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A sedimentary DNA perspective about the influence of environmental and food-web changes on the microbial eukaryotic community of Lake Biwa

Eric Capo[1] | Maïlys Picard[1] | Kai Nakane[2] | Michinobu Kuwae[2] | Stefan Bertilsson[3] | Maiko Kagami[4] | Xin Liu[5] | Yoichiro Sakai[6] | Narumi Tsugeki[7]

[1]Department of Ecology and Environmental Science, Umeå University, Umeå, Sweden

[2]Center for Marine Environmental Studies, Ehime University, Matsuyama, Japan

[3]Department of Aquatic Sciences and Assessment, Swedish University of Agricultural Sciences, Uppsala, Sweden

[4]Faculty of Environment and Information Sciences, Yokohama National University, Yokohama, Japan

[5]Guangxi Academy of Marine Sciences, Guangxi Academy of Sciences, Nanning, Guangxi, China

[6]Lake Biwa Environmental Research Institute, Otsu, Japan

[7]Faculty of law, Matsuyama University, Matsuyama-shi, Ehime, Japan

Correspondence
Eric Capo, Department of Ecology and Environmental Science, Umeå University, Umeå, Sweden.
Email: eric.capo@umu.se

Narumi Tsugeki, Faculty of Law, Matsuyama University, Matsuyama-shi, Ehime, Japan.
Email: ntsugeki@g.matsuyama-u.ac.jp

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Abstract

1. The impacts of environmental change on Lake Biwa have been explored for decades, with water monitoring and palaeolimnological studies revealing how environmental forcing, including climate warming, eutrophication, water level manipulation and human manipulation of fish populations, has influenced the food web of Lake Biwa. However, these studies have rarely accounted for microbial food-web components. This knowledge gap is mostly due to the lack of time series spanning more than a couple of decades, coupled with the high taxonomical expertise required to identify organisms belonging to very diverse groups.
2. The use of a sedimentary DNA approach allows for the reconstruction of past changes in the diversity, composition and structure of the microbial eukaryotic community of aquatic systems. The application of 18S metabarcoding has been proven successful to describe the response of unicellular eukaryotes (protists) and aquatic fungi in lake ecosystems, encompassing a large taxonomic and functional diversity such as phototrophs, heterotrophs and mixotrophs.
3. We applied 18S metabarcoding to 31 sediment core samples from Lake Biwa, spanning the past 100 years and explored the response of microbial eukaryotic communities to changes in multiple environmental stressors, including nutrient levels, lake water level, climate, as well as fish and zooplankton biomass for the period 1973–2010.
4. We found that the manipulation of the water level and changes in fish community composition were the primary factors impacting (indirectly) the structure of the lake microbial eukaryotic community with minor, but significant, effects of climate warming and phosphorus levels. Co-occurrence network analysis highlighted the potential food web impacts on the microbial eukaryotic community, suggesting that organisms from this compartment were impacted by both bottom-up and top-down processes.

Eric Capo and Maïlys Picard shared first-authorship.

KEYWORDS
18S metabarcoding, climate, eutrophication, fish, lakes, microbial eukaryotes, sedimentary DNA, water management

1 | INTRODUCTION

Lake Biwa is one of the oldest existing lakes on Earth, formed about 430 thousand years ago (Hampton et al., 2018; Satoguchi, 2020). It is home to 62 endemic species (Nishino, 2012) and the main source of freshwater for 14 million people in the Kinki region (Japan). However, since the industrial revolution in the 1800s, multiple anthropogenic stressors have impacted this ecosystem. For example, Lake Biwa became eutrophic in the 1960s (Hyodo et al., 2008; Ogawa et al., 2001; Tsugeki et al., 2003), when regional development was carried out as part of a national plan to expand industry, commerce and residential areas at the expense of forests and agricultural lands (Nakamura, 2002; Yamamoto & Nakamura, 2004). Successful nutrient control led to re-oligotrophication from the 1990s (Kawanabe et al., 2020). These shifts in trophic status had marked effects on the phyto-, zooplankton and fish assemblages (Hsieh et al., 2011; Kawanabe et al., 2020; Tsugeki et al., 2010). In addition, water temperature has increased in Lake Biwa since the late 1980s due to ongoing global warming (Hayami & Fujiwara, 1999). Combined with moderate eutrophication, this led to frequent and severe hypoxic episodes, which have putatively caused episodic mass mortality of the endemic freshwater goby isaza (*Gymnogobius isaza*; Itai et al., 2012). Finally, hydrological modifications have been in place since 1992 to decrease the lake water level to mitigate repeated flooding of the region around Kyoto (Kawanabe et al., 2020). These modifications have had marked impacts on Lake Biwa's planktivorous fish whose numbers have declined due to littoral areas drying out in their spawning grounds (Yamamoto et al., 2006). Changes in these fish populations might have further consequences for other food-web components in Lake Biwa via predation pressure (Dur et al., 2022; Liu et al., 2020).

Studies to date have mostly investigated the dynamics of phytoplankton, zooplankton and fish communities, either from water samples or material in sediment cores (Hsieh et al., 2010, 2011; Kawanabe et al., 2020; Liu et al., 2020; Tsugeki et al., 2003, 2010). For example, the amounts of diatom frustules and pigments in sedimentary archives have increased since the onset of eutrophication (1960s to 1980s) and stabilized upon meteorological changes (warmer winters), accompanied by a shift in the dominant species (Tsugeki et al., 2010). Cladoceran remnants (*Daphnia*) have increased in parallel, suggesting that this population is constrained and controlled by bottom-up processes such as nutrient availability (Hsieh et al., 2011; Tsugeki et al., 2003). Furthermore, a comparison of data from sediment cores and planktonic water samples indicated that *Daphnia* are also affected by climate change, as *Daphnia galatea* can now live year-long in the water column due to warmer winters (Tsugeki et al., 2009). Such long-term time-series obtained through palaeoecological data are particularly valuable for investigating the natural responses of ecosystems to environmental change, as they provide us with a baseline to understand how—and to what extent—aquatic ecosystems are impacted by superimposed human-induced stressors (Alsos et al., 2024; Lin et al., 2024).

Classical palaeoecology proxies such as macro- and microscopic remains (e.g. diatoms, cysts and spores) can provide important information about the long-term trends of specific biological groups (diatoms, cladocerans, chironomids, Battarbee et al., 2011; Gregory-Eaves & Smol, 2024). However, these proxies are only applicable to taxa with persistent and identifiable remains, with information about the remaining biological diversity being out of reach. Recent game changers are the improvements in DNA sequencing capacity and deeper understanding of genetic signals from sedimentary archives (Capo et al., 2023; Picard et al., 2024). These methodological advances have paved the way for sedimentary DNA records to establish a baseline and reconstruct the temporal variability in both community diversity and structure, thereby complementing the existing historical records. Over the past decade, sedimentary DNA approaches have been successfully used in palaeolimnological contexts to study the past dynamics of various communities, such as microbial eukaryotes (Keck et al., 2020; Yan et al., 2024) and cyanobacteria (Heathcote et al., 2024; Nwosu et al., 2023; Picard et al., 2022). In the case of Lake Biwa, sedimentary DNA archives have recently been used to track past changes in *Daphnia* (Tsugeki et al., 2022) and copepod populations (Nakane et al., 2023). While methodological advances are still required to comprehend what the molecular signal recovered from sedimentary archives truly represents, numerous recent papers have highlighted the reliability of such approach to reconstruct past changes in various biological groups (Huston et al., 2023; Thorpe et al., 2023).

