Paleoecological evidence for a multi-trophic regime shift in a perialpine lake (Lake Joux, Switzerland)

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Figures 2,3,6,7 use color in print

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4 **Abstract**

5 Freshwater ecosystems are under new and increasing threats from anthropogenic change.

6 Ability to detect and predict consequences of environmental perturbations on ecosystem

7 function and water quality is limited by the lack of empirical data over relevant time scales.

8 Paleoecological records present a unique opportunity to broaden understanding of ecological

9 transitions over decadal to millennial timescales. This study tested the occurrence of regime

10 shifts to track changes throughout the lake food web beyond the typical instrumental era,

11 using both "traditional" paleoecological proxies (e.g., microzoo-plankton, zoobenthos, and

12 pigments) and more recently developed molecular genetic methods (sedimentary DNA). We

13 used sediment cores from the perialpine Lake Joux (Swiss Jura), where the history of human

14 settlement and land-use practices in the catchment has been well documented since the

15 Medieval period. Paleoecological evidence revealed an abrupt and unprecedented biological

16 reorganization in the second half of the 20th century, following several centuries of relatively

17 stable communities despite growing human pressure. Time-varying autoregression

18 computed using dynamic linear modelling identified this transition, triggered by the onset of

19 rapid cultural eutrophication in the 1950s, as a true regime shift. Since this time, despite

20 decades of re-oligotrophication, biotic communities of Lake Joux have not returned to pre-

21 disturbance composition, most likely due to other confounding factors, including climate

22 warming, that may prevent the lake from returning to an earlier equilibrium state.

23 Paleoecological reconstruction further suggested that cladocerans responded earlier to

24 disturbance, which is highly relevant for lake monitoring and management strategies.

25

26 **1. Introduction**

Lake ecosystems are changing at an increasing rate as a consequence of global climate change, as well as regional and local environmental and anthropogenic disturbances (Jenny et al., 2020). Threats to lake ecosystems arise from the accumulation of local and global human stressors, but also from their potentially non-linear and sudden responses to the stressors after years of relative stability. Such transitions are referenced as regime shifts, and can occur without easily detectable early-warning signals (Scheffer et al., 2001). Regime shifts typically occur at multiple trophic levels, after exposure to discrete or continuous stressors (Bestelmeyer et al., 2011; Randsalu-Wendrup et al., 2016). That happens abruptly and has prolonged consequences on ecosystem structure and function (McGowan et al., 2005) that would delay the efficiency of management actions (Andersen et al., 2009; Randsalu-Wendrup et al., 2016; Scheffer et al., 2001). Detecting the occurrence and quantifying the strength of such major shifts in lakes is fundamental to identify causes of catastrophic changes and to help develop management strategies for the protection and the conservation of freshwaters.

Regime shifts raise important management issues, yet there are few documented records of true regime shifts, especially for deep lakes. Our ability to detect regime shifts and to determine their underlying causes is currently limited by the lack of long-term high-resolution data (Smol, 2010; Taranu et al., 2018). Lacustrine sediments provide unique archives of climatic, environmental and ecological changes that constitute an alternative option for the retrieval of information from the past (Gregory-Eaves and Beisner, 2011). Paleolimnological records archive evidence for major events such as regime shifts through community changes. Therefore, paleolimnological approaches have potential to shed light on the dynamics of environmental and biological change over decadal to millennial time scales.

Subfossil-based paleolimnological proxies that are readily preserved in the sediment over thousands of years include diatoms, cladocerans and chironomids (Leavitt and Hodgson, 2001; Watts and Maxwell, 1977). Used widely to track various facets of lake

54   histories, such as primary production (Deshpande et al., 2014; Leavitt and Hodgson, 2001;

55   Makri et al., 2019), these proxies can support assessment of long-term responses of

56   biological communities to key environmental drivers. These drivers include changes in

57   nutrient regime (eutrophication and re-oligotrophication) (Hall et al., 1999) or pH (Battarbee,

58   1991), climate change (e.g., Walker *et al.*, 1991; Perga *et al.*, 2015; Bruel *et al.*, 2018) and

59   human impacts (Battarbee and Bennion, 2011; Dubois et al., 2017).

60   More recently developed molecular-based proxies have further opened sedimentary

61   archives to ecologists by enabling the investigation of all organisms across the tree of life.

62   Such proxies include those that do not leave identifiable morphological remains or fossils,

63   such as microbes and microeukaryotes (Capo et al., 2021; Domaizon et al., 2017; Taberlet et

64   al., 2012; Thomsen and Willerslev, 2015). Sedimentary DNA (*sed*DNA) approaches applied

65   to lakes have led to successful ecological reconstructions of primary producers at deeper

66   taxonomic resolutions compared to photosynthetic pigments. They have made possible the

67   estimation of their genetic diversity (Capo et al., 2016; Coolen et al., 2006; Domaizon et al.,

68   2013; Monchamp et al., 2018, 2019a). Further, studies combining both *sed*DNA and other

69   paleolimnological proxies have proven useful to validate temporal trends in lake biodiversity

70   (Coolen et al., 2006; Stoof-Leichsenring et al., 2012; Tse et al., 2018; Wirth et al., 2013).

71   Both paleoecological and neolimnological studies on the changing dynamics of

72   systems usually cover a limited range of diversity or trophic levels (for example, pigments

73   (Makri et al., 2019); cladocerans (Bruel et al., 2018)). Processes leading to an ecosystem-

74   wide shift, on the other hand, result from intricate relationships at multiple trophic levels. The

75   coupling of molecular genetics and more classical paleolimnological approaches can help

76   evaluate the evolutionary response of aquatic organisms to changing environments and to

77   uncover trophic interactions (Ellegaard et al., 2020). So far, multi-trophic assessments of

78   biodiversity dynamics at a lake scale have not been available.

79   Lake Joux, a mountain lake located in the Swiss Jura, is particularly well suited to

80   paleoecological work. The history of human activities in the catchment, back to the early

81   settlement of humans (~300 CE), is well known. Regular monitoring of the lake since the

82   1980s provides decades of valuable information on biological communities and water quality.

83   Similar to other perialpine lakes (Tolotti et al., 2018), Lake Joux has undergone

84   eutrophication over the second half of the 20th century (max. 35 µg total phosphorus (TP)/L),

85   with consequences on the zoobenthos and phytoplankton composition documented by

86   contemporary monitoring.

87   To improve ability to detect and to quantify the consequences of regime shifts over

88   the entire ecosystem, this study addressed the following questions. First, how did ecological

89   communities change in Lake Joux during the last millennium. Second, to what extent do

90   identified changepoint(s) qualify as critical transitions or regime shifts? What is the character

91   of the changes? For example, were the changes restricted to some biological compartments

92   or did it spread/ripple across all trophic levels? In brief, we aimed at qualifying and

93   quantifying biological change attributable to local human activities, specifically land use and

94   pollution in the watershed.

95

96   **2. Study Area**

97   Lac de Joux (Lake Joux) is located in the Swiss Jura at 1004 m above sea level (m a.s.l.). It

98   is the largest of four lakes that lie in the Joux Valley, which is flanked by 1300-1600 m a.s.l.

99   mountains (Figure 1a). Table 1 lists the lake's main morphological and limnological features.

100

4
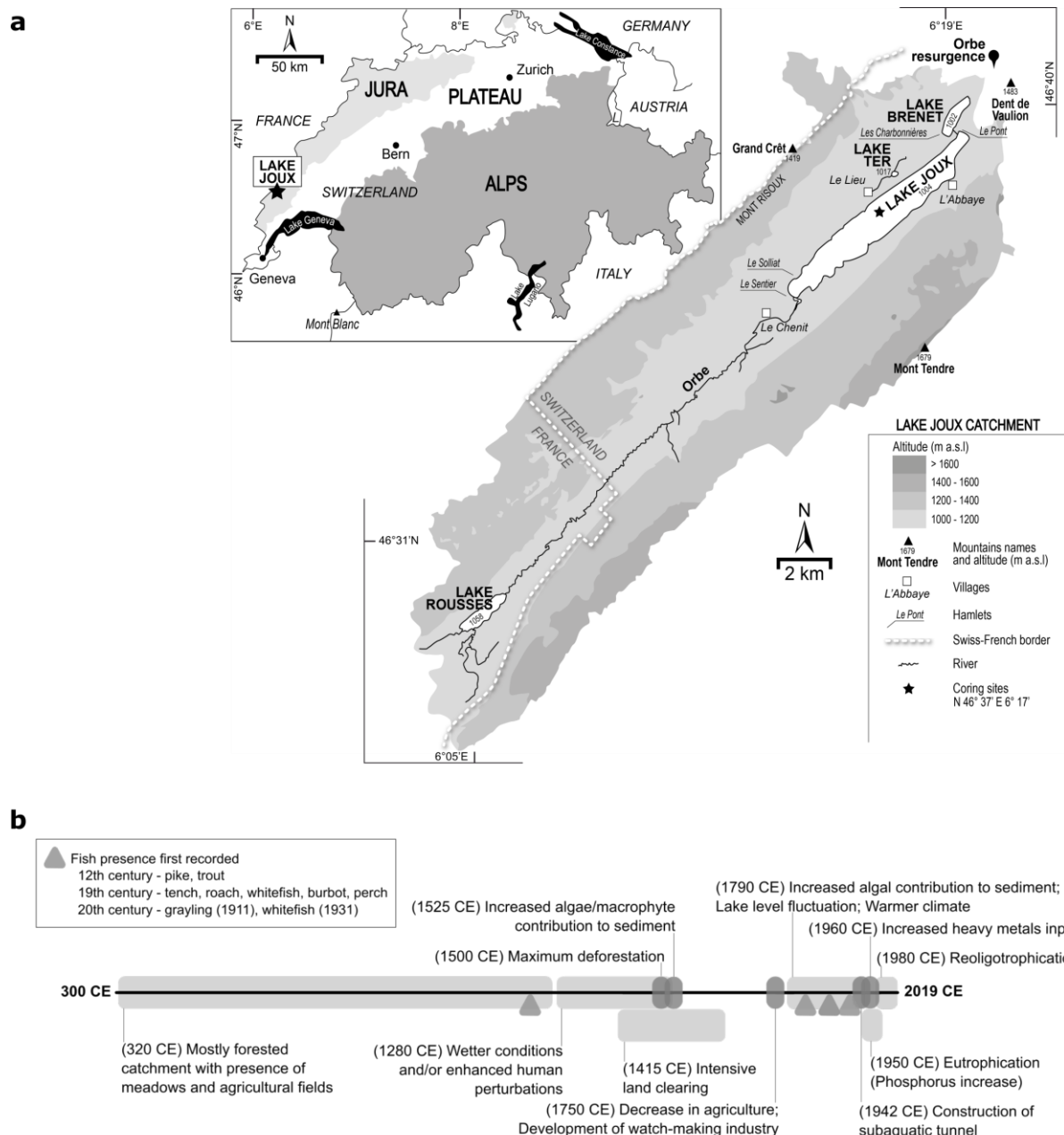
Figure 1. a) Map of Lake Joux catchment showing the location of the lake and the coring sites (modified from Lavrieux *et al.* 2017). The inset identifies the geographical location of the lake in the perialpine area. All sediment cores were collected within 20 m of the original coring site location. b) Timeline showing major climatic and human-driven events in Lake Joux and its catchment documented since the early presence of humans in the region (historical information derived from Lavrieux et al., 2017 and references therein).

