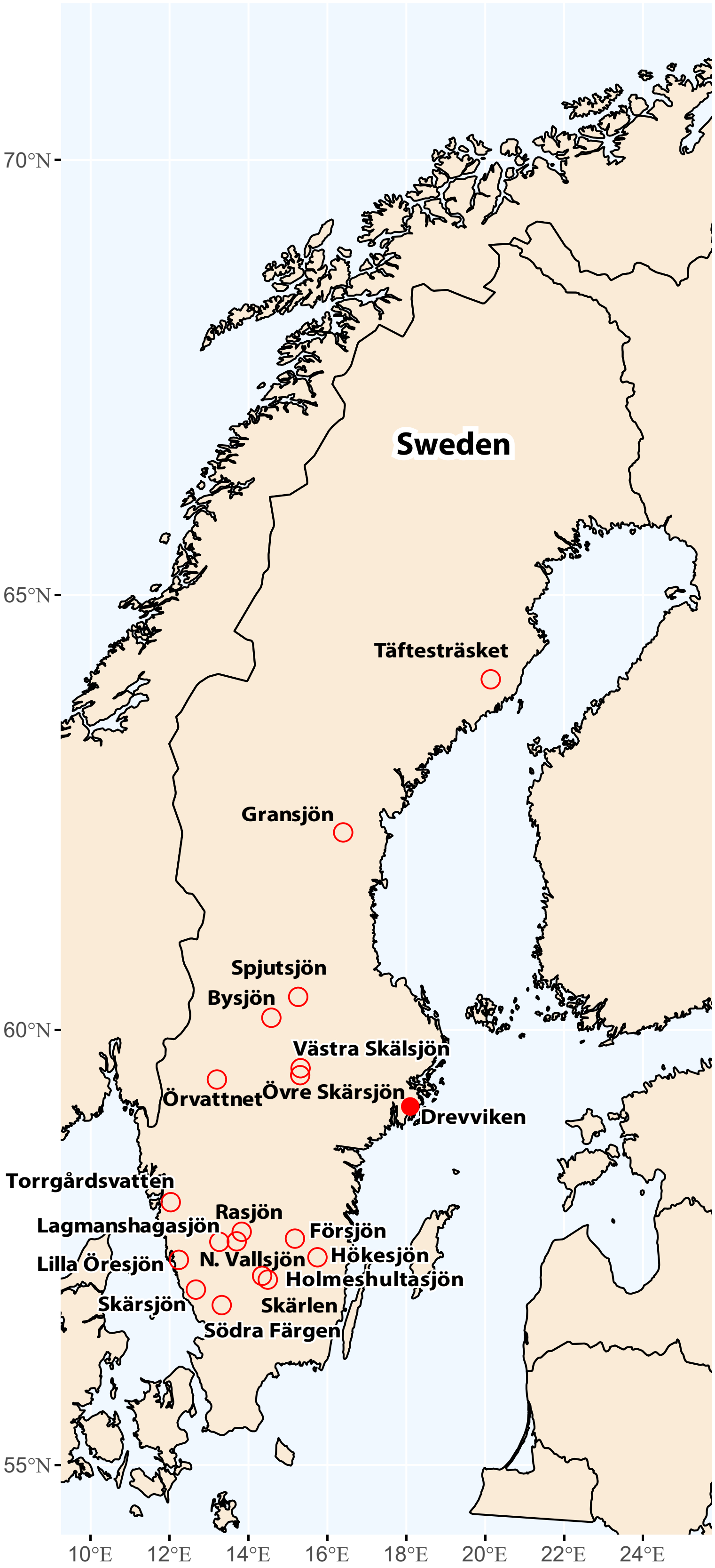
In this study, we combined stable isotopes of chironomid remains and sedimentary ancient DNA of MOB in sediment cores covering different spatial and temporal scales to unravel the drivers of biogenic CH_4 contribution to chironomid biomass in boreal lakes. We hypothesized that oxygen concentrations in lakes are a good predictor of MOB abundances in sediments and the incorporation of CH_4-derived carbon into the chironomid biomass. We also hypothesized that, due to the known decoupling between lake water quality and benthic processes in lakes recovering from eutrophication, nutrient reduction only slightly affects MOB community composition and CH_4-derived carbon incorporation to the chironomid biomass.

Materials and methods

Study sites

In total, 18 relatively small (area range 39–528 ha) and deep (maximum water depth range 15.4–32 m) lakes with relatively small catchments (range 1.31–40.3 km^2) located in Sweden have been selected in this study (Fig. 1). Monthly mean water quality data during the summer period were retrieved from the Swedish National Monitoring Program database (https://miljodata.slu.se/mvm/), including light absorbance at 420 nm, calcium and magnesium concentrations, chlorophyll *a* water concentrations, water conductivity, total organic carbon concentrations, pH, Secchi depth, nutrient concentrations, and bottom water oxygen concentrations (when available, 16 out of 18 lakes). Since the uppermost sediment layers represent several years, mean values for all environmental variables were calculated for the last 5 available years, and average values are further presented

Fig. 1 Location of the study region showing the sampling sites. Open symbols refer to the lake only included in the 19-lake dataset, whereas the closed symbol refers to Lake Drevviken



by (Belle et al. 2022). The lakes cover large environmental gradients in water transparency (Secchi depth 1.5–8 m; total organic carbon concentrations 4–19.7 mg l^{-1}; light absorbance at 420 nm ranging from 0.02 to 0.51), and total phosphorus concentrations (ranging from 3.6 to 37.8 µg l^{-1}), thus allowing study of the relationships between environmental conditions and carbon flows in lake food webs.