Lake Biwa is an ideal site to study long-term changes in microbial eukaryotic communities under reduced predation pressure and to disentangle the likely interactive effects of shifts in trophic status, warming and water level management on this community. Although the long-term dynamics of most trophic levels have been studied in Lake Biwa, heterotrophic protists, which include grazers, parasites and symbiotic organisms, have been overlooked until now. Understanding if/how this community has changed would complement our understanding of Lake Biwa's food web and nutrient cycling, and therefore highlight how resilient the lake is in the face of multiple environmental stressors. To this end, we amplified and sequenced a portion of the V7 region of 18S rRNA genes extracted from sediment samples covering the past 100 years of Lake Biwas history. This data was aligned with zooplankton and planktivorous fish survey data from a previous study (Liu et al., 2020) and environmental monitoring data (Hikone local Meteorological Observatory). The specific aims were to study how the microbial eukaryotic community has changed over the last century in Lake Biwa and whether shifts in Lake Biwa microbial community could be linked to known environmental stressors, thus deepening our understanding of the severity of these stressors.

2 | MATERIALS AND METHODS

2.1 | Study site and core collection

Sediment core samples were collected on 17 August 2017. Two 30-cm-long sediment cores were collected (LB2 and LB7) at the anchoring site using the research vessel, Hasu, belonging to the Center for Ecological Research, Kyoto University (Figure 1, details are shown in Tsugeki et al., 2022). A gravity corer with an inner diameter of 10.9 cm and a length of 30 cm was used to retrieve the sediment cores, which were subsequently sectioned in 1-cm thick slices using an upright extruder with pins. During the sectioning, several millimetres from each layer that were influenced and thus possibly compromised by the splitting process were carefully removed from the entire surface of the split core samples using a knife. The acrylic pipes, knives and cutting apparatus were cleaned with 0.6% sodium hypochlorite, tap water and Milli-Q water to avoid DNA cross-contamination. Each sliced sample was transferred into aluminium zipper bags (opaque) and frozen at −80°C until analysis.

2.2 | Sediment dating and geochemistry

The LB7 core was used for sediment dating and other analyses, while the LB2 core was used for sedimentary DNA. Details of the chronological method used for the LB7 core and the resulting dating model were published in Tsugeki et al. (2021). The age-depth model of the LB2 core was indirectly estimated from the ^{210}Pb-based constant rate of supply (CRS) age-depth model (Appleby and Oldfield, 1978) of the LB7 core. The peaks or troughs in the depth profiles of chlorophyll pigments and magnetic susceptibilities were used for the stratigraphic correlations between the LB2 and LB7 cores and time constraints to date each depth of LB2 core (Figure S1). Three time constraints from the pigments including a trough at 21.5 cm depth in LB7 and 20.5 cm depth in LB2 (estimated date: 1953), a peak at 13.5 cm depth in LB7 and 12.5 cm depth in LB2 (1983) and decreased a peak at 5.5 cm depth in LB7 and at 4.5 cm in LB2 (2007) were identified. Similarly, two time constraints from the magnetic susceptibility including a peak at 17.5 cm depth in LB7 and at 16.5 cm depth in LB2 (estimated date: 1969) and a trough at 9.5 cm depth in LB7 and at 7.5 cm depth in LB2 (1996) were identified. Details of the method used for chlorophyll a pigments and magnetic susceptibility determination are described in Tsugeki et al. (2021).

2.3 | Environmental and biological parameters

Meteorological data including air temperature, precipitation, wind velocity and sunshine duration were obtained from Hikone local Meteorological Observatory located on the east coast of the north

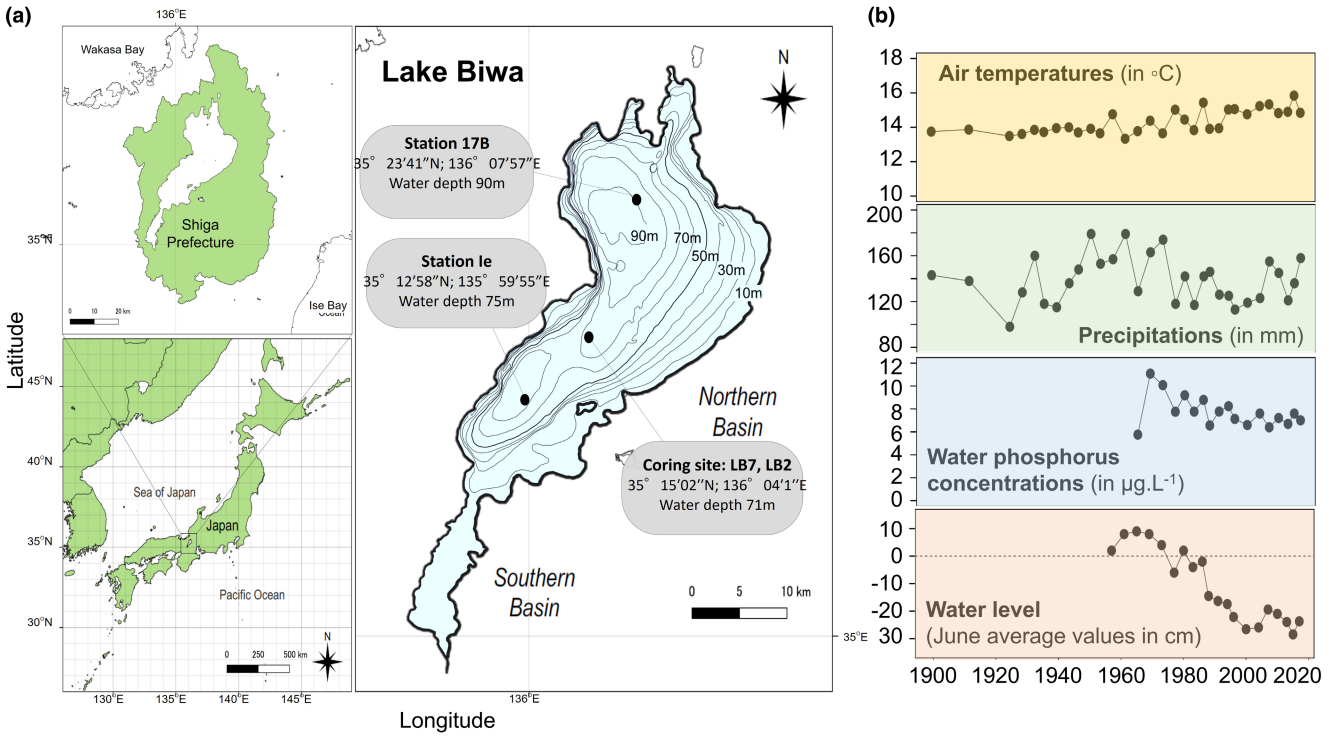

FIGURE 1 (a) Map of Lake Biwa with the location of sampling sites. (b) Temporal changes in environmental parameters: Air temperatures, precipitations (in mm), water phosphorus concentration (in µg L^{-1}), water level (in June average values in cm). The map was made using the National Land Numerical Information data-download service (https://nlftp.mlit.go.jp/ksj/index.html) created by the Ministry of Land, Infrastructure, Transport and Tourism Japan and using a map data (http://www.naturalearthdata.com), sponsored by NACIS.