110　Table 1. Morphological and limnological characteristics of Lake Joux. (Data from Fiaux *et al.*,
111　2006; Lods-Crozet *et al.*, 2006)

Lake elevation (m a.s.l.)	1001.5 - 1005.0
Lake area (km^2)	8.7
Lake volume (10^6 m^3)	132
Maximum depth (m)	33
Average depth (m)	21
Water retention time (yrs.)	0.83
Catchment area (km^2)	211

112

113　*2.1. Local Human impact*

114　Major environmental and anthropogenic forces (summarized in Figure 1b) have influenced

115　the physical and chemical structure of Lake Joux, and in turn, the lake ecology over the last

116　millennium (Fiaux et al., 2006; Lavrieux et al., 2017). The history of human presence in this

117　catchment goes back to 6850 cal yr BP, continuous since the roman period, including the

118　establishment of a monastery (5-6th century) and later an abbey (12-16th century; see

119　Lavrieux et al., 2017 and references therein). The Valley became only extensively settled in

120　the late Middle Ages, however, followed by intensive industrial development starting in the

121　late 17th century (Lavrieux et al., 2017). Although human presence in the catchment dates

122　back to the Neolithic, anthropogenic impact on the environment remained relatively low until

123　the 14th century, when soils were drained and forest cleared (Lavrieux et al., 2017). The

124　sedimentary record of Lake Joux only started recording anthropogenic perturbations and of a

125　warmer climate around 1280 CE (Lavrieux et al., 2017). Intensive land clearing started

126　around that time and reached a maximum around 1500 CE. The period between the 18th and

127　the mid-20th century was marked by overall warmer climatic conditions and in 1777, the

128　failure of a newly built dike, which caused a sudden decline in the water level of ~3.6 m

129　within 24 hrs, an event that is imprinted in the sediments (Lavrieux et al., 2017; Nicole,

130　1840). Concomitantly, development of the watch-making industry in the region increased

131　inputs of heavy metal (Iron (Fe), Zinc (Zn), Lead (Pb)) into the lake (Lavrieux et al., 2017).

132　　　More recently, construction of a subaquatic tunnel between Lake Joux and Lake

133　Brenet in the middle of the 20th century restricted water flow and led to the development of

134 standing water areas along the northeastern shoreline. These changes in lake dynamics

135 might have promoted eutrophic conditions. Lake Joux shares a common perturbation history

136 with other perialpine lakes--that is, rapid eutrophication in the 1950s followed by a re-

137 oligotrophication phase beginning around the 1980s. In the early 1950s, total phosphorus

138 (TP) concentration in Lake Joux waters averaged around 10 µg/L (Bosset, 1961). Increasing

139 human presence in the catchment and possibly changes in lake dynamics stemming from the

140 tunnel construction increased TP concentrations rapidly, with concentrations reaching 35

141 µg/L in 1979. This increase was followed by a re-oligotrophication phase starting in 1988-89

142 after phosphate-containing detergents were banned and sewage treatment and management

143 of manure were improved. Data from lake monitoring documented the re-oligotrophication

144 process (from 35 µg TP/L in 1979 to 12 µg TP/L in 2015) (Lods-Crozet et al., 2006) and

145 recorded ages for the sediment cores analysed in this study, covering both the eutrophication

146 and re-oligotrophication phases (Figures S1-S2).

147 *2.2. Fish stocking*

148 Over the last eight centuries, Lake Joux's fish community underwent large changes, likely

149 profoundly affecting the ecosystem as a whole. Historical records indicate that fish were

150 introduced into Lake Joux during medieval times (Fiaux et al., 2006). Records of the Abbey

151 of Lac de Joux mentioned the presence of both trout (*Salmo trutta*) and pike (*Esox lucius*) in

152 the 12th century (Nicole, 1840). These species were noted for their use as tax payment, and it

153 is unclear if other, less valued species were also present at that time. By the late 19th

154 century, five additional species were recorded (Fatio, 1890, 1882): tench (*Tinca tinca*), roach

155 (*Rutilus rutilus*), whitefish (*Coregonus* sp.), burbot (*Lota lota*), and perch (*Perca fluviatilis*). Of

156 these, the year of introduction (1982) is only known for whitefish, according to Fatio (1890).

157 In the 20th century, further introductions and additional stocking took place, e.g., of grayling

158 (*Thymallus thymallus*) in 1911 and whitefish in 1931 (Fiaux et al. 2006), increasing the

159 number of historically recorded fish species in Lake Joux to 14 (Périat and Vonlanthen,

160 2015).

2.3. Climate.

Climate has varied substantially in the Lake Joux region over the past millennium. The Little
Ice Age (LIA) period (ca. 1300 – 1750 CE) followed a prolonged warm period known as the
Medieval Climate Anomaly and is characterized in European mid-latitudes by a colder and
wetter climate (Magny et al., 2008). Substantial warming of surface waters has been
observed over the 20th century in mid-latitude temperate lakes as a consequence of climate
change (Woolway et al., 2019, 2017).

3. Methods

To pursue the research questions posed, we used an integrated approach combining
paleolimnological and *sed*DNA proxies to reconstruct the multi-trophic dynamics (i.e.,
decomposers; bacteria, primary producers; photosynthetic plankton, primary consumers;
cladocerans and chironomids, and secondary consumers; fish) over a period of ~1000 years
in Lake Joux. We then used a change-point analysis to identify major shifts in communities
and tested for the occurrence of regime shifts using a modelling approach.

3.1. Sediment coring, subsampling, and dating

We collected four sediment cores at the deepest point of Lake Joux (Lat. N 46° 37', Long. E
6° 17') in 2016 and 2017 using a gravity corer. We measured magnetic susceptibility on all
cores at intervals of 0.5 cm using a GEOTEK multi-sensor core logger (Geotek Limited,
Daventry, UK). The JOU16-02 core was extruded at 1 cm intervals, and the sediment layers
were divided into two subsamples. We used one subsample collected from the inner part of
the core for analysis of chlorophyll and carotenoid pigments. The other subsample provided
identification and counts of cladoceran and chironomid remains. Cores JOU17-01, JOU17-02
and JOU17-03 were opened longitudinally in the laboratory prior to sediment subsampling.
JOU17-01 and JOU17-02 used for identification and counts of fish scales and bones, and
JOU17-03 for bacterial DNA analyses. Sediment subsamples for DNA analyses were
collected at intervals of 1 cm from one half of the core using sterile disposable instruments in

188 a room where no DNA work had been done before. We further cleaned surfaces and

189 instruments with hypochlorite solution. Subsamples were collected in sterile Eppendorf tubes

190 and transported to the DNA clean laboratory facility where we conducted DNA extractions .

191 The four core profiles (JOU16-02, JOU17-01, JOU17-02 and JOU17-03) were correlated

192 using magnetic susceptibility (Loizeau et al., 1997; Thompson et al., 1975) with a reference

193 core (JOU13-02) previously collected at the same location and dated with radiocarbon (^{14}C)

194 and ^{210}Pb (Lavrieux et al., 2017) (Figure S1).

195 3.2. Bacterial and cyanobacterial DNA analysis

196 The *sed*DNA extractions were performed in batches of 7 samples with the addition of one

197 negative extraction blank to each batch using the PowerSoil DNA isolation kit (Mo Bio

198 Laboratories Inc., Carlsbad, California, USA) following strict ancient DNA laboratory

199 procedures (Monchamp et al., 2018, 2016). We performed two independent DNA extractions

200 per layer using 0.5 g to 1 g wet sediments each. We obtained between 2.8 and ~60 ng

201 DNA/µL from each layer cut. Primer pairs Univ519-F and Univ802-R (Claesson et al., 2009;

202 Wang and Qian, 2009) were used to amplify a 284 bp-long fragment within the V4 region of

203 the 16S rRNA gene. The primers were supplemented with unique tags of 8 nucleotides and

204 with an additional 2 to 4 random bases to increase heterogeneity in amplicon length

205 (Monchamp et al., 2016). Polymerase chain reaction (PCR) was done in triplicate using 12

206 ng of template DNA per 40 µL reaction, and both extraction and PCR blanks were added to

207 each plate. The products of PCR triplicates (including the blanks) were pooled and purified

208 using the Illustra GFX PCR DNA and Gel Band Purification Kit (GE Healthcare, Little

209 Chalfont, United Kingdom). The final product was eluted in Tris–EDTA buffer (10 mM) and

210 quantified using a Qubit 2.0 fluorometer (Thermo Fisher Scientific) using the high sensitivity

211 reagent kit. Finally, we pooled the 48 tagged libraries in equimolar concentrations and added

212 three negative PCR controls were added in a final pool at a concentration of 30 ng/µL, which

213 was sent to the Fasteris AG sequencing facilities (Geneva, Switzerland) for indexing and

214 paired-end sequencing (2 × 250 bp) on an Illumina MiSeq platform.