Among these sites, Lake Drevviken was selected to test whether management actions taken to overcome anthropogenic eutrophication have similar effects on CH_4 cycling and its biological transfer to chironomid larvae. Lake Drevviken is located in Stockholm's suburbs and the lake suffered from excessive inputs of nutrient concentrations which led to an increase of algal productivity in the 1970s and 1980s due to an ineffective wastewater treatment plant. At the beginning of 2000, the wastewater of the area was connected to Stockholm's sewage network, and monitoring data over the 1982–2020 period showed similar temporal dynamics in total phosphorus and chlorophyll *a* water concentrations, with a dramatic decrease starting at the beginning of 2000.

Sediment coring and dating techniques

In June–July 2020, one surface sediment core was retrieved from the deepest point of each selected lake using a gravity corer (9 cm diameter: UWITEC). All sediment cores were also vertically split into two halves in the laboratory, and the uppermost 1-cm-thick sediment layer of each core and the first 30 cm of the Lake Drevviken sediment core were immediately collected and stored at − 20 °C for DNA extraction or + 4 °C for other analyses.

The sediment core from Lake Drevviken was dated by ^{210}Pb and ^{137}Cs at Liverpool University's Environmental Radioactivity Laboratory. Sub-samples were analysed for ^{210}Pb, ^{226}Ra, and ^{137}Cs by direct gamma assay, using Ortec HPGe GWL series well-type coaxial low background intrinsic germanium detectors (Appelby et al. 1986). ^{210}Pb was determined via its gamma emissions at 46.5 keV, and ^{226}Ra by the 295 keV and 352 keV γ-rays emitted by its daughter radionuclide ^{214}Pb, following 3 weeks of storage in sealed containers to allow radioactive equilibration. ^{137}Cs was measured by its emissions at 662 keV. Corrections were made for the effect of self-absorption of low-energy γ-rays within the sample (Appleby and Oldfield 1992). Dates were calculated using the CRS model (Appleby and Oldfield 1978). Discrepancies with any clearly defined ^{137}Cs dates were resolved using the methods outlined in (Appleby 2002). The results were previously discussed in Belle et al. (2022).

Nitrogen stable isotope of sedimentary organic matter ($\delta^{15}N_{OM}$) was used to estimate the anthropogenic inputs of nutrients to the lake (Gu 2009) where higher anthropogenic nutrient inputs lead to an enrichment of the heavier isotope (^{15}N) thus inducing an increase in $\delta^{15}N_{OM}$ values. $\delta^{15}N_{OM}$ was analysed using an isotope ratio mass spectrometer interfaced with an elemental analyser (EA-IRMS) at SLU Stable Isotope Laboratory (Umeå, Sweden). Results were expressed as the deviation (δ) with atmospheric nitrogen as a reference standard: $\delta^{15}N$ (‰) = ($[R_{sample}/R_{standard}]$—1) × 1000, where $R = {}^{15}N/{}^{14}N$. Sample measurement replications from internal standards (wheat and maize flours) produced analytical errors (1σ) of ± 0.2% for $\delta^{15}N$ values ($n = 24$).

Analysis of methanotroph community using sedimentary ancient DNA

DNA extraction and polymerase chain reaction (PCR) were conducted in separate clean laboratories in UV-treated laminar flow cabinets with high-efficiency particulate air filtration. DNA was extracted from 0.25 g of three parallel samples of each sediment sample by using the DNeasy PowerSoil Kit (Qiagen) following the manufacturer's instructions. A negative extraction control was included in every extraction. The concentration of DNA was measured using NanoDrop ND-1000. The DNA concentration of Lake Drevviken samples was normalized to 0.25 ng.µl^{-1} using PIRO Pippeting robot (Dornier, Lindau, Germany) according to the manufacturer's instructions. All DNA samples were stored at − 80 °C until PCR amplification.

The bacterial 16S rRNA gene (regions V3–V4) was amplified using the universal prokaryotic primers 341F (5'-CCTACGGGNGGCWGCAG-3') and 805R (5'-GACTAC HVGGGTATCTAATCC-3'; Herlemann et al. 2011). Amplification of bacterial communities was conducted in 20 µl volumes and in three replicates. The PCR mixture comprised 10 µl of 2 × Phusion Master Mix with high-fidelity buffer and 0.75 µl of BSA (20 mg ml^{-1}; Thermo Scientific, Vilnius, Lithuania), 1 µl of each forward and reverse primer (10 µM), 1 µl of DNA (4 µl of DNA for Drevviken core samples), and nuclease-free water (Solis BioDyne, Tartu, Estonia). The amplification programme with Phusion High-Fidelity DNA polymerase (Thermo Scientific) included an initial denaturation at 98 °C for 30 s; 29 cycles of denaturation for 10 s at 98 °C, annealing for 30 s at 55 °C, elongation for 15 s at 72 °C; final elongation at 72 °C for 10 min and storage at 4 °C. The relative quantity of amplicons was visualized on 1% agarose gel (Bioatlas; 1xTAE buffer). PCR products from the three replicates were pooled and diluted 20×, followed by the dual-indexing (i7/i5) method. The indexing step PCR reaction (20 µl) contained 10 µl of 2 × Phusion Master Mix with High-Fidelity buffer (Thermo Scientific), 2 µl of each of the indexes (5 µM), 1 µl of PCR amplicons, and nuclease-free water (Solis BioDyne). The indexing PCR programme included 2 min at 98 °C; followed by 12 cycles of 20 s at 98 °C, 30 s at 60 °C, 30 s at 72 °C; and a final elongation of 5 min at 72 °C. Negative controls (no added DNA) were included in each step. Samples were sequenced using the Illumina MiSeq 2 × 250 base pairs (bp) platform at the FIMM (Institute of Molecular Microbial Medicine Finland, Helsinki, Finland, or Asper Biotech, Tartu, Estonia).