basin of Lake Biwa (Figure 1). At Hikone observatory, air temperature, precipitation and sunshine duration have been recorded since the late nineteenth century, while wind velocity has only been recorded since 1951 (Hikone Meteorological Observatory). In this study, the annual mean was calculated based on the monthly average of precipitation, sunshine duration and wind speed. These annual mean values were used in the statistical analyses described later on. To investigate a possible effect of water level manipulation on the pelagic system through food-web interactions, we used the monthly average of water level at Toriigawa station in June (Shiga Prefecture 1963–2018). The annual average of total phosphorus (TP) concentration in the upper water column (0–20 m) collected at the northern basin was used to estimate the trophic status. The data (1962 to 2017) were obtained from two different sources depending on the period: TP concentrations from 1962 to 1981 were based on the compiled data collected at station Ie (Figure 1) by Kyoto University (Fujinaga & Hori, 1982) while TP concentrations from 1978 to 2017 were collected at station 17B (Figure 1) by the Shiga Prefectural Institute of Public Health and Environmental Science. TP concentration was not measured in 1965, therefore the average between 1964 and 1966 was used instead. As noted in the previous report (Hsieh et al., 2010), the TP data during the overlapped period (1978–1980) varied in similar ways between the two data sources. Therefore, we used their average during that period.

The annual fish catch data of seven planktivorous fish was used as a proxy for fish predation pressure. Since there are no data showing changes in fish biomass, we used the main species of fish caught as an indicator of changes in fish abundance. The species are ayu/sweetfish (*Plecoglossus altivelis*), isaza/endemic goby (*Gymnogobius isaza*), the common carp (*Cyprinus carpio*), the crucian carp (*Carassius carassius*), moroko/endemic minnow (*Gnathopogon caerulescens*) and wakasagi/Japanese pond smelt (*Hypomesus nipponensis*). The catch data were provided by the Shiga Prefectural Fisheries Experimental Station (SPFES). The biomass (in g dry weight m^{-2}) of the dominant zooplankton species was recorded from 1973 to 2010 based on the monthly samples collected in four layers at depths up to 75 m (0–10, 10–20, 20–40 and 40–75 m) with a vertical net in the north basin of Lake Biwa (mouth diameter, 22.5–25 cm; mesh size, 95 μm; more details in Liu et al., 2020). This includes counts of *Bosmina longirostris*, *Cyclopoida* spp., *Daphnia galeata*, *Daphnia pulicaria*, *Diaphanosoma orientalis* and *Eodiaptomus japonicus*.

2.4 | DNA extraction, PCR and sequencing

All laboratory procedures relating to the DNA analysis were carried out in a dedicated clean DNA laboratory and followed strict protocols to ensure analytical quality, that is, working under sterile conditions, using sterile disposable labware and storing subsamples and DNA extracts at −20°C. DNA extraction was performed from 31 samples corresponding each to different sediment layers according to methods described in previous studies (Kuwae et al., 2020). In brief, 9 g of each sediment sample was incubated at 94°C for 50 min in a 9 mL alkaline solution comprising 6 mL of 0.33 M sodium hydroxide and a 3 mL Tris-EDTA buffer (pH 6.7). After centrifugation at 10,000***g*** for 60 min, 7.5 mL of the supernatant of the alkalized mixture was neutralized with 7.5 mL of 1 M Tris–HCl (pH 6.7). After adding 1.5 mL of 3 M sodium acetate (pH 5.2) and 30 mL absolute ethanol, the solution was preserved at −20°C for more than 1 h and then centrifuged at 10,000***g*** for 60 min. The pellet was transferred into a bead tube from a soil DNA extraction kit (Power Soil DNA Isolation Kit, Qiagen, Germany). The 'Experienced User Protocol 2 to 22' of the Power Soil DNA Isolation Kit was followed. Finally, 200 μL of the DNA solution was obtained and stored at −20°C until PCR analysis. DNA extracts from each sample (~25 ng) were then used to PCR amplify the V7 region of the 18S rRNA gene by targeting a 260-bp-long fragment using the general eukaryotic primers 960f (5′-GGCTTAATTTGACTCAACRCG-3′) and NSR1438 (5′-GGGCATCACAGACCTGTTAT-3′) as in Capo et al. (2017). Each PCR was performed in a total volume of 25 μL containing 2.5 μL of 10× reaction buffer, 0.625 μL of DreamTaq DNA Polymerase (Thermofisher, Waltham, MA, USA), 0.5 μL of 10 mM dNTP (deoxyribonucleotide triphosphate) and 1.25 μL of each primer (500 nM). The amplification conditions consisted of an initial denaturation at 94°C for 10 min, followed by 35 cycles of 1 min at 94°C, 1 min at 55°C and 1 min 30 s at 72°C and terminated by a final 10-min extension at 72°C. Negative controls performed during the DNA extraction step showed no band on agarose gel after PCR amplifications. Two negative controls were performed during the PCR amplification step. Only DNA extracts obtained from sediment layers were further used for the next steps. Sample-specific molecular barcode combinations for the forward and reverse primers were performed to reduce the frequency of analytical cross-contamination between PCR products. The molecular inventories were multiplexed using 31 of the 225 possible molecular barcode combinations with 15 forward and 15 reverse tagged primers. The PCR products were pooled at equal volumes purified using the QIAquick PCR Purification kit (Qiagen, Carlsbad, CA, USA). Library preparations (Illumina TruSeq PCR-free) and paired-end (2 × 250 bp) sequencing (MiSeq Illumina instrument) were performed at SciLifeLab (NGI, Stockholm, Sweden).

2.5 | Bioinformatics

Raw sequence reads were analysed using the procedure outlined in Capo et al. (2017). Briefly, the paired-end reads were merged and cleaned (no undefined bases, minimum sequence length of 200 bp, no sequencing error in primers and removal of chimeric sequences) using the UPARSE and UCHIME tools (Edgar, 2013; Edgar et al., 2011), resulting in a total of 2,876,110 sequence reads (hereafter, DNA reads) distributed across the 31 samples. These reads were subsequently grouped into 5228 OTUs (Operational Taxonomic Units) at 95% sequence identity. The taxonomic annotation was performed by BLAST against the SSURef SILVA database NR108 (Pruesse et al., 2007) amended with lacustrine DNA reads originating from previous studies (Lefranc et al., 2005; Lepère

et al., 2006; Mangot et al., 2013). This database is provided with the PANAM2 pipeline (https://github.com/panammeb/PANAM2). We removed all the OTUs affiliated to metazoans, embryophytes (242 OTUs, 29,403 reads), fungi (1741 OTUs, 974,226 reads; except chytrids whose infection burden significantly influence the dominant phytoplankton dynamics at this lake; Kagami et al., 2006; Song et al., 2021), Cercozoa nuclear (39 OTUs, 4876 reads), Cryptophyta nucleomorph (12 OTUs, 3706 reads) and unclassified eukaryotes (3 OTUs, 745 reads) in order to consider only known microbial eukaryotes in our downstream analyses. Rarefaction curves (Figure S2) were performed for the molecular inventories using the function *rarecurve* from the vegan R package (Oksanen et al., 2019). To make the analyses more robust, we further removed OTUs represented by less than four DNA reads across the entire dataset. The 31 resulting samples were rarefied to 12,238 DNA reads, matching the molecular inventory with the least number of reads.