215   3.3. Bioinformatics and sequence data processing.

216   We performed quality control and processing of the sequence data following a modified

217   protocol developed at the Genetic Diversity Centre (ETH Zürich) (see Monchamp *et al.,*

218   2016). Primers and adapters were trimmed and sequences were clustered in operational

219   taxonomic units (OTUs) with a 97% similarity threshold using the UPARSE workflow (Edgar,

220   2013). Taxonomic assignment of OTUs was based on the SILVA database (v128) (Quast et

221   al., 2013). Finally, OTU reference sequences were aligned using PyNAST (Caporaso et al.,

222   2010) and a phylogenetic tree was estimated using FastTree (Price et al., 2010). Sequences

223   assigned to phyla other than Bacteria, as well as poorly known bacterial phyla were removed

224   from the dataset. Rarefication of the number of sequences in each sample (146,851 reads

225   per sample) avoided biases related to uneven sequencing depth across samples. After

226   filtering, 425 OTUs remained, consisting of bacteria and cyanobacteria.

227   3.4. Chlorophyll and carotenoid pigments

228   We extracted sedimentary pigments to determine the presence of algae and photosynthetic

229   bacteria (Leavitt and Hodgson, 2001) using a mixture of acetone: methanol: water (80:15:5)

230   at -4 ºC, filtered with a PTFE 0.2 µm filter and dried down under nitrogen gas. After

231   dissolution in a 70:25:5 solution of acetone, ion-pairing reagent (0.75 g of tetra butyl

232   ammonium acetate and 7.7 g of ammonium acetate in 100 ml water) and methanol were

233   injected into an Agilent 1200 series high performance liquid chromatography (HPLC) unit.

234   The mobile phase comprised Solvent A (80:20 methanol: 0.5 M ammonium acetate), solvent

235   B (9:1 acetonitrile: water) and solvent C (ethyl acetate) with a Thermo Scientific ODS

236   Hypersil column (205 x 4.6 mm; 5 µm particle size). The separation conditions were initially

237   100% solvent A ramping to 100% solvent B over 4 mins, and then to 75% solvent C for 34

238   mins, held for 1 min followed by a return to initial conditions. Total runtime was 43 mins (a

239   modification of Chen et al. (2001)). Eluted pigments passed through a photo-diode array

240   detector and UV-visible spectral characteristics were scanned between 350-750 nm.

241   Quantification based on scanning peak areas at 435 nm was calibrated to a set of

242   commercial standards (DHI Denmark). Pigment concentrations are reported as molecular

243   weights per dry weight of sediment.

244   Chlorophyll-a, β-carotene, and pheophytin-a are overall indicators of phytoplankton

245   biomass and myxoxanthophyll, oscillaxanthin, cantaxanthin, echinenone, and aphanizophyll

246   are indicators of cyanobacterial biomass. We used diatoxanthin to estimate siliceous algae

247   (diatoms and chrysophytes), alloxanthin for cryptophytes, and lutein, chlorophyll-b and

248   pheophytin-b for chlorophytes. UV-absorbing compounds derived the UVR index, a proxy for

249   water clarity, using the following equation: (UVR pigment/alloxanthin + diatoxanthin+

250   zeaxanthin + lutein))*100 (Leavitt et al., 1997). Higher UVR values signified increased UV

251   exposure, a consequence of increased water clarity. Separation conditions sometimes co-

252   elute lutein and zeaxanthin. In Lake Joux sediments, these compounds showed clear

253   separation, enabling quantification.

254   3.5. Subfossil cladocerans and chironomids

255   We analyzed cladoceran remains according to Frey (1986). Briefly, 2hr-baths of

256   hydrochloric acid (HCl 10%) and potassium hydroxide (KOH 10%) enabled sediment

257   samples to extirpate carbonates and to deflocculate organic matter. At each step, we sieved

258   the remaining sediment materials using a net of 31 µm mesh. In each sample, >400

259   cladoceran remains (head shields, shells, post-abdomens, post-abdominal claws, mandibles,

260   and others) were counted and identified. The determination keys of Szeroczynska and

261   Sarmaja-Korjonen (2007) and an Olympus BX41 microscope at 100-200 x magnification

262   enabled identification of the cladoceran remains. We determined *Bosmina* (*B. longirostris, E.

263   longispina, E. coregoni*) and Chydorids to the species level, and *Daphnia* to the genus level.

264   We sieved the remaining defloculated sediment materials using a 150 µm

265   mesh.Chironomid head capsules were gently collected under a stereomicroscope and

266   mounted for microscopic observations using Aquatex© mounting agent. Brooks *et al.* (2007)

267   provided guidance for identifying head capsule remains.

268   3.6. Fish remains

269   We subsampled JOU17-01 and JOU17-02 sediment cores at 2.5 cm intervals. Subsamples

270   were washed through a 250 µm sieve and sieve fractions were screened for fish remains

271   (i.e., scales and bones) using a stereomicroscope. In total, we screened 4453 cm^3 of

272   sediments. Scales were classified as either percid (i.e., perch (*Perca fluviatilis*), as no other

273   percid species has been recorded in Lake Joux) or non-percid scales.

274   3.7. Community composition

275   The R v.3.3.2 (R Core Team, 2013) provided the software for data analysis. The *phyloseq*

276   package in Bioconductor (McMurdie and Holmes, 2013) imported, filtered, rarefied, and

277   analyzed the DNA sequence data. The *changepoint* package (Killick et al., 2016)

278   (https://github.com/rkillick/changepoint.git) enabled identification of significant shifts in

279   community composition over time.Hierarchical constrained clustering was performed using

280   the *rioja* package with the Constrained Incremental Sums of Squares (CONISS) algorithm

281   (Grimm, 1987) to delimit and visualize biostratigraphic zones in the sediment profiles.

282   Pigment concentrations, as well as chironomid and cladoceran relative abundance values

283   were square root-transformed before clustering. We also applied a Hellinger transformation

284   before the principal component analysis (PCA) (Legendre and Gallagher, 2001), which were

285   used to visualize the temporal trajectories of each assemblage. To test whether temporal

286   dynamics of the communities relate to the change in lake trophic status, we extracted PC1

287   scores for use as proxy for pigment, cladoceran and chironomid composition. We plotted the

288   trajectory of each proxy against a time series for total phosphorus (TP) comprising data from

289   direct measurements in water for the period ~1961 – 2015, as well as simulated values for

290   sediment layers prior to 1961. For the simulation, a mean TP concentration of 10 ±3 µg/L

291   was used based on the premise that the lake was oligotrophic until the mid-20th century.

292   Because TP measured in recent sediments did not reflect the known re-oligotrophication of

293   the lake since the 1980s, we did not use the sedimentary TP data in any of the analyses. In

294   addition to the community plots, the concentrations of individual pigments, the percentage of

295   chironomids and the abundance of cladocerans were plotted against the TP time-series to

examine temporal dynamics at a more refined taxonomic scale and potentially determine

"key taxa" that might constitute early indicators of disturbance.

3.8. Regime shifts and critical transitions

To test for the presence and the time of regime shifts and evidence of critical transitions

throughout Lake Joux sediment cores, we applied time-varying autoregression using Online

Dynamic Linear Models (DLMs) (Carpenter and Kinne, 2003; Pole et al., 1994; Taranu et al.,

2018) to the sedimentary pigment record. Briefly, DLMs are statistical methods that allow

parameters to slowly change with addition of new data points (Carpenter et al., 2014).

"Online" implies that the models are automated and instantaneous such that the statistic of

interest (e.g., autoregression coefficient) is updated recursively at each step of the time

series (or in this case, each sediment core interval) (Ives and Dakos, 2012). This method

allows the user to detect any sharp change in the statistic, and thus an early detection of an

impending critical transition, or in the case of paleoecological data series, to retrace back in

time when the system would experience a critical transition. Given that lower sample size,

resolution and density were previously shown to degrade critical transition signals (Thomas,

2016), we chose pigments as proxy because we had a better temporal resolution throughout

the sediment core, with 68 sediment layers analyzed compared to 26 layers for cladocerans

and chironomids. To detect critical transitions, we examined the rate with which the system

returned to its equilibrium state. When working with observed time series, approximating the

recovery rate (or dominant eigenvalue of the variance-covariance matrix of a given

dynamical model) by fitting an AR(1) model is common. An AR(1) coefficient near zero

implies that the state variable returns rapidly towards the mean, but as the AR(1) coefficient

approaches 1, this tendency to return towards the mean disappears and we have evidence

of a critical transition. To determine which algal pigment provided the best and earliest

indicator of a change in state, we ran univariate models to find the dominant eigenvalues,

summed them, and then calculated their ratio (Carpenter et al., 2011). The pigment with the

highest ratio represented the best indicator.

4. Results

As detailed below, the data analysis revealed significant changes in communities at multiple levels across the food web. These changes reflect the ecosystem's response to human and climate changes, including the physical and chemical properties of the lake. The shift from oligotrophic to eutrophic status, (Bosset, 1961) and the following re-oligotrophication of the lake (Lods-Crozet et al., 2006) reported in the literature, is partly reflected in the concentrations of total phosphorus measured in sediments (Figure S2) and in the primary producer community dynamics conveyed by photosynthetic pigments (Figures 2 and S3).

4.1. Temporal dynamics across trophic levels

4.1.1. Photosynthetic plankton

The analysis detected sedimentary pigments in each of the sixty-nine layers investigated corresponding to ~1000 to 2015 CE. We excluded the layer between 50 and 51 cm downcore because of a sudden peak in pigment concentration This peak is possibly due to the presence of terrestrial plant or macrofossil material and is therefore very likely an outlier.

Pigment analysis revealed compositional changes down core. In the oldest sediments, pigment concentrations were generally low, and remained stable over several centuries (Figure 2). The pheophytin a/chlorophyll a preservation index (not shown) shows an expected increase in preservation of materials in the upper 6 cm of the core (i.e., since ~1980). Increases in lutein, chlorophyll-b and pheophytin-b pigments between the years ~1000 and 1200 CE, were indicative of enhanced growth of chlorophytes (green algae) during this time frame. The next major change occurred centuries later, in the 1800s, when the okenone-like pigments characteristic of purple-sulphur bacteria (phylum Proteobacteria) were no longer detected after having been found sporadically in older sediments (Figure 2). This result suggests that purple-sulphur bacteria disappeared or became too rare for detection via pigment analysis in the sedimentary archive after the 1800s. Finally, in the 1900s, the concentration of some chlorophyte- and cyanobacteria- associated pigments increased slightly in the decades preceding the onset of eutrophication in the mid-1950s.