The paired-end demultiplexed data were analysed using QIIME 2 version 2021.8.0 (Bolyen et al. 2019). The sequencing resulted in 8,413,049 reads with a median of 105,305 reads per sample and 11,472,035 reads with a median of 121,034 reads per sample for Drevviken core dataset. DADA2 package (Callahan et al. 2016) denoise-paired method was used to denoise paired-end sequences, dereplicate them, filter out chimeras, and finally construct a feature table of amplicon sequence variants (ASVs). Based on quality plots generated using 10,000 randomly selected reads, forward and reverse reads of Drevviken core data were truncated with denoise paired to 247 and 231 bp, respectively. Surface sediment reads did not require truncating. ASVs were taxonomically classified using the feature-classifier plugin in QIIME 2 with the pre-trained (uniform naive Bayes classifier trained on full-length 16S) SILVA database version 138.1 (Quast et al. 2013; Kaehler 2022).

The number of reads attributed to ASVs present in negative, extraction, and sampling controls was used to detect potential sequencing errors and contaminants. Reads in controls were summed for each ASV, its proportional representation in total read count per ASV was calculated, and ASVs with > 20% were excluded. For the remaining ASVs, the read sum in controls was subtracted from the corresponding samples. All Archaea, Eukaryota, unidentified ASVs, and unidentified Bacteria were discarded. In addition, ASVs that had a frequency < 2 across all samples within a dataset were excluded from further analyses. The known methanotrophic activity of the different ASVs was derived from existing literature (Borrel et al. 2011; Kalyuzhnaya et al. 2019; Guerrero-Cruz et al. 2021), and the relative proportions of the different MOB types within the MOB community and relative to total sequences were then calculated and consisted of two aerobic main groups [MOB type I (affiliated to γ-proteobacteria) and MOB type II (affiliated to α-proteobacteria] and one anaerobic group (Methylomirabilia), which differ in terms of ecological requirements and physiological differences (Hanson and Hanson 1996; Billard et al. 2015; Yang et al. 2019).

Chitinous remains analysis

Carbon stable isotope analyses were performed on chironomid head capsules belonging to the fourth instar of morphotypes of *Sergentia coracina* type, *Chironomus anthracinus* type, and *Chironomus plumosus* type identified using (Brooks et al. 2007). For the sorting of chitinous remains, sediment samples were deflocculated in NaOH (10%) solution, pre-treated using washing with HCl (10%) solution, and sieved through a 100-µm mesh according to (van Hardenbroek et al. 2010). Head capsules were sorted under a dissection microscope until approximately achieving a minimal mass of 30 µg for chironomid remains. Carbon stable isotopic composition of chitinous remains ($\delta^{13}C_{HC}$) was analysed using an isotope ratio mass spectrometer interfaced with an elemental analyser (EA-IRMS) at our Stable Isotope Laboratory (Umeå, Sweden). Results were expressed as the delta notation with Vienna Pee Dee Belemnite as a standard: $\delta^{13}C\ (‰) = [(R_{sample}/R_{standard})—1] \times 1000$, where $R = {}^{13}C/{}^{12}C$. Sample measurement replications from internal standards (wheat and maize flour) produced analytical errors (1σ) of ± 0.15% for $\delta^{13}C$ values ($n = 14$).