2.6 | Data analysis

A change point analysis was performed on univariate data using the function *e.divisive* from the R package ecp (parameters: R = 999, alpha = 1, min.size = 2; James et al., 2023). A principal coordinate analysis (PCoA) was performed applying the function *wcmdscale* to a Bray–Curtis dissimilarity matrix built with the function *vegdist* from the OTU relative abundance table. The *env.fit* function (permutations = 999, type = series) was applied to the Bray–Curtis dissimilarity matrices to evaluate the significance of relationships between the microbial eukaryotic community structure and the selected environmental stressors. The functions *wcmdscale*, *vegdist* and *envfit* belong to the *vegan* R package (Oksanen et al., 2019). To study associations, that reflect either potential similarity in environmental niche preferences or real biological interactions, between microbial eukaryotes, zooplankton and planktivorous fish species, a network analysis was performed using relative OTU abundance data with a focus on the period 1973–2010. In order to reduce the complexity of each dataset used for the network analysis, OTUs with less than 25 DNA reads (0.2% of the number of DNA reads per sample) were excluded. Relative abundance OTU data was standardized with Hellinger transformation (function *decostand*; Oksanen et al., 2019) while zooplankton and fish abundance data were not transformed. The network was constructed following the workflow applied in Capo et al. (2017) with the WGCNA R package (Langfelder & Horvath, 2008). Briefly, a signed network of clustered OTUs was created using the function *adjacency* and a minimum of eight nodes (OTUs) per module, and power five was used as the threshold value. The visualization of the network was done using the software Gephi (Bastian et al., 2009). It included the edges with Pearson's correlation coefficient equal to or higher than 0.7. Furthermore, an eigenvalue analysis for each module was performed, where each module was assigned a singular value decomposition (SVD) of abundance called module eigenvalue. These eigenvalues were correlated with environmental factors through a modularity-detection algorithm, to reveal potential correlation of the environmental conditions with the modular structure (Deng et al., 2012).

3 | RESULTS

3.1 | Environmental changes over time in Lake Biwa

Air temperatures near Lake Biwa increased gradually over the study period, especially since the late 1980s with average values at 14.8 ± 0.6°C versus 14.0 ± 0.5°C before (Figure 1). In contrast, the precipitation values were higher (141.9 ± 23 mm) prior to the 1980s and only reached average values of 133 ± 14 mm after this period. In the north basin of Lake Biwa, total phosphorus concentration increased in the late 1960s with values up to 11.1 $\mu g L^{-1}$ and then gradually decreased in more modern times (7 $\mu g L^{-1}$ in 2017). The water level of Lake Biwa was first manipulated in 1992, leading to an overall decrease in water level over time (up to −28 m in 2015). In terms of the composition of the fish community, the community before early 1990s was dominated by ayu, isaza, common carp, crucian carp and moroko species, but shifted to a community dominated by ayu, crucian carp and wakasagi after the water level manipulation (Figure 2b). Over the period 1973–2010, *E. japonicus* was the dominant crustacean zooplankton, accounting for 65% of the total biomass on average, followed by Cyclopoida spp., *D. galeata* at 16% and 15%, respectively (Figure 2b). *D. orientalis*, *B. longirostris* and *D. pulicaria* exhibited proportions lower than 2% during most of the observed years. Both *E. japonicus* and *D. galeata* showed similar trends, being stable until 1994, and then increasing during the following years. Although *D. pulicaria* occurred first after the late 1990s, it temporarily disappeared following the period, but then appeared again from the early 2000s.

3.2 | Past diversity of Lake Biwa microbial eukaryotic community

The sedimentary DNA analysis identified 2037 OTUs distributed across 45 microbial eukaryotic groups, from the 31 sediment core samples spanning 100 years of the lake history. The number of OTUs observed in a single sample ranged from 170 to 596 OTUs, with the lowest and highest values recorded in 1924 and 1990, respectively (Table S1). Among the 44 taxonomic groups, 10 groups accounted for more than 74% of the total richness (number of OTUs) and 88% of the total number of DNA reads (Table 1). These groups included strict heterotrophs such as cercozoans (Cercozoa), ciliates (Ciliophora), chytrids, bicosoecideans (Bicosoecida) and perkinseans (Perkinsea). Phototrophs such as diatoms (Bacillariophyta) and chlorophytes (Chlorophyta), groups containing heterotroph, phototroph and mixotrophic taxa such as dinophytes (Dinophyceae), and unclassified alveolates (Alveolata) and stramenopiles (Stramenopiles) OTUs were also found among the 10 most abundant taxonomic groups. Altogether, the 10 most abundant OTUs accounted for more than 39% of the total number of reads. They included precisely identified OTUs such as a ciliate (OTU2702, Spathidiidae, *Arcuospathidium* sp.), a diatom (OTU488, *Aulacoseira* sp.) and a chlorophyte (OTU1327, Chlorophyceae, *Mychonastes* sp.) (Table 2). The remaining seven OTUs were taxonomically identified at higher taxonomic rank within alveolates, stramenopiles, chlorophytes