351   Around 1959 (± 3 years), total pigment concentration suddenly increased by 4-fold and

352   remained high thereafter (Figures 2 and S4). The change-point analysis identified a

353   significant transition in photosynthetic pigments a few years later, between ~1963 and 1969,

354   when total pigment concentration increased from around 9.6 to 74.2 nmol/g sediment dry

355   weight. This change-point was also identified by CONISS (Figure 2). During the same period,

356   cyanobacteria-specific pigments became prominent and some of them (e.g.,

357   myxoxanthophyll) appeared for the first time.

358

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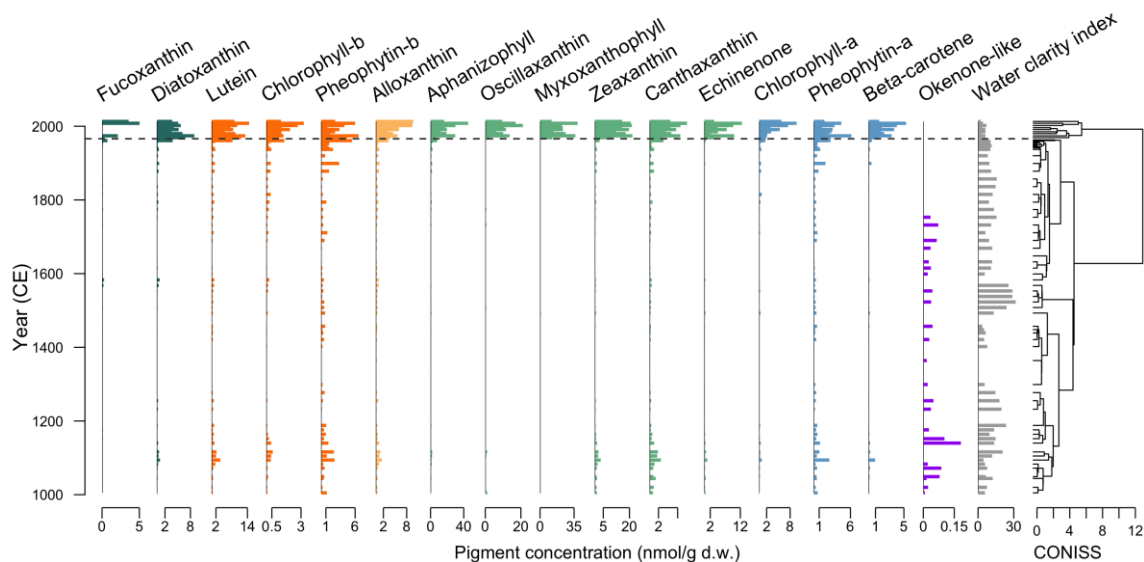
360   Figure 2. Stratigraphy of Lake Joux showing the concentration of the main sedimentary

361   pigments in sixty-eight sediment layers. Colors represent the main group of phytoplankton or

362   bacteria associated with specific pigments (dark green; siliceous algae, orange;

363   chlorophytes, yellow; cryptophytes, green; cyanobacteria, blue; all algae, purple; purple

364   sulfur bacteria). The last column (grey) shows the water clarity index. The dendrogram at the

365   far right informs about similarity in pigment composition across sediment layers. Two distinct

366   clusters (delimited in the plot by the horizontal dashed line; transition ca. 1963 – 1969) are

367   determined by CONISS.

368   *4.1.2. Bacteria*

15

369   DNA analysis showed variations in the composition and relative abundance of the main

370   bacterial phyla throughout the sediment core (Figure 3a). The main change was observable

371   in the early 1900s, when the relative proportions of Verrucomicrobia and Proteobacteria

372   increase. At the same time, the relative abundance of reads assigned to Planctomycetes

373   began to decline. Overall, Firmicutes accounted for a relatively low proportion of the reads,

374   but they become rarer in the uppermost sediment layers. The proportion of Bacteroidetes

375   and Actinobacteria sequenced reads increased in the 1900s, but both phyla represented

376   generally a low proportion of bacteria. A slight decrease in relative abundance of

377   Acidobacteria was detectable between 1200 and 1700 CE, before a slight increase towards

378   the end of the 1800s. Finally, we discovered 43 cyanobacterial operational taxonomic units

379   (OTUs) in the 47 samples collected for DNA analysis. Although cyanobacteria generally

380   accounted for a small proportion of all bacterial phyla, a rapid increase in cyanobacteria

381   abundance was detectable around the middle of the 20th century (Figure 3b). Thereafter,

382   cyanobacteria sequences remained abundant in all subsequent sediment layers, with the

383   maximum number of reads recorded in the sample corresponding approximately to 2002 –

384   2006 (between 3 and 4 cm-depth in the JOU17-03 sediment core). Cyanobacterial OTUs

385   were also supportive of an observed increase in cyanobacterial pigments in the 1950s

386   (Figure S4b).

387   *4.1.3. Chironomids (benthic community)*

388   A total of 11 chironomid taxa or groups were detectable in the JOU16-02 sediment core, and

389   three main clusters emerged based on the dendrogram (Figure 4). In the period between

390   ~1000 and 1650 CE, *Tanytarsus* spp. *Microspectra* sp., *Sergentia* sp. and Tanypodinae were

391   dominant. At the end of the 1600s, the proportion of *Paracladoperma* sp. and Pantaneurini

392   increased in addition to *Tanytarsus* spp. and *Microspectra* sp..This was not a significant

393   transition, however, based on the change-point analysis. A single significant transition in the

394   chironomid assemblage was detectable between ~ 1953 – 1959 (10 - 12 cm downcore). This

395   recent transition marked the shift from a community dominated by *Tanytarsus* spp. and

396   *Microspectra* sp. towards an assemblage dominated by *Chironomus* sp..

397
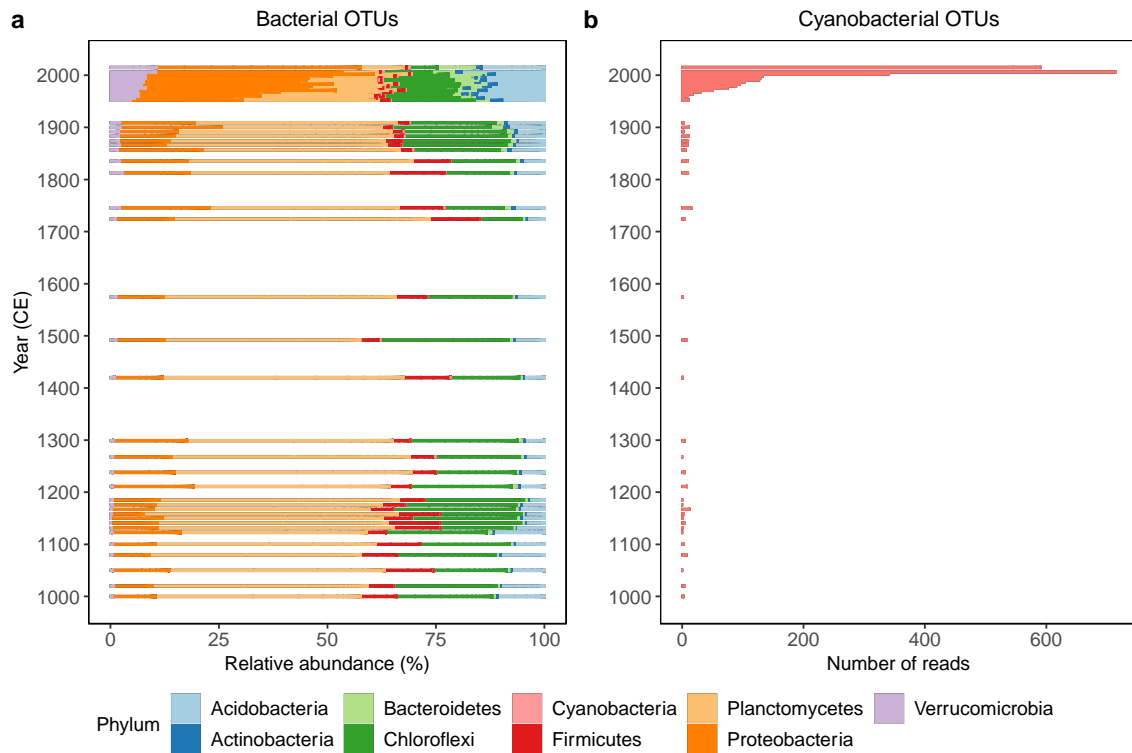
Figure 3. Downcore profiles showing a) the relative percentage of sequences assigned to the main bacterial phyla, and b) the abundance of cyanobacterial sequences recovered from the JOU17-03 sediments core (both plots are based on rarefied read counts to account for sequencing depth).

402

Figure 4. Stratigraphy of chironomids in the JOU16-02 sediment core expressed as percentage over total abundance. The horizontal dashed line identifies the significant transition (1953 – 1959) in community composition identified by CONISS.