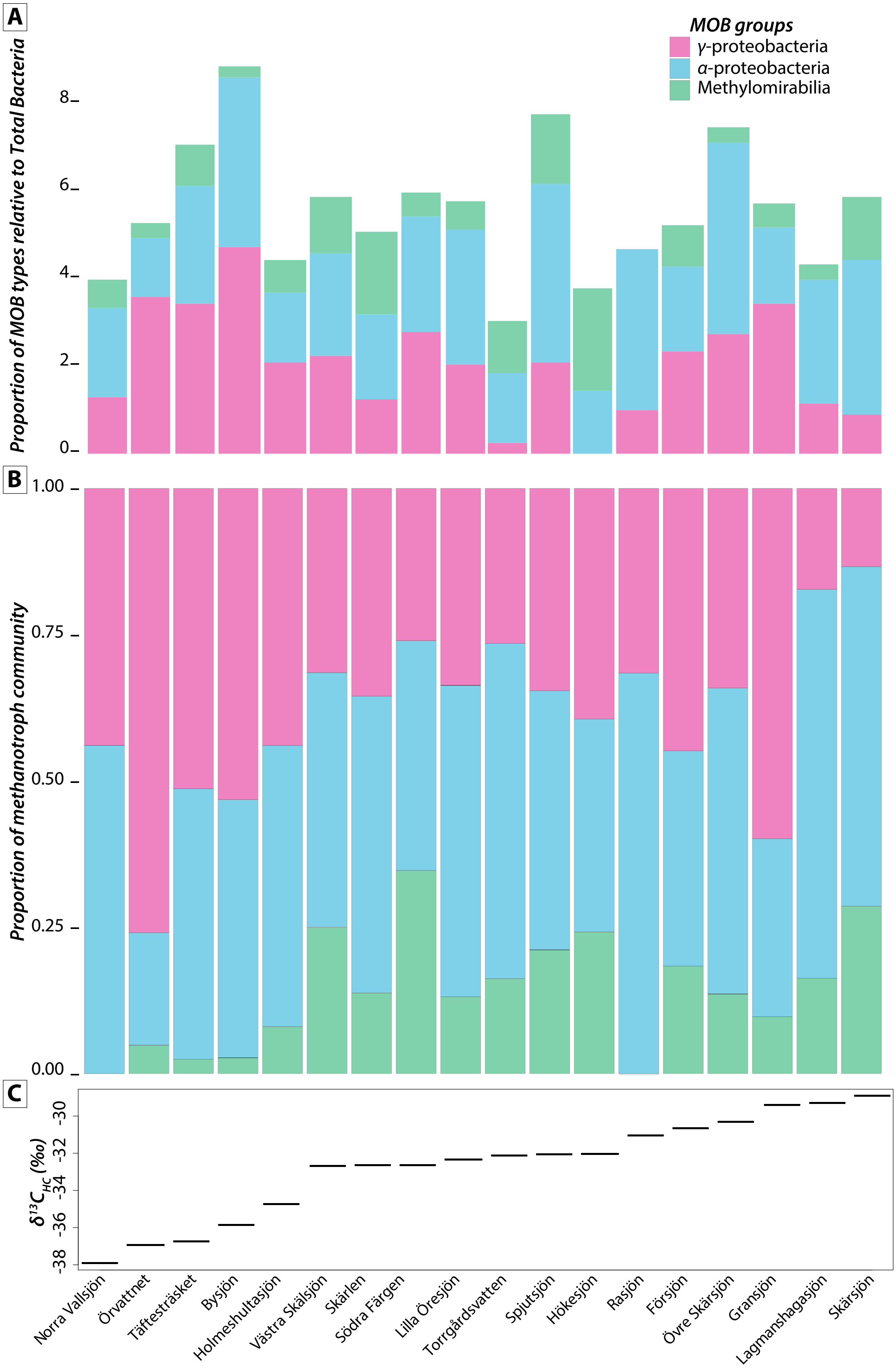
For sediment samples from Lake Drevviken, chironomid head capsules were also hand-sorted from sediment samples of wet sediment (ca. 17 g WW) following Walker (2001) and mounted between microscope slides. Chironomid community composition was identified under a microscope using Brooks et al. (2007) and Rieradevall and Brooks (2001) for Tanypodinae. Data are expressed as relative abundances, and only taxa occurring in at least two samples, with a maximum relative abundance of > 2%, were included in further analysis. A principal component analysis was performed on the chironomid data, and the scores of the second PC axis (expressing the percent variance explained) were used as indicators of the temporal changes in chironomid community composition.

Data analysis

We used generalized additive models (using *mgcv* package for R; Wood 2011) to determine whether $\delta^{13}C_{HC}$ values were associated with bottom oxygen concentrations and proportions of different MOB groups within both MOB community and relative to total sequences. To examine whether management actions influenced Lake Drevviken food webs (from community composition to carbon flows), temporal trends in water chemistry, sediment composition, chironomid, and MOB communities were assessed using boxplots, and a set of non-parametric Kruskal-Wallis tests (using *ggpubr* package for R; Kassambara 2023). All statistical analyses and plots were performed using the R 4.1.1 software (R Core Team 2021).

Results

Using the spatial dataset, we investigated changes in the predominance of main MOB groups in the sediment samples of lakes for which chironomid remains were found in sufficient numbers. MOB community of sediment samples predominated by MOB type I (γ-proteobacteria) or/and MOB type II (*α*-proteobacteria), and small changes


A
MOB groups
γ-proteobacteria
α-proteobacteria
Methylomirabilia
Proportion of MOB types relative to Total Bacteria
0
2
4
6
8
B
Proportion of methanotroph community
0.00
0.25
0.50
0.75
1.00
C
δ13C_HC (‰)
-38
-36
-34
-32
-30
Norra Vallsjön
Örvattnet
Täftesträsket
Bysjön
Holmeshultasjön
Västra Skälsjön
Skärlen
Södra Färgen
Lilla Öresjön
Torrgårdsvatten
Spjutsjön
Hökesjön
Rasjön
Försjön
Övre Skärsjön
Gransjön
Lagmanshagasjön
Skärsjön

◂**Fig. 2** **A** Proportions of the different groups of methanotrophic bacteria groups relative to total sequences and **B** within the methanotrophic bacteria community and corresponding **C** carbon stable isotopic compositions of chironomid head capsules ($\delta^{13}C_{HC}$; %). The $\delta^{13}C_{HC}$ values are ranked from the most negative to the most positive ones