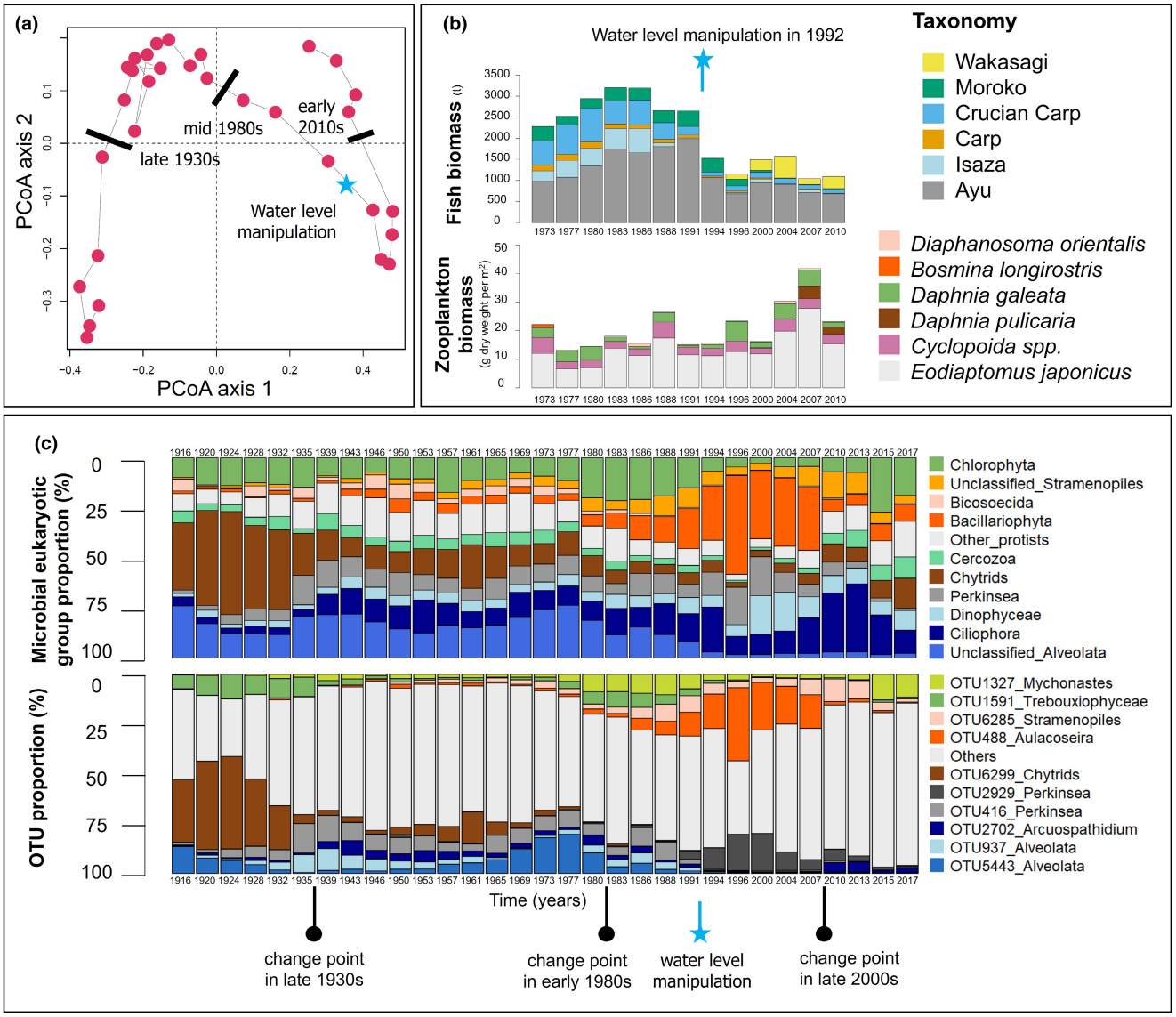

FIGURE 2 Temporal changes (a) in the structure of the microbial eukaryotic community over 100 years (b) in the fish and zooplankton biomass (c) in the relative abundance of microbial eukaryotic groups (upper panel) and the 10 most abundant OTUs (lower panel). Change points, detected by ecp analysis, are indicated by black lines (a) and black dots (c) and the start of water level manipulation by a blue star.

and chytrids. An additional BLAST search on the nucleotide sequences of these seven OTUs enabled a better identification for the chrysophyte OTU6285 (Stramenopiles, Chrysophyceae) and chlorophyte OTU1327 (Trebouxiophyceae, *Choricystis* sp.) (Table 2).

3.3 | Temporal changes of the microbial eukaryotic community

The structure of the microbial eukaryotic community from Lake Biwa changed over time as illustrated by the PCoA analysis. The change-point analysis detected three major shifts in community structure as displayed in Figure 2a, which were consistent with changes in broader community composition (Figures 2c and S3, Table S1). The first shift in the late 1930s was indicated by a higher OTU richness (262–349 OTUs) and higher proportion of ciliate and perkinsean OTUs (i.e. OTU2702 *Spathidiidae* (*Arcuospathidium* sp.) and OTU416 Perkinsea (Perkinsea_1), respectively), as well as a lower proportion of chytrid reads (i.e. OTU6299, chytrids) (Figure 2c). This first shift was also marked by lower proportions of the chlorophyte OTU1591 (Trebouxiophyceae, *Choricystis* sp.) and higher proportions of the unclassified alveolate OTU937. The second shift in the early 1980s was characterized by higher number of OTUs (363–596 OTUs) and higher proportion of the diatom OTU488 (*Aulacoseira* sp), the chrysophyte OTU6285 (Stramenopiles), but, noticeably higher proportion of the chlorophytes OTU1327 (*Mychonastes* sp.) and OTU1591 (*Choricystis* sp.) since early 1980s. The third shift happened in the late 2000s and was mainly characterized by lower proportions of the diatom OTU488 (*Aulacoseira* sp.) and perkinsean OTU2929, and higher

TABLE 1 Number of DNA reads and OTUs for each of the 45 taxonomic groups found within the microbial eukaryotic community of Lake Biwa. Bold letter: One addressed in the main text.

Microbial eukaryotic groups		#DNA reads	#OTUs
Alveolata	Apicomplexa	7629	64
	Ciliophora	49,393	313
	Dinophyceae	25,164	189
	Perkinsea	32,023	49
	Other_Alveolata	57,906	75
Amoebozoa	Centramoebida	69	11
	Tubulinea	2530	28
Cryptophyta	Cryptomonadales	188	3
	Cryptophyta_2	109	3
	Cryptophyta_3	15	2
	Cryptophyta_4	644	10
	Pyrenomonadales	1566	8
	Other_Cryptophyta	96	6
Haptophyta	Coccolithales	5	1
	Phaeocystales	55	1
	Prymnesiales	1812	11
	Zygodiscales	165	2
	Other_Haptophyta	231	4
Heterolobosea	Schizopyrenida	138	5
Opistokonta	Choanoflagellida	83	7
	Chytrids	62,148	227
	Opistokonta_incertae_sedis	70	3
Euglenozoa	Euglenida	163	11
	Kinetoplastida	52	3
Rhizaria	**Cercozoa**	20,970	225
	Other_Rhizaria	3097	71
Rhodophyta	Bangiophyceae	268	8
	Florideophyceae	98	5
Stramenopiles	**Bacillariophyta**	39,899	136
	Bicosoecida	11,246	61
	Chrysophyceae	6543	73
	Dictyochophyceae	2	2
	Eustigmatophyceae	4928	9
	Labyrinthulida	373	9
	MAST	158	7
	Oomycetes	3399	39
	Pinguiophyceae	13	1
	Pirsonia	4814	5
	PX_clade	518	6
	Raphidophyceae	517	6
	Synurophyceae	2502	20
	Other_Stramenopiles	17,385	86
Viridiplantae	**Chlorophyta**	51,371	158
	Streptophyta	5355	67
	Other_Viridiplantae	382	7
Total		**416,092**	**2037**

TABLE 2 Taxonomy of the 10 most abundant OTUs. Additional BLAST searches have been conducted to confirm or help resolving taxonomic identification.

OTUs	Taxonomy	Total read proportion (%)	Identity (%)	BLAST
OTU5443	Alveolata; environmental_samples	7.34	89.2	na
OTU937	Alveolata; environmental_samples	4.35	86.1	na
OTU2702	Alveolata; Ciliophora; Spathidiidae; Arcuospathidium	3.97	94.3	*Foissnerides/Spathidium* sp.
OTU416	Alveolata; Perkinsea; Perkinsea_1	8.26	94.5	na
OTU2929	Alveolata; Perkinsea; Perkinsea_2	4.52	81.6	na
OTU6299	Fungi; Chytridiomycota; environmental_samples	12.90	84.8	na
OTU488	Stramenopiles; Bacillariophyta; Aulacoseiraceae; Aulacoseira	8.64	95.3	*Aulacoseira granulata*
OTU6285	Stramenopiles	5.06	92	Uncultured Chrysophyceae
OTU1591	Viridiplantae; Chlorophyta; Trebouxiophyceae	6.05	93.2	*Choricystis* sp.
OTU1327	Viridiplantae; Chlorophyta; Chlorophyceae; Mychonastes	6.03	81	*Mychonastes* sp.