4.1.4. Cladocerans (littoral and pelagic communities)

407   Twenty-eight Cladocera species were identifiable in the sediments of Lake Joux. For

408   simplicity, we discuss the dynamics of the eight main taxa identified. The overall temporal
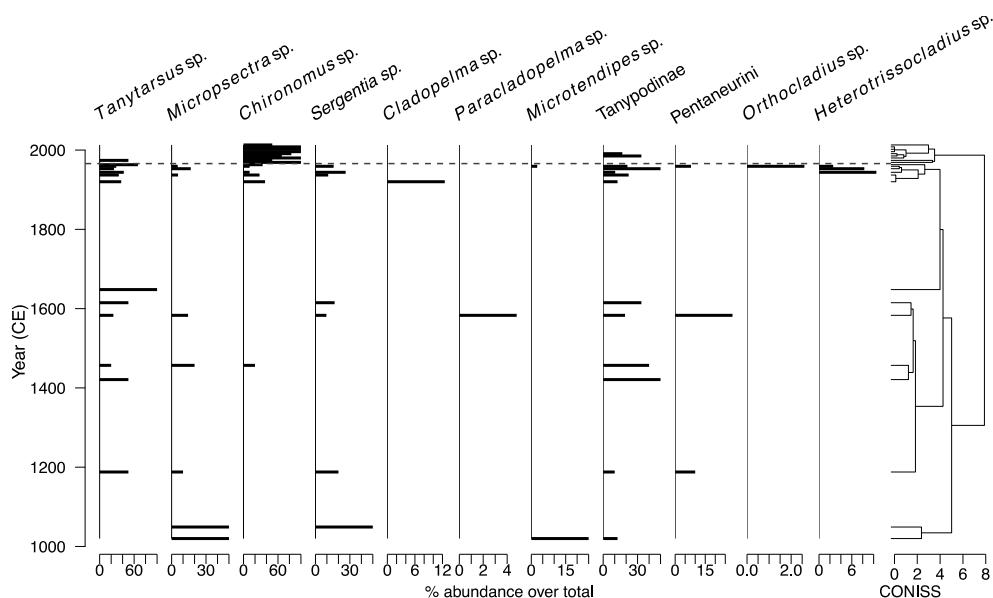
409   trends in the cladoceran assemblage resembled the trajectories observed in chironomids. A

410   single significant shift in cladocerans was revealed by the change-point analysis at the same

411   time as chironomids (i.e., between 1953 and 1959; Figure 5). Earlier changes in species

412   composition were visible, however throughout the core. Between ca. 1016 and 1118 CE (61

413   – 68 cm depth), the pelagic *Eubosmina longispina* and two littoral species, *Sida crystallina*

414   and *Rhynchotalona falcata* (Chydorids) were abundant. Between ca. 1187 and 1950 CE,

415   *Daphnia longispina* replaced *E. longispina* in the pelagic compartment. Later, during the

416   height of eutrophication between 1958 and 1980, the littoral species *Bosmina longirostris*

417   was present in higher abundance and *Eubosmina coregoni* appeared for the first time. The

418   high abundance of *E. coregoni* correlated inversely with the abundance of *Eurycercus* spp.

419   (not shown). *Eurycercus* spp. are littoral species capable of migrating within sediments due

420   to vulnerability and pressure associated with fish predation (Beklioglu and Jeppesen, 1999).

421   We also observed a slight decrease in *Daphnia* size, from ~170 µm to ~140 µm (non-

422   significant; not shown) which is stable from 10 cm-depth (early 1960s) to the core surface.

423   Finally, in the most recent time period (~1980 – 2015), *Bythotrephes longimanus*, a large

424   predatory pelagic species that was found throughout the sediment core was replaced by

425   *Leptodora kindtii*, which played a similar ecological role (predatory and pelagic), but that

426   does better in warmer (Palmer et al., 2001) and more hypoxic (Sorensen and Branstrator,
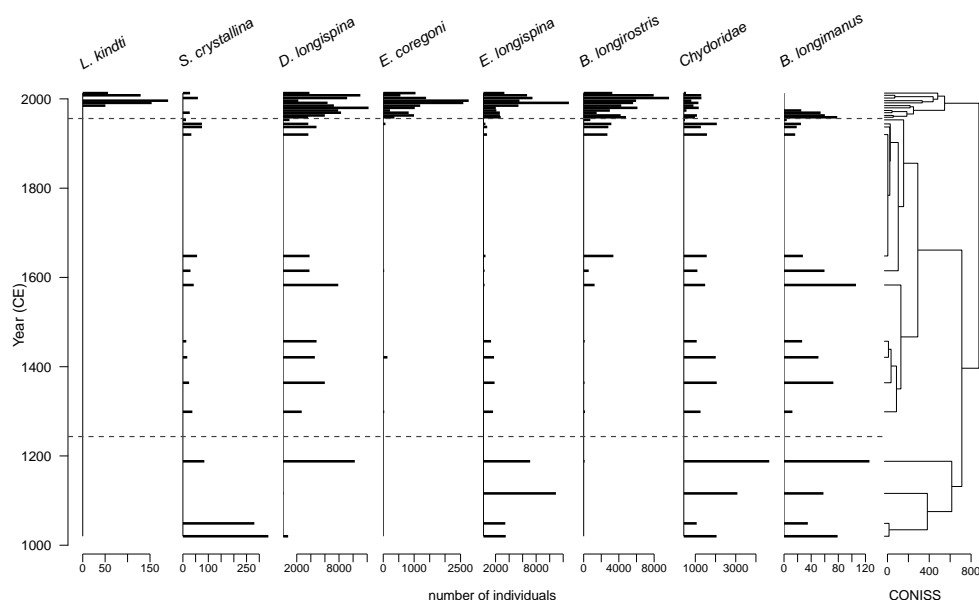
427   2017) waters.

428

429 Figure 5. Stratigraphic plot of the main cladocerans observed in the JOU16-02 sediment

430 core. Abundance was expressed as number of individuals per gram of organic matter. The

431 uppermost dashed line marks the only statistically significant transition in cladoceran

432 assemblages as identified by the change-point analysis and CONISS (transition 1953 –

433 1959). The earlier transition (~1188 – 1299 CE; not significant) is shown for reference.

434
435   *4.1.5. Fish*

436 Fish remains were generally low in abundance, although an increasing trend was observable

437 towards the upper portion of the sediment core (except for the top-most interval between 7.5

438 and 2.5 cm-depth (~1987- 2008)). The large majority of the remains were percid scales (n =

439 73; Table S1). Only two non-percid scales and three bones were found but could not be

440 identified further.

441 4.2. Regime shift

442 Because dominant modifications to the lake biota were only detectable from the mid 20th

443 century, changes in total phosphorus (TP) appear as the dominant structuring force over the

444 study period. To verify this, we extracted the scores of the main PCA axis (which explained

445 between 28 and 41% of the variance in community composition) and plotted them against a

446 time-series of TP concentrations. The results suggested a relationship between TP and the

447   composition and abundance of pigments, cladocerans, and chironomids that varies through

448   time (Figure 6). When the temporal trajectory of individual proxies (i.e., each taxon or

449   individual pigment concentration) was plotted against TP (Figure S3, S5 and S6), similar

450   general trends were observed in the majority of cladocerans, chironomids, and pigments,

451   supporting the relationship between TP and species turnover in the PCA.

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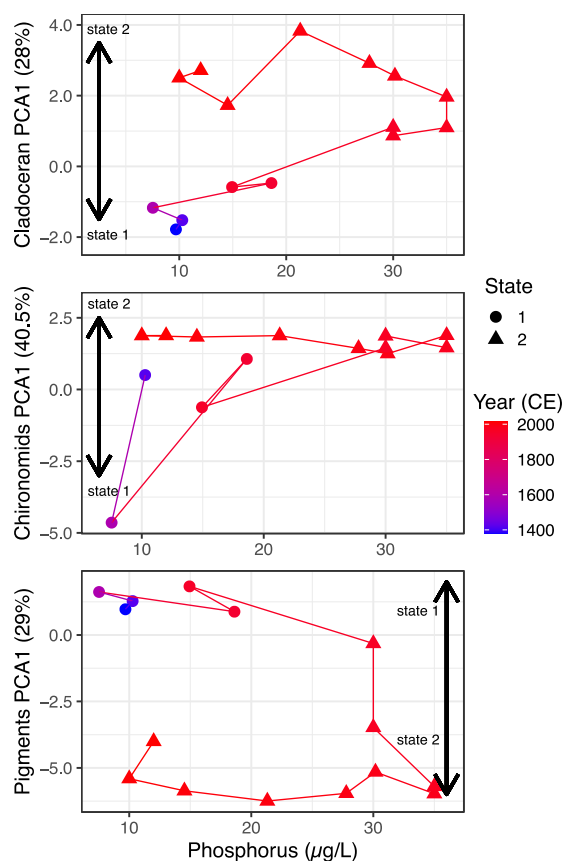
453   Figure 6. Temporal trajectories of PCA scores for Cladocerans, Chironomids, and pigments

454   (axis 1) plotted against a gradient of total phosphorus concentrations. Arrows indicate the

455   direction of the transition from ecological state 1 (baseline, pre-eutrophication; circles) to

456   state 2 (eutrophication and re-oligotrophication phases; triangles).

457   Applying time-varying autoregression using Online Dynamic Linear Models (DLMs) to

458   the time-series of echinenone pigment concentration provided the best indicator for the

459   presence and the timing of regime shifts and critical transitions in Lake Joux over the last ca.

460   1000 years. The sharp increase in the intercept (Figure 7) identified a recent regime shift

461   during 1963 to 1969 CE. This observation was further supported by the abrupt changes

462 notable in the pigment composition (Figure 2) and the turnover of cladocerans and

463 chironomids (Figures 4 and 5). The pronounced increase in eigenvalue (eigenvalue > 1;

464 Figure 7, bottom panel) provides evidence of critical transition.

465
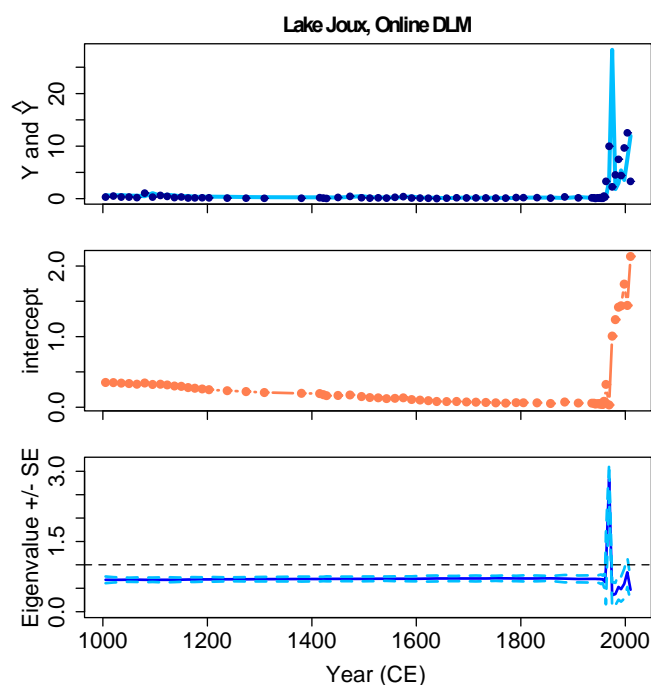
466 Figure 7. Main results of the online dynamic linear model (DLM) for Lake Joux echinenone

467 pigment (n = 68). In the top plot, the time series is shown in dark blue dots and the goodness

468 of fit is the clear blue line. The middle plot shows the intercept in orange dots, and the bottom

469 plot shows the eigenvalues (blue line) and standard deviation dotted light blue line.