in community composition and relative abundances were observed between lakes (Fig. 2A and 3B). Variability in $\delta^{13}C$ values of chitinous remains ranged from -37.9% to -29.3%, largely exceeding those of analytical errors and trophic fractionation uncertainties (Fig. 2B). Overall, when $\delta^{13}C_{HC}$ values were ranked from the most negative values to the most positive ones (as shown in Fig. 2B), two contrasting patterns occurred between the two main MOB groups: MOB type II proportion tended to display an increasing trend, while MOB type I proportion followed a slightly decreasing one. Therefore, $\delta^{13}C_{HC}$ values showed more negative values (more ^{13}C-depleted) associated with higher proportions of MOB type I (Spearman's correlation test, $p=0.05$, $r=0.46$; γ-proteobacteria) and lower proportions of MOB type II (Spearman's correlation test, $p=0.31$, $r=0.25$; Fig. 2A, B). When bottom oxygen concentration data were present, chironomid remains of the targeted morphotypes were found in sufficient numbers in 16 lakes (out of 18 lakes, see "Methods"), and $\delta^{13}C_{HC}$ values were found significantly and positively associated with bottom oxygen concentrations ($R^2=0.35$, $p<0.1$) with chironomid head capsules being ^{13}C-depleted in hypoxic lakes (Fig. 3A). Furthermore, GAM results also showed that $\delta^{13}C_{HC}$ values were strongly and negatively associated with the development of MOB type I within the MOB community ($R^2=0.30$, $p=0.01$; Fig. 3B) and relative to total sequences ($R^2=0.15$, $p=0.01$; Fig. 3C). Furthermore, no clear pattern in $\delta^{13}C_{HC}$ values can be observed between morphotypes, as $\delta^{13}C_{HC}$ values seemed to be distributed along the gradients regardless of their morphotypes (Fig. 3).

A total of 454 chironomid head capsules were retrieved from the 30 sediment layers of the Lake Drevviken sediment core, with counts ranging from 8 to 28 HC per sample. As expected, due to historical and present-day ecological status, chironomid remains concentrations in sediments were extremely low (much below the commonly used minimum of 50 head capsules) and temporal changes in chironomid community composition should therefore be interpreted cautiously. Nevertheless, 17 morphotypes were selected for inclusion in further statistical analysis. The first two PC axes applied to chironomid data accounted for 14.6% and 13.5% of the total variance, respectively (Fig. 4A), and the PC2 axis largely reflected the predominance of *Tanytarsus lugens/mendax* type and *Chironomus plumosus* type, therefore indicating an oxygen gradient. Across the core, two contrasting trends occurred between the two morphotypes with the highest relative contributions: *T. lugens/mendax* type displayed an increasing trend, while *C. plumosus* type showed a decreasing one (Fig. 4B), and PC2 axis scores showed an increasing trend over time. Nitrogen stable isotope of sedimentary organic matter ($\delta^{15}N_{OM}$) was also measured to estimate the inputs of nutrients to the lake, and $\delta^{15}N_{OM}$ values ranged from 4.64–8.4%, followed by a gradual decrease over time (Fig. 4B). Furthermore, PC2 axis scores were negatively correlated with $\delta^{15}N_{OM}$ values (Pearson product-moment correlation, $r=0.25$. $p<0.001$), with high $\delta^{15}N_{OM}$ values leading to higher developments of the oxygen-tolerant *C. plumosus*-type and lower developments of the oxygen-sensitive *T. lugens/mendax* (Fig. 4B), suggesting a positive influence on decreasing nutrient inputs on chironomid community.

Total phosphorus concentrations ranged from 36–181 μg. l^{-1}, with the highest concentrations reported before 2000 (Fig. 4), and similar significant changes were also observed for chlorophyll *a* water concentrations, $\delta^{15}N_{OM}$ values, and PC2 axis scores (Fig. 4). Comparison between MOB communities before/after 2000 showed significant trends in community composition characterised by a slight decrease in MOB type II proportion after 2000 while MOB type I proportion instead increases (Fig. 4), although the MOB community is still strongly predominated by MOB type I (up to 80%; Fig. 4). Head capsules of *C. plumosus* type were found in sufficient number for isotopic analysis in only six sediment layers. The $\delta^{13}C_{HC}$ values of *C. plumosus* type found in Lake Drevviken sediment core were extremely low (ranging -54.4 – -42.7%), and temporal pattern in $\delta^{13}C_{HC}$ values did not show significant change over time as previously reported for water chemistry, sediment composition, MOB, and chironomid community composition (Fig. 4).

Discussion

Our study confirms the potential of using combined analyses of stable isotopes of chironomid remains and sedimentary ancient DNA of MOB to provide unique insights into CH_4 cycling in lake food webs. Results support previous findings suggesting that chironomids growing in boreal hypoxic lakes are more subsidized by methanotrophic bacteria belonging to the γ-proteobacteria class (MOB type I) compared to well-oxygenated lakes. However, the applied space-for-time approach failed to provide any reliable information on whether CH_4 cycling follows the same pathway in forward and reverse directions. Using downcore reconstruction, results suggest that mechanisms underlying the pathways of CH_4-derived carbon in aquatic food webs instead differ during the perturbation and recovery phases of eutrophication.