Parameters	PCoA1	PCoA2	r^2	Pr(>r)	
Annual air temperature	0.89379	−0.44848	0.7105	0.008	**
Annual precipitations	−0.99935	−0.03592	0.1974	0.333	
Water phosphorus concentrations	−0.99999	0.00370	0.4952	0.029	*
Water level	−0.96491	0.26258	0.8292	0.002	**
Eodiaptomus japonicus	0.90670	−0.42178	0.3175	0.130	
Daphnia galeata	−0.00122	−1.00000	0.1375	0.482	
Cyclopoida sp.	−0.28714	0.95789	0.0389	0.801	
Diaphanosmoma orientalis	0.44069	−0.89766	0.6019	0.014	*
Daphnia pulicaria	0.33676	−0.94159	0.3712	0.072	
Bosmina longirostris	0.90136	−0.43307	0.1123	0.562	
Ayu	−0.22258	0.97491	0.4834	0.064	
Crucian carp	−0.99244	0.12274	0.8419	0.001	***
Hon-Moroko	−0.35097	0.93639	0.7927	0.002	**
Isaza	−0.98915	0.14693	0.5126	0.023	*
Wakasagi	0.46247	−0.88664	0.6428	0.006	**
Common carp	−0.95329	0.30207	0.9221	0.001	***

TABLE 3 Env.fit results showing relationships between the structure of the microbial eukaryotic community and environmental and biological parameters. Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1.

proportions of ciliates OTUs, including OTU2702 (*Arcuospathidium* sp., Spathidiidae) (Figure S3).

3.4 | Causal drivers of the changes in the microbial eukaryotic community

With a focus on the period 1973–2010, temporal correlations were observed between a few selected environmental stressors—annual air temperature, annual precipitation, water phosphorus concentration, water level, zooplankton and fish species biomass—and the structure of the microbial eukaryotic community (Table 3). Changes in water level and biomass of all selected fish species were both significantly correlated to the composition of the microbial eukaryotic community, while no to low correlations were found with the biomass of dominant zooplankton species.

A co-occurrence network was created to study in further detail the temporal associations between microbial eukaryotes, zooplankton and fish taxa over the period 1973–2010. The network consisted of 453 nodes (taxa) linked by 3020 edges (Pearson's rank correlation calculated from [relative] abundance data, keeping only edges with values superior or equal to 0.5; Figure 3a). A total of 15 modules (clusters of highly connected taxa) were detected in the network (Table S2). The temporal changes in the relative abundances of certain modules (i.e. m03, m10 and m13) were found to be significantly correlated to changes in environmental parameters (Figure 3b). The crucian carp, isaza, moroko and certain alveolates, perkinseans and chlorophytes OTUs from module m03 showed lower proportion in the 1990s and were negatively correlated to air and water temperature and positively correlated to phosphorus concentration and water level. The m10 eigenvalues were negatively correlated with water phosphorus concentration, water level and sunshine duration but positively with air and water temperature and wind velocity. A higher proportion of m10 taxa were identified as diatoms and perkinseans before the 1990s and thereafter this module had a higher influence from unclassified

stramenopiles (Figure 3c). Finally, m13 eigenvalues were positively correlated to air and water temperature and wind velocity and negatively correlated with water level and phosphorus concentration. The taxa from m13 were dominated by wakasagi, dinophytes, diatoms and chytrids having higher biomass/proportions from the 1990s.

4 | DISCUSSION

4.1 | Sedimentary DNA to describe the past dynamics of lake microbial eukaryotes

The analysis of the DNA found in a sediment core revealed historical changes in the microbial eukaryotic community of Lake Biwa. Non-photosynthetic taxa (i.e. ciliates, perkinseans and chytrids) represented up the vast majority of DNA reads. Phytoplankton represented c. 10% of micro-eukaryotes in the 1910s and increased up to 60% in the 2000s. The increase in phytoplankton over the last decades matches trends observed in other lakes (Capo et al., 2017; Ibrahim et al., 2021).

The reliability of the sedimentary DNA approach to reconstruct past changes in lake biota has been evaluated by several recent works (see Capo et al., 2021; Barouillet et al., 2023 for synthesis). There are still uncertainties about whether sedimentary DNA reconstructs the signal from microbial eukaryotes from lake sediment, lake water or external inputs. Several protists are indeed able to thrive in organic-rich surface sediments and are an important part of benthic food webs (Coolen & Shtereva, 2009; Forster et al., 2016). However, their ability to thrive in subsurface sediments depleted in electron acceptors is still understudied compared to bacteria and archaea with certain groups able to survive in deeper sediment zones (Vuillemin et al., 2023). For instance, resurrection of protists from marine subsurface sediments has been successfully performed for certain amoebae, ciliates, diatoms or dinoflagellates (Delebecq et al., 2020;