470

471 **5. Discussion**

472 5.1. Dynamics of the transition and regime shift

473 The paleolimnological data from this study provided distinctive biostratigraphic signatures

474 that suggests resilience of the lake over several hundreds of years followed by an

475 ecosystem-level response to environmental forcing (González Sagrario et al., 2020). These

476 changes are consistent with a possible transition from the Holocene to a proposed

477 Anthropocene, with a stratigraphic signal around the mid-20th century of the Common Era.

478 This transition marks an intensification of human impacts causing unprecedented changes to

479   the Earth system (Syvitski et al., 2020; Zalasiewicz et al., 2020). Earlier disturbances can

480   certainly have an impact, but they are usually more limited and potentially reversible. Mid-20th

481   century eutrophication surpassed all other disturbances observed in the catchment of large

482   lakes in the perialpine area (Tolotti et al., 2018). Loss of resilience caused by long-term

483   perturbation, however, may increase vulnerability to further stressors such as eutrophication

484   (Bruel et al., 2021; Scheffer et al., 2001). Multiple perturbations of Lake Joux since the

485   settlement of the first humans in the catchment (Figure 1b) may have contributed to increase

486   the lake vulnerability to eutrophication leading to a regime shift.

487   Despite decades of re-oligotrophication leading to a return to pre-eutrophication total

488   phosphorus concentrations (11 – 13 µg/L; Lods-Crozet *et al.* (2006)), Lake Joux has not

489   returned to its pre-disturbance ecological state. As such, across the same concentrations of

490   total phosphorus, biological assemblages in Lake Joux exist in two states: pre-eutrophication

491   and re-oligotrophication states (Figures 6 and 7). Although this study was not designed to

492   test whether the permanence of the observed modifications to Lake Joux's communities, no

493   clear indications was apparent whether the lake might return to its previous configuration in

494   the near future. However, the trajectory of chironomids estimated, based on PCA, suggests

495   that the recent zoo-benthos configuration was more similar to some of the pre-eutrophication

496   assemblages compared to the communities at the height of eutrophication. Confounding

497   effects of additional environmental disturbances, including climate change, might explain the

498   delay in water quality recovery despite reduced nutrient loadings in Lake Joux since the

499   1980s (Bruel et al., 2018; Perga et al., 2015). Enhanced warming of surface waters has been

500   observed in perialpine lakes and elsewhere (Livingstone, 2003; Woolway et al., 2019, 2017).

501   Warming has direct and indirect effects on lake processes (e.g., stratification length and

502   strength, hypoxia, phosphorus release from sediment, calcium carbonate precipitation) and

503   in turn, these alterations to the physical and chemical structure of lakes have consequences

504   on the lake ecology (phenology or species life history traits).

505   5.2. Biodiversity change

506   Alterations to Lake Joux physical and chemical characteristics due to eutrophication

507   are visible through biotic changes at multiple trophic levels. Sedimentary pigments and

508   bacterial *sed*DNA analyses revealed an increase in primary productivity during the

509   eutrophication period (~1950 – 1980), leading to a phase of reduced water clarity. The

510   decline in the UV-Index (Figure 2) with the onset of eutrophication may be a response to

511   higher phytoplankton biomass, which creates shade for organisms that live deeper in the

512   water column, such as photosynthetic bacteria (Lami et al., 1991; McGowan, 2017). This

513   change in the lake's ecology also contributed greatly to modify the composition of primary

514   producers. A major change observed in phytoplankton assemblage was the establishment of

515   the invasive cyanobacterium *Planktothrix rubescens*, as conveyed by monitoring data and

516   supported by the analysis of pigments. *P. rubescens* often forms metalimnetic (deep)

517   blooms, which firstly may be undetected by some monitoring programmes. Metalimnetic

518   phytoplankton are generally more readily incorporated into the sediment record intact,

519   however, as they have smaller distances to travel when sinking and are less exposed to

520   photodegradation (Cuddington and Leavitt, 1999). The presence of *P. rubescens* in Lake

521   Joux was first reported in 1972 (Bosset, 1981) and since 1979, local environmental agencies

522   have regularly reported high biomasses of this filamentous cyanobacterium. *Planktothrix*

523   (both *P. agardhii* and *P. rubescens*) remained the dominant phytoplankton in the lake over

524   the following decades despite the ongoing reduction of pollution by phosphorus (Lods-crozet

525   *et al.*, 2006 and references therein). *P. rubescens* is a potentially toxic cyanobacterium that

526   has recently widened its geographical distribution in the perialpine area (Ernst et al., 2009;

527   Jacquet et al., 2005; Monchamp et al., 2018), posing a threat to water quality in several

528   lakes. It thrives in deep and stratified lakes with moderate light and moderate nutrient

529   availability (Dokulil and Teubner, 2000). *P. rubescens* is also more suited than other

530   phytoplankton to the warmer conditions and the current meso- to oligo- trophic state

531   characterizing Lake Joux (Lods-Crozet et al., 2006), as well as, other lakes in the region

532   (Monchamp et al., 2019b, 2018; Posch et al., 2012).

533   DNA metabarcoding can provide estimates of the proportion of bacterial taxa within

534   communities, which enables detection of species turnover and investigation of community

535   responses to environmental gradients (e.g., Bálint et al., 2018; Stoeck et al., 2018). The time

536   of the drastic increase in the proportion of cyanobacteria agrees with the sedimentary

537   pigments signal and is further validated by lake monitoring data (Lods-Crozet et al., 2006).

538   This study did not detect other drastic turnover in the rest of the bacterial assemblage over

539   the period of high disturbance, but rather gradual changes in the proportion of the main phyla

540   (e.g., Planctomycetes and Proteobacteria; Figure 3). The increase in relative abundance of

541   Verrucomicrobia, Protebacteria and Bacteroidetes indicates a community better adapted to

542   eutrophic conditions (Huang et al., 2017). The concomitant decrease in the proportion of

543   Chloroflexi, many of which generally thrive in the oxygenated hypolimnion of deep lakes

544   (Okazaki et al., 2018), agrees with the more hypoxic conditions that prevail in the bottom

545   waters of Lake Joux.

546   Modifications in the zoobenthos assemblage (Figure 4) also reflect warmer and more

547   eutrophic conditions reported in Lake Joux over the second half of the 20th century. Around

548   1953 – 1959, the community shifted from a taxa adapted to cold and well-oxygenated waters

549   towards an assemblage dominated by *Chironomus sp.*, which is tolerant of warmer waters

550   and possibly hypoxic conditions. Our findings corroborate previous reports of the presence of

551   oligochaete worms and chironomid larvae at the lake bottom after the onset of eutrophication

552   (Lang and Reymond, 1996, 1993).

553   In the cladoceran assemblage (Figure 5), the replacement of *Eubosmina longispina*

554   by *Daphnia longispina* in the pelagic compartment around 1250 CE may indicate a period of

555   slight eutrophication, as *D. longispina* has higher stoichiometric requirements (Elser et al.,

556   2001). More recently, the replacement in the pelagic compartment of *Bythotrephes*

557   *longismanus* by the heat-tolerant *Leptodora kindtii* towards the end of the 1970s – early

558   1980s concurred with enhanced warming of surface waters.

559   Given the generally low abundance of fish scales and bones in the sediment, and the

560   coarse taxonomic resolution achieved (Table S1), these data were, however, not sufficient to

561   infer the dates of introduction of species, or changes in their abundance over time. Records

562   of fish catches showed that whitefish was most abundant between 1959 and 1998, while

563   perch comprised a maximum of 15 % of catches during this period (Naceur and Büttiker,

564   1999). We report a drastic decrease in perch populations between 1971 and 1984 as well as

565   decrease in average productivity of whitefish since 1987 in Lake Joux. This overall decline in

566   whitefish productivity has been hypothetically linked to the regular development of high

567   biomass of the cyanobacterium *Planktothrix rubescens* (previously *Oscillatoria rubescens*)

568   since the end of the 1970s (Naceur and Büttiker, 1999). To our knowledge, however,

569   validation of this hypothesis has not yet occurred. Other factors affecting whitefish

570   productivity, and that have been observed in other lakes of the region, include intraspecific

571   competition and the timing and duration of the lake thermal stratification (Eckmann et al.,

572   1988). The reduction in *Daphnia* size (not shown), often used as a proxy for high fish

573   predation pressure, as well as the sudden increase in abundance of the small *Eubosmina*

574   *coregoni* during the eutrophication phase also concur with increased fish predation. *E.*

575   *coregoni* is also tolerant to the presence of filamentous cyanobacteria, which gives this taxa

576   a competitive advantage (Mayer et al., 1997). Interestingly, the main change-point in

577   cladoceran and chironomid assemblages were detected slightly earlier (~1953 – 1959) than

578   the transition in phytoplankton (1963 – 1969). Although a first change was detectable in

579   pigment composition between 1959 and 1961, the community shift towards the second

580   ecological state (post-eutrophication) were apparently more gradual and slightly delayed

581   compared to zooplankton and micro-zoobenthos. Because the time series of pigments,

582   cladocerans, and chironomids were all derived from the same sediment core (JOU16-02), we

583   can rule out discrepancies in timing due to age model correlation. Our observation is

584   consistent with other studies in perialpine lakes, where cladocerans systematically showed

585   an early response to increased total phosphorus before diatoms signaled any change (Alric

586   et al., 2013; Bruel et al., 2018). Cladocerans are highly responsive to climate, nutrient

587   availability as well as fish predation pressure. In the case of Lake Joux, cladocerans have

588   been simultaneously exposed to both top-down (fish predation) and bottom-up (nutrients)

589   processes in addition to warming, and the accumulation of external stressors may have led

590   to a stronger and potentially early response. Similarly, chironomids are extremely sensitive to

591   environmental factors, including oxygen concentration and temperature (Walker, 2001),

592   making them good indicators of pollution and climate change.