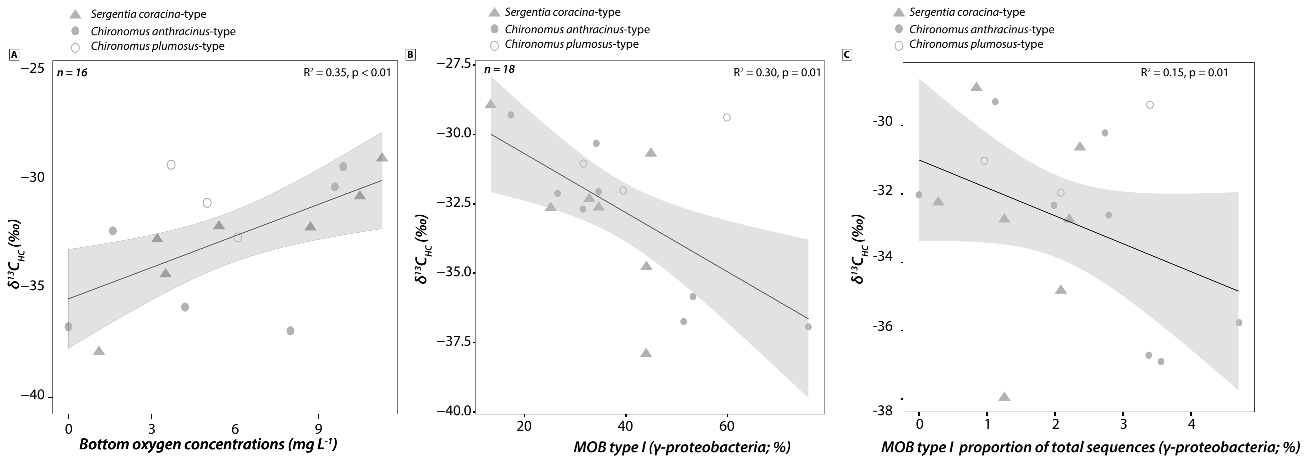


Fig. 3 Relationship between carbon stable isotopic compositions of chironomid head capsules ($\delta^{13}C_{HC}$; %) and **A** bottom oxygen concentrations (mg l^{-1}) and **B** percentages of γ-proteobacteria (MOB type I) within the methanotrophic bacterial community. The number of lakes differs from panels A and B because of the availability of bottom oxygen concentration data (see Methods). Open circles refer to *Chironomus plumosus*-type head capsules whereas closed circles represent *Chironomus anthracinus*-type head capsules, and closed triangles represent those of *Sergentia coracina* type

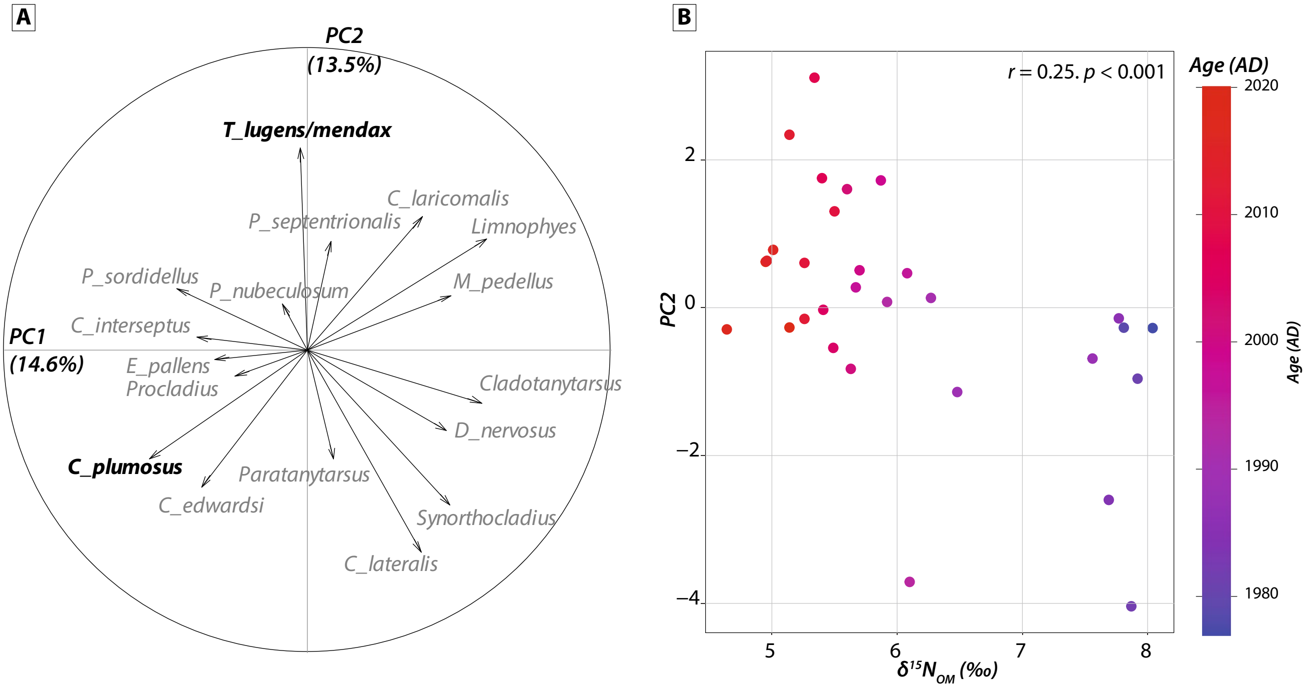


Fig. 4 **A** Correlation circle representing variable contributions to the first two PC axes of the principal component analysis performed on chironomid data identified in Lake Drevviken. A colour gradient was used to indicate sample age. **B** Relationships between stable nitrogen isotopic composition of sedimentary organic matter ($\delta^{15}N_{OM}$; %) and scores of the second PC axis (PC2)