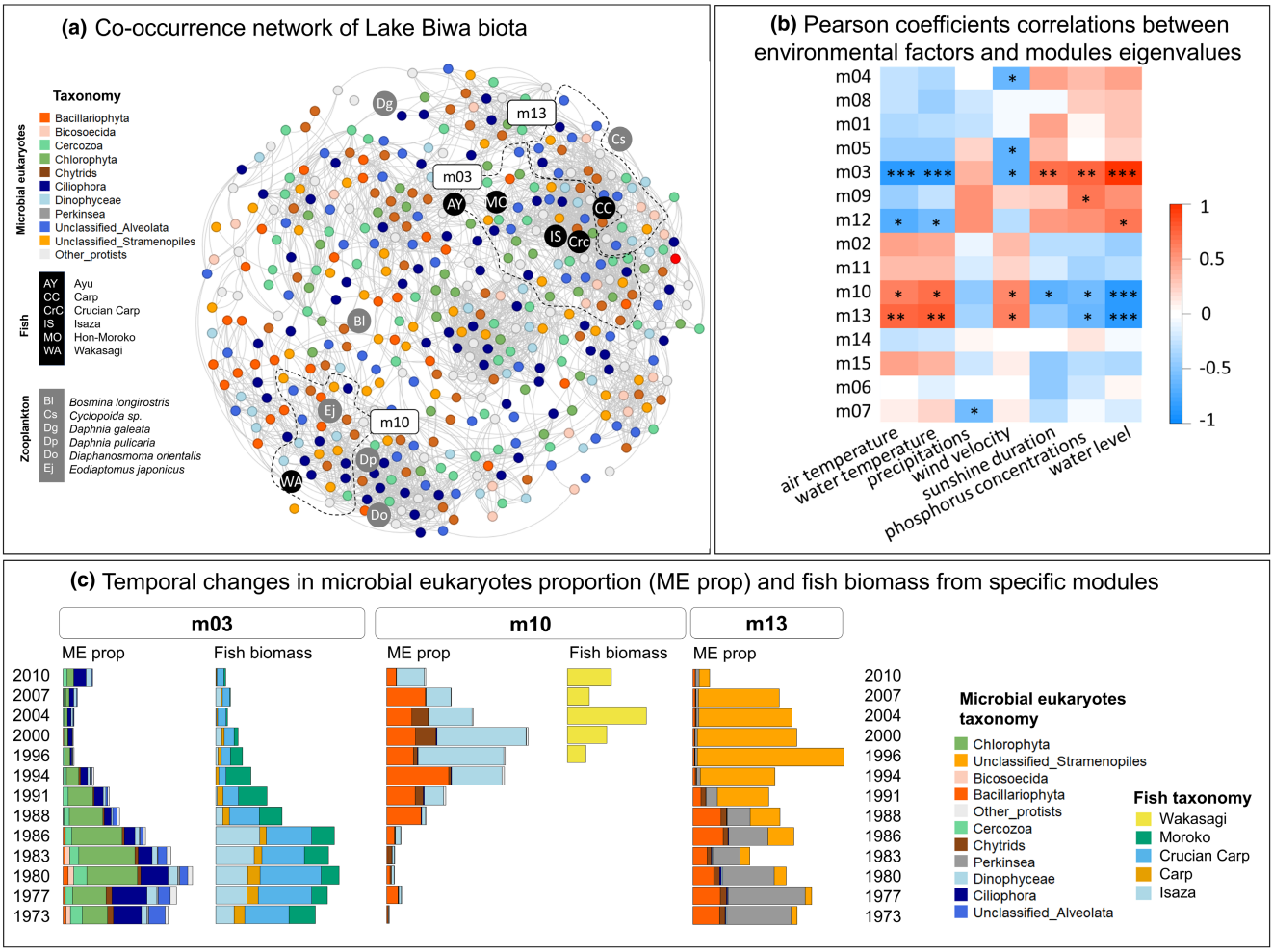

FIGURE 3 (a) Network analysis based on temporal abundance data between 1973 and 2010 showing co-occurrence patterns between microbial eukaryotes, zooplankton and fish taxa in Lake Biwa. (b) Outputs of Pearson's coefficient correlations between environmental factors and the eigenvalues on the network modules based on Mantel's tests. The correlation degree between each module and each environmental factor is visualized with colour scale (right side of the heatmap) and the significance level (*p* value) is indicated with the following significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05. (c) Temporal dynamics pattern (biomass or proportion) of taxa found in the modules m03, m10 and m13.

Ellegaard et al., 2020; Sanyal et al., 2022). Comparatively to marine environments, the presence of active protists in lake subsurface sediments has been scarcely studied.

Previous works suggest that the molecular signal from microbial eukaryotes found in sediments offer a good reflect of past planktonic community (Capo et al., 2015; Gauthier et al., 2021). Although relative abundance data from OTUs associated with unclassified alveolates and fungi need to be analysed cautiously, for photosynthetic organisms, the sediment molecular signal is usually considered to reflect their past distribution in the ecosystem studied (Capo et al., 2023). In Lake Biwa, the higher proportion of dinophytes observed from 1990s are concomitant with higher biovolumes measured from a pelagic station of the North Basin of Lake Biwa and lower read proportion of chlorophytes between 1990s and 2010s were consistent with lower green algae biovolumes measured for the same period (Ichise et al., 2020; Tsugeki et al., 2009). In contrast, diatom DNA reads assigned to the genus *Aulacoseira* (including OTU488) were found in higher proportions between 1980s and late 2000s (compared to other periods). Similarly, diatom biovolumes at the North Basin of Lake Biwa (Ichise et al., 2020) and sediment diatom frustules from *Aulacoseira* spp. (*A. granulata*, *A. ambigua*) (Tsugeki et al., in preparation) increased continuously after the late 2000s. This difference in findings with different approaches may be due to the difficulty in lysing diatom frustules without a tailored DNA extraction method, as diatom studies generally perform overnight lysis and add proteinase K (e.g. Dulias et al., 2017). A shorter and less powerful lysis (present study) may yield less diatom sedDNA from recent and undamaged diatom cysts, which would explain that less diatom reads are observed than expected in recent samples. Finally, a major outbreak of the chrysophyte *Uroglena americana* which occurred the first time in the North Basin of Lake Biwa in May 1977 (Ichise et al., 2020) and genetically characterized by Ishikawa et al. (2005), was not robustly captured in our DNA analysis despite several OTUs were characterized as unclassified chrysophytes (Table 1). Despite such discrepancies, we expect that the signal from, at least, photosynthetic taxa amplified in this study reflects historical trends from Lake Biwa, albeit with less diatom reads than diatom frustules found in the core. As for other protists, more studies are needed to determine which taxa can survive in deeper lake sediments, for example whether saprotrophic taxa (e.g. chytrids) reflect past conditions or if they can adapt and multiply in lake sediments.

4.2 | Marked historical changes in Lake Biwa highlighted by microbial eukaryotes

Lake Biwa has been under severe anthropogenic pressures over the past century (Kawanabe et al., 2020). In our study, changing points detected in the structure of the microbial eukaryotic community from Lake Biwa were detected in the late 1930s, early 1980s and late 2000s, aligning with the main environmental changes that have happened in the lake over the past century.

The first shift identified by the change point analysis occurred in the late 1930s, notably marked by changes in richness and the proportion of certain microbial eukaryotic groups. Since the beginning of the 20th century, the water level of Lake Biwa has been controlled to prevent risks of floods (Nishino, 2012, 2020). The lowering of the water levels starting in the mid-1940s created shallow littoral zones (called naiko) used as paddy fields. The reduction in the water surface had an impact on the fish biomass that used these littoral zones as spawning sites with the exception of ayu whose life does not depend on naiko. Modifications in the proportion of DNA reads associated with chytrids and perkinseans, both groups of parasites (Jobard et al., 2020; Kagami et al., 2014), suggest that this shift may reflect a modification in the abundance and composition of planktonic and/or fish assemblages and confirm the links between the manipulation of water level, fish community and microbial eukaryotic communities.

The second shift detected by change point analysis in early 1980s was in-details analysed by network topology analysis to study co-occurrence patterns of planktivorous fish, dominant zooplankton and microbial eukaryotes taxa in Lake Biwa over 38 years (from 1973 to 2010), such co-occurrences being either the reflect of similar environmental preferences or real biological interactions. Interestingly, most planktivorous fish and microbial eukaryotes showed strong co-occurrence patterns related to environmental factors, with strongest correlations to air and water temperatures, phosphorus concentration and water level. Enhanced nutrient loading to lakes, sometimes leading eutrophication, is well recognized as a major driver of changes in lake food webs including diversity and composition of microbial eukaryotic communities (Capo et al., 2017; Huo et al., 2022; Ibrahim et al., 2021). In Lake Biwa, an increase of water phosphorus concentration of lake waters, used as a proxy for eutrophication, was reported in the 1960s and 1970s associated with enhanced primary productivity (Hayakawa et al., 2012). Lake primary productivity then declined in the mid-1980s, concomitant with a decrease in phosphorus concentrations, largely due to human regulations related to nutrient loading from the catchment area (Hayakawa et al., 2012; Hsieh et al., 2010; Hyodo et al., 2008).