593   5.3. Methodological aspects

594   A strength of this study lies in the combination of multiple paleolimnological and *sed*DNA-

595   based proxies that allows investigation of temporal dynamics of the ecosystem as a whole,

596   and cross-validation of multiple markers. Congruent patterns through time and across

597   proxies increase the robustness of paleo-reconstructions. Previous studies have described

598   methodological concerns related to *sed*DNA metabarcoding (Pedersen et al., 2015; Ruppert

599   et al., 2019). A main concern in *sed*DNA studies is the degradation and the loss of DNA

600   molecules over time. Although DNA has been shown to preserve over several millennia in

601   sediments (Pedersen et al., 2015), some limitations due to taphonomic processes after burial

602   have been identified (Gilbert et al., 2005). Deep cold lakes with anoxic bottoms, prevalent in

603   perialpine lakes, provide excellent conditions for DNA preservation (Boere et al., 2011;

604   Coolen et al., 2004). To reduce potential biases related to DNA degradation, we targeted a

605   short gene fragment and used technical replicates. In addition, we visually inspected the

606   sequence data for patterns that might point towards temporal *sed*DNA degradation. For

607   example, a sudden disappearance of specific taxa or a loss of the molecular signal in older

608   sediment layers could indicate DNA damage or degradation. Sedimentary pigments are also

609   prone to degradation over time, which can bias the ecological signal. The pheophytin

610   a/chlorophyll a ratio in Lake Joux sediments indicated a recent increase in the preservation

611   since ~1980 CE, however, which is at least two decades after the general increase in

612   pigment concentration is measured in both the sedimentary pigments and DNA. Finally, our

613   multi-proxy survey allowed cross-validation of the sequencing and the sedimentary pigment

614   data in corresponding sediment layers.

615   Despite the success of using fish remains from marine and freshwater sediments to

616   infer changes in ecosystem state or community composition (e.g., Davidson et al., 2003;

617   Dieleman et al., 2019; O'Connell and Tunnicliffe, 2001), many confounding factors are still

618   poorly understood. For example, species with more strongly mineralized, and therefore better

619   preserving, bones and scales might be relatively overrepresented in the subfossil record

620   (Kalvoda et al., 2009). Additionally, perch, roach and dace (*Leuciscus leuciscus*) were

621   reported in higher densities in the littoral zone of Lake Joux, in contrast to whitefish, which

622   was found to be most abundant in the pelagic zone (Périat and Vonlanthen, 2015). This

623   might partially explain the general scarcity of fish remains in Lake Joux pelagic sediments

624   despite a recent comprehensive census (Périat and Vonlanthen, 2015) illustrating the

625   predominant presence of perch and reporting both roach and dace as common in Lake

626   Joux. Future studies that reconstruct fish diversity through time using lake sediments could

627   improve the approach by screening larger volumes of sediment, ideally also including core

628   sites representing more littoral communities. Extending the *sed*DNA approach to fishes could

629   also increase detection sensitivity and chronological resolution, especially for poorly

630   preserving species.

631   **6. Conclusions**

632   The analyses presented above provide answers to the three research questions asked in this

633   paper. First, our unique multi-level paleoreconstruction dataset compiles important biological

634   change over the last 1000 years in Lake Joux and reveals enhanced human impact on the

635   ecosystem over the second half of the 20th century, mainly linked to cultural eutrophication.

636   Consequently, there was a rapid turnover in community composition in phytoplankton,

637   Cladocera, and chironomid assemblages, with taxa adapted to eutrophic waters becoming

638   dominant over original species configuration. Second, the PCA suggests that the major

639   change in nutrient regime led to the regime shift (~1963-1969) and apparent bi-stability

640   between two states, as also conveyed by the dynamic lineal model. Lastly, the results further

641   suggest that the reconfiguration in Lake Joux is not restricted to some biological

642   compartments, but rather spreads across trophic levels, at least partially. The lack of a

643   complete fish dataset does not allow conclusions on the extent of the regime shift at the top

644   of the food chain (i.e., in fish communities), but the concomitant changes reported at all other

645   trophic levels investigated provide evidence for reorganization of the lake's ecology at

646   multiple levels (both in primary producers and primary consumers).

647   Although earlier disturbances might have increased vulnerability of the lake to

648   eutrophication, the rapid increase in phosphorus concentrations, triggered the regime shift at

649   a moderate concentration (~20 – 35 µg/L) of total phosphorus. The fact that the lake has not

650   returned to its pre-eutrophication state in over >70 years of re-oligotrophication leads to the

651   conclusion that the lake has shifted to an alternative stable state and its biological

652   configuration is not easily reversible. In the case of Lake Joux, this might mean recurring

653   cyanobacterial blooms in the future. More generally, the shift to an alternative stable state

654   typically has major consequences on water quality and ecosystem services that humans

655   derive from lakes. This study adds to the body of evidence suggesting that a key aspect to

656   effective management strategies is maintaining resilience of ecosystems in their desired

657   state. To achieve this, a need exists to improve understanding of the underlying mechanisms

658   of critical transitions that may reveal early-warning signals through paleoecological records.

659   Ecosystem-wide paleoecological approaches, demonstrated in this study, provide a wealth of

660   biological and genetic information that can significantly improve ability to quantify the rate

661   and amplitude of change in ecosystems under anthropogenic forcing.

662   **Data availability**

663   The sequence data produced in this study have been deposited in the European Nucleotide

664   Archive (ENA) with the study accession number PRJEB36317.

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Supplementary information

Paleoecological evidence for a multi-trophic regime shift in a perialpine lake (Lake Joux, Switzerland)

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Supplementary Table

Table S1. Abundance of fish subfossils found in the sediment cores JOU17-1 and JOU17-2.

| core	interval_top [cm]	interval_bottom [cm]	perch_scales	non_perch_scales	fish_bones
	0	2.5	12	0	2
	2.5	5	17	1	0
	5	7.5	16	0	0
	7.5	10	5	0	0
	10	12.5	1	1	1
	12.5	15	3	0	0
	15	17.5	0	0	0
	17.5	20	0	0	0
	20	22.5	0	0	0
	22.5	25	0	0	0
	25	27.5	0	0	0
	27.5	30	0	0	0
	30	32.5	0	0	0
	32.5	35	0	0	0
	35	37.5	0	0	0
JOU_17_1	37.5	40	0	0	0
	40	42.5	0	0	0
	42.5	45	0	0	0
	45	47.5	0	0	0
	47.5	50	0	0	0
	50	52.5	0	0	0
	52.5	55	0	0	0
	55	57.5	0	0	0
	57.5	60	0	0	0
	60	62.5	0	0	0
	62.5	65	0	0	0
	65	67.5	0	0	0
	67.5	70	0	0	0
	70	72.5	0	0	0
	72.5	75	0	0	0
	75	77.5	0	0	0
	0	2.5	0	0	0
	2.5	5	10	0	0
	5	7.5	6	0	0
	7.5	10	2	0	0
	10	12.5	1	0	0
	12.5	15	0	0	0
	15	17.5	0	0	0
	17.5	20	0	0	0
	20	22.5	0	0	0
	22.5	25	0	0	0
	25	27.5	0	0	0
	27.5	30	0	0	0
	30	32.5	0	0	0
	32.5	35	0	0	0
	35	37.5	0	0	0
JOU_17_2	37.5	40	0	0	0
	40	42.5	0	0	0
	42.5	45	0	0	0
	45	47.5	0	0	0
	47.5	50	0	0	0
	50	52.5	0	0	0
	52.5	55	0	0	0
	55	57.5	0	0	0
	57.5	60	0	0	0
	60	62.5	0	0	0
	62.5	65	0	0	0
	65	67.5	0	0	0
	67.5	70	0	0	0
	70	72.5	0	0	0
	72.5	75	0	0	0
	75	77.5	0	0	0
	77.5	80	0	0	0