Drivers of variations in $\delta^{13}C$ values of chironomid remains

By reporting positive relationships between bottom oxygen concentrations and $\delta^{13}C_{HC}$ values, results suggest that chironomids growing in hypoxic lakes are more subsidized by MOB compared to well-oxygenated lakes, therefore supporting previous findings in other boreal (Premke et al. 2010) and temperate lakes (Jones et al. 2008). Results also suggest that $\delta^{13}C_{HC}$ values were negatively associated with higher abundances of MOB type I (γ-proteobacteria) in lake sediments and relative to other MOB groups. These results are well in line with previous findings suggesting a higher contribution of the MOB type I relative to other MOB groups to the chironomid biomass (Eller et al. 2005; Sanseverino et al. 2012), a pattern also reported from paleolimnological investigations (Belle et al. 2014). MOB type I are indeed known to grow mainly in the uppermost centimetres of lake sediments (Schubert et al. 2011; Kojima et al. 2012) where chironomid larvae also grow and feed. Other MOB groups instead colonize deeper sediment layers (e.g. *ca*. 15–20 cm depth for MOB type II; He et al. 2012) and might therefore not be available for chironomids. Furthermore, results report the predominance of MOB type I and MOB type II over other studied MOB groups (Fig. 2A), as previously reported in similar boreal (Rissanen et al. 2018) and temperate lakes (Eller et al. 2005; Rahalkar et al. 2009; Billard et al. 2015) or sediments of a boreal estuary (Graham et al. 1993). We also reported opposite trends between MOB type I and MOB type II, the latter showing higher proportions in more oxygenated lake sediments, likely because of the competitive advantage of MOB type II to exhibit a higher ability to access nutrients under nutrient-poor conditions (Wise et al. 1999; Myllykangas et al. 2020), whereas MOB type I exhibit a more eutrophic strategy than MOB type II (Ho et al. 2013; Nijman et al. 2021). Our study therefore confirms that CH_4-based food webs could be considered one of the symptoms of anthropogenic eutrophication, being enhanced by hypoxic conditions (Belle et al. 2015; Schilder et al. 2017). However, this space-for-time substitution cannot allow us to unravel the pathway of CH_4 cycling in lakes recovering from eutrophication.

Downcore reconstruction was used to investigate the response of the CH_4-based food web to mitigation-induced nutrient reduction, focusing on Lake Drevviken, a lake heavily impacted by excessive anthropogenic nutrient loadings before the 2000s. A drastic reduction of total phosphorus water concentrations (with a decrease of up to 75%) was observed at the beginning of 2000, and a similar change was observed for chlorophyll *a* concentrations, suggesting a drastic decrease in algal productivity. Temporal changes in chironomid community composition were characterised by the increasing development of the oxygen-sensitive taxa *T. lugens/mendax* type, while the relative abundances of hypoxic indicator taxa *C. plumosus* type instead decreased (Little and Smol 2001; Verbruggen et al. 2011a, b). These taxonomic changes could reflect better oxygen conditions at the bottom of Lake Drevviken after 2000. The MOB community of Lake Drevviken was largely predominated by MOB type I throughout the core (up to 80%), and slightly increasing proportions of MOB type I were revealed after 2000. However, MOB type I are known to inhabit the uppermost centimetres of lake sediments, and the observed change in MOB community occurred at 15 cm depth in the core. It is, therefore, difficult to rule out the potential influence of living MOB on the ancient DNA signal. Finally, extremely low $\delta^{13}C_{HC}$ values were reported throughout the core (from − 54.4 to − 42.7‰ typically matching the range of MOB values; Fiskal et al. 2021), and these values could only be explained by the substantial incorporation of MOB into chironomid biomass as the lowest $\delta^{13}C$ of all other potential food resources was largely exceeded. The high proportion of MOB type I and the very low $\delta^{13}C_{HC}$ values are also well in line with our previous finding showing a negative relationship between these two variables (see also Fig. 3B). Results therefore suggest a steady and substantial incorporation of MOB in chironomid biomass in Lake Drevviken throughout the study period despite drastic reductions in nutrient concentrations and strong evidence of biological recovery of algal and chironomid communities. Our study suggests that Lake Drevviken food webs are still dominated by CH_4-based processes, and no significant changes in response to the drastic reduction in nutrient concentrations can be evidenced. Mechanisms underlying the pathways of CH_4-derived carbon in aquatic food webs are likely not the same during perturbation and recovery trajectories. Further investigations are, however, still needed to better understand how the human-induced CH_4-based food webs could also follow the theoretical framework on regime shift and hysteresis (see also Scheffer et al. 2009). Our study demonstrated the urgent need to fill the knowledge gap in the link among anthropogenic eutrophication, management actions, and carbon processing within the food webs (in particular CH_4 cycling).

Implication for ecological assessment and restoration targets

Numerous tools have been developed to mitigate anthropogenic eutrophication, ranging from external measures to reduce nutrient loading to internal measures designed to change in-lake nutrient availability and bind nutrients in lake sediments (Jilbert et al. 2020). All these actions contribute to successfully improving lake water quality (Jeppesen et al. 2012), but contrasted efficiency has been documented for benthic processes, and similar observation was expected for CH_4 cycling. At the beginning of 2000, management actions were taken to counteract the effects of anthropogenic eutrophication, and the sewage network was connected to a new wastewater treatment plant leading to clear and immediate effects on the water chemistry of Lake Drevviken and algal and chironomid community composition (see also Fig. 4). Overall, this management action appeared to be a successful experience from the chemical and biological recovery perspective (Fig. 5). However, these changes were not followed by any change in carbon flows within chironomid larvae. Environmental conditions favouring CH_4-based food webs of benthic habitats, such as high nutrient inputs and low levels of oxygenations, were likely still prevailing