In our study, the period of the late 1980s when the lake was re-oligotrophied was identified as one of the changing points for the community. However, the temporal changes in water phosphorus concentrations was not detected as a major driver of changes in Lake Biwa microbial eukaryotic community (Table 3). A potential explanation is that despite an increasing trend, phosphorus concentration in the water column of Lake Biwa (11 $\mu g L^{-1}$) is low compared to other previously studied systems for example, Lake Bourget (120 $\mu g L^{-1}$, Capo et al., 2017), Lake Constance (86 $\mu g L^{-1}$, Ibrahim et al., 2021) or Lake Chao (256 $\mu g L^{-1}$, Chen et al., 2013). Nevertheless, OTU1591 (chlorophytes, Trebouxiophyceae) was more predominant from early 1980s to early 1990s, consistent with a marked change in phosphorus concentrations. Members of this family has not been reported by microscopic phytoplankton counting for this lake (Hsieh et al., 2010), and were recently identified as an endosymbiotic algae associated with ciliates (Hoshina et al., 2018). In the present study, higher proportions of ciliates (OTU2702) were observed during this period.

Therefore, the higher proportions of Trebouxiophyceae (OTU1591) might be related to higher proportions in the early 1980s to early 1990s though it is not clear why the Trebouxiophyceae were in higher proportions in the late 1890s to mid-1930s when the ciliates proportion was seemingly very low.

In the 1970s–1990s, levees along the lake shore, dams and enclosing bands on the river flowing to the lake have been constructed reducing further spawning migration of fish from Lake Biwa to naiko. Additionally, indigenous fish communities from Lake Biwa have been shown to be strongly influenced by the introduction of alien fish species large-mouth bass (*Micropterus salmonides*) and bluegill (*Lepomis macrochirus*), with adverse effects on the indigenous fish communities from the 1980s (Maehata, 2020). Furthermore, modifications in the water level control since 1992 had a negative impact on fish spawning and thus some fishes, including crucian carp and common carp, decreased because of the water level manipulation (Yamamoto et al., 2006). Indeed, the lowering of the water level from 1992 appears to have been a major stressor explaining the major shift observed in richness and structure of the microbial eukaryotic community in the mid-1980s. This lowering of the water level exerted a strong control of the fish assemblage with a shift from a community dominated by ayu, isaza, common carp, crucian carp and moroko species to ayu, crucian carp and wakasagi species with further consequences for other levels of the food webs. The shift in fish assemblages explained 84% of the temporal variation in read proportion of OTU416 (Perkinsea). This link could be explained by the fact that this specific perkinsean is a parasite of phyto- or zooplanktonic species (Jobard et al., 2020; Mangot et al., 2011), with modified abundance related to changes in top-down pressures, or directly fish species (Freeman et al., 2017).

Another part of the network analysis indicated a possible impact of the food web on microbial eukaryotes. Changes in zooplankton biomass only co-occurred with wakasagi catch and higher proportions of ciliates. Pearson's correlations indicated weak links to environmental factors (mainly lake level change), but the main driver for this co-occurrence may be a shift in the food web when wakasagi started to self-recruit in rivers near Lake Biwa (after 1995, not tested by Pearson's correlations). A previous study has identified ayu biomass as a main driver of changes in zooplankton biomass (Liu et al., 2020), but this was not observed in the present network analysis, despite ayu being the most abundant fish taxa. These results may be due to the addition of microbial eukaryotes in the analysis, since Liu et al. (2020) only compared zooplankton metrics (biomass and body size) to ayu CPUE. Furthermore, other studies have observed a shrinking of the food web (i.e. less trophic levels) in the offshore zone of Lake Biwa since the lake's levels have decreased (Okuda et al., 2020). Changes in the zooplankton community and in specific microbial taxa may be therefore be due to fish predation modified by water level changes. Additionally, a vertical net sampling which a mesh size 95 μm was used to study the zooplankton community, may not capture all zooplanktonic taxa equally; this would explain the low correlations observed between zooplankton biomass and the microbial eukaryotic community structure changes.

The third shift indicated by the change point analysis took place in the late 2000s, marked by lower proportions of diatoms and perkinseans and higher proportions of ciliates. In the 2010s, the increase of large desmids, *Micrasterias hardyi* was reported (Hodoki et al., 2020). The reason for the increase of *M. hardyi* has not been clarified, but may be related to zooplankton communities, as *M. hardyi* was too large to be eaten by zooplankton. Because of the lack of zooplankton data after the 2010s, no other statistical analysis could be made on the causes of the changes in the microbial eukaryotic community at this time. It is possible that the impact of global warming, largely auto-correlated with changes in water levels in Lake Biwa, has synergistic and long-term impacts on the microbial eukaryotes in this lake.

Overall, the findings of the present study confirmed that manipulation of the water level in Lake Biwa are likely to be primary cause of food-web changes over the past 100 years, in addition to nutrient levels. The present study highlights that microbial eukaryotes have also been significantly impacted by re-oligotrophication, warmer waters and lake level decrease, with strong implications for the resistance and resilience of the Lake Biwa ecosystem. Beyond changes in taxonomy, sedimentary DNA records imply shifts in the functionality of microbial eukaryotes, seen as shifts in the proportions of primary producers (diatoms and chlorophytes), grazers and phagotrophs (ciliates), and saprotrophs (chytrids). Microbial eukaryotes are a key part of lake ecosystems, sand they should be included alongside other communities to gain a better understanding of the complexity of lake food webs. In the case of Lake Biwa, future studies should also include rotifers and larger fish such as the introduced largemouth bass, bluegill and trout, to help disentangle the top-down effects from bottom-up effects. Due to the large size of Lake Biwa, having data from multiple sites (north vs. south, deep vs. shallow) could also reveal local shifts that are currently drowned in the present dataset. Altogether, such a dataset would deepen our knowledge about how much Lake Biwa biota is resistant and resilient to the multiple environmental forcing that it is facing.

5 | CONCLUSIONS

Lake biological communities are influenced by multiple stressors, including human-induced modifications of environmental conditions that have occurred over the last century. However, observational data obtained from before environmental perturbations are scarce and such baseline observations are required to fully understand the impacts of environmental changes on aquatic biota and their ecosystem services. Using a molecular palaeolimnological approach, our study showed that human manipulation of the water level, the shifts in fish community appear as primary drivers of changes in its microbial eukaryotic community over the past century, surpassing the parallel effects of increased phosphorus loading to this lake. Our work illustrates the need of incorporating microorganisms in the components of lake food webs to fully understand how top-down

and bottom-up pressures impact these food webs. The recent advances of sequencing and bioinformatics from water column and sedimentary archives will undoubtedly augment our knowledge about lake biota and their responses to environmental changes.

AUTHOR CONTRIBUTIONS

Conceptualization: EC, NT; SB. Developing methods: EC, NT. Data analysis: EC, MP, KN, XL, YS, NT Preparation of figures and tables: EC, MP, NT. Conducting the research, data interpretation, writing: EC, MP, KN, MK, SB, MK, XL, YS, NT.

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CONFLICT OF INTEREST STATEMENT

Authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Raw sequences data have been made available on FigShare (https://doi.org/10.6084/m9.figshare.14575488). Metadata are shared in Table S1.

ORCID

Eric Capo https://orcid.org/0000-0001-9143-7061
Maïlys Picard https://orcid.org/0000-0002-8124-5741
Michinobu Kuwae https://orcid.org/0000-0002-7747-5421
Stefan Bertilsson https://orcid.org/0000-0002-4265-1835
Maiko Kagami https://orcid.org/0000-0003-3086-390X
Xin Liu https://orcid.org/0000-0002-6660-4486
Narumi Tsugeki https://orcid.org/0000-0003-1236-906X

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SUPPORTING INFORMATION

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