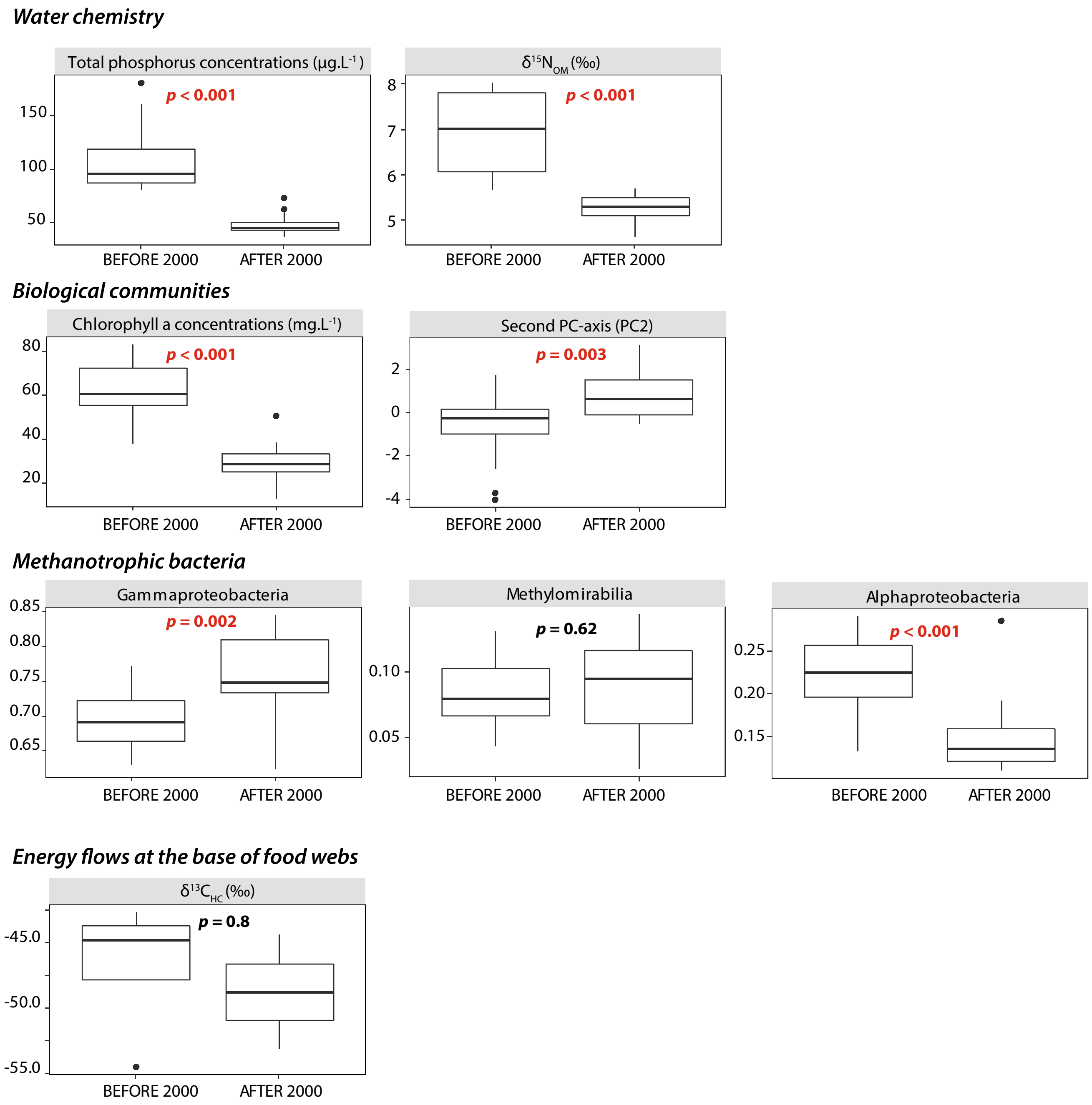


Fig. 5 Boxplots showing temporal trends before/after the new wastewater treatment plant in Lake Drevviken watershed was built in 2000 in monitoring data of water concentrations of hlorophyll *a* (expressed in mg l^{-1}) and total phosphorus (expressed in µg l^{-1}) measured in Lake Drevviken, sedimentary organic matter ($\delta^{15}N_{OM}$; %) and scores of the second axis of a principal component analysis performed on chironomid data (PC2), proportions of the different groups of methanotrophic bacteria groups found in sediment samples, and stable carbon isotopic composition of chironomid remains ($\delta^{13}C_{HC}$; %). *P* values from the Kruskal-Wallis test are noted on each panel, and *p* values below the level of significance (<0.05) are highlighted in red

after 2000, and the management action did not succeed in changing these conditions. Our results therefore suggest that the study of carbon flows within food webs may be used to support the management of freshwater ecosystems by providing relevant indications on ecosystem integrity and may be used to quantify the effectiveness of management actions taken to counteract the effects of anthropogenic eutrophication on different compartments of lake ecosystems.

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Authors contribution SB and VK designed the study; SB, EO, HT and VK analysed the samples. SB wrote the paper with substantial input from all co-authors.

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Data availability Data will be available upon reasonable request.

Declarations

Conflict of interest The authors declare that they have no competing interests.

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