Supplementary figures
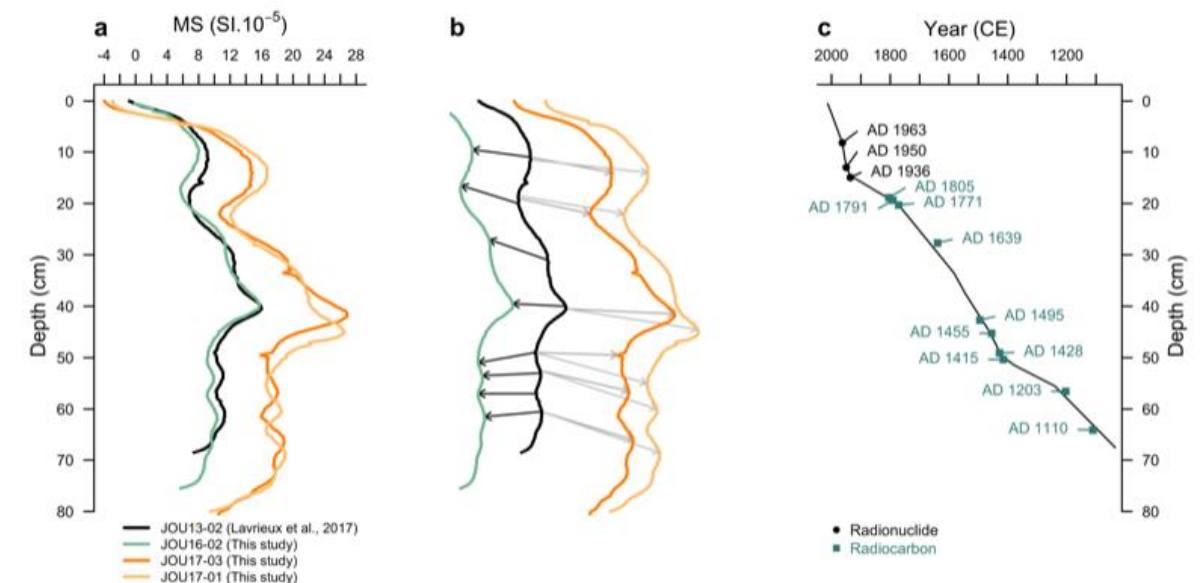
Figure S1. (a) Magnetic Susceptibility (MS) of the reference core JOU13-02 (Lavrieux et al., 2017) and the cores JOU16-02, JOU17-01, and JOU17-03 (this study). (b) Correlation between remarkable points. (c) Age-depth model for JOU16-02 derived from the correlation of MS measurements with the dated reference core. Differences in MS values between cores are explained by errors in the calibration.
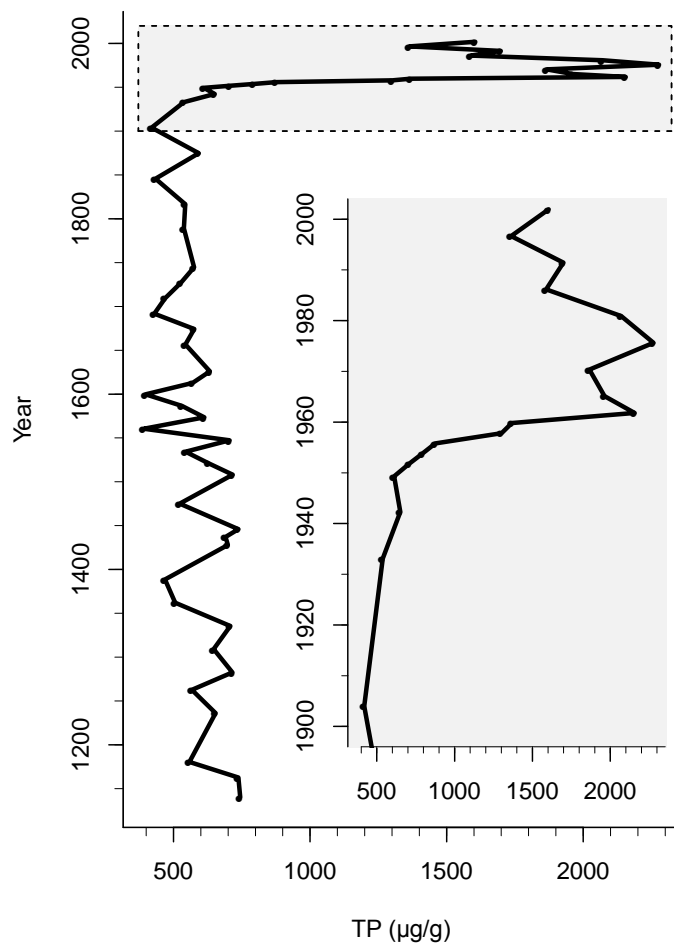
Figure S2. Time-series of total phosphorus (TP) concentrations (μg/g dry sediment) measured in the JOU13-02 sediment core. These are corrected values from Lavrieux et al. (2017), as we identified errors in the original sedimentary TP concentrations. A close-up of the recent eutrophication and re-oligotrophication phases (in the dashed grey box) is shown.
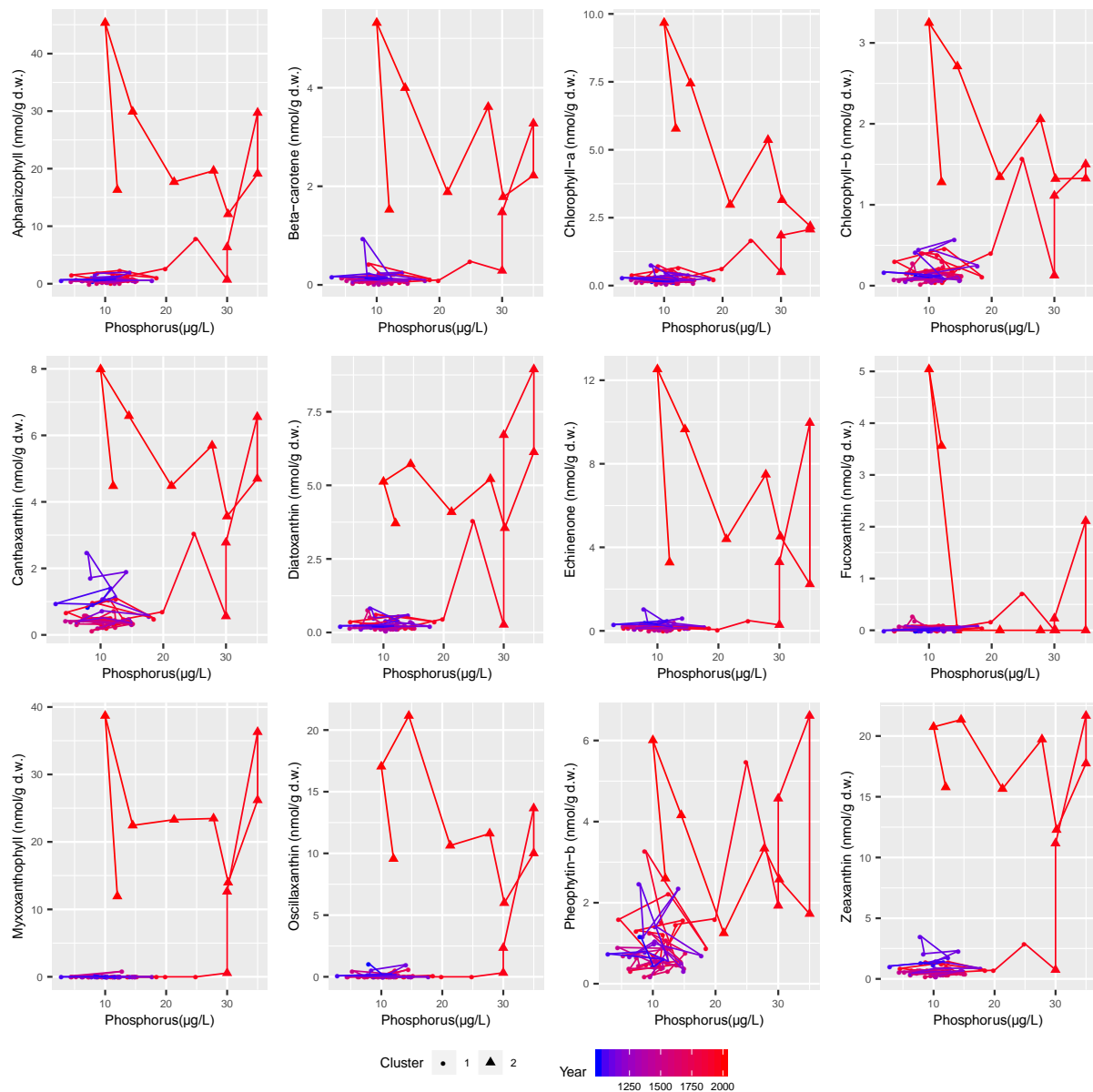
Figure S3. Temporal trajectory of the main photosynthetic pigments plotted against total phosphorus (TP) concentrations. A general increase in pigment concentration is observed when phosphorus concentration reaches between 20 and 30 µg/L. TP concentrations reflect the major eutrophication event beginning in the mid-1900s and the re-oligotrophication phase from the end of the 1980s in Lake Joux. For similar TP concentrations, the recent pigment concentrations (re-oligotrophication phase) are generally higher compared to the pre-eutrophication period (before the mid-1950s), indicating that the recent reduction of TP input into the lake has not led to a return to pre-disturbance conditions.
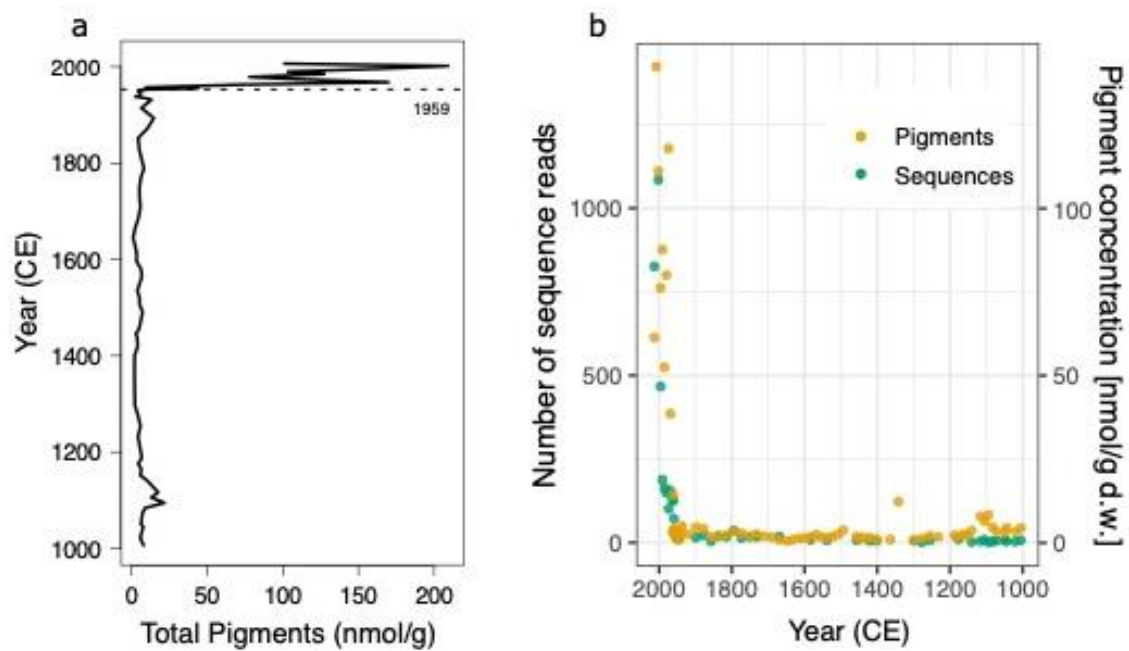
Figure S4. a) Total sedimentary pigment concentration in the JOU16-02 core plotted against time. The dashed line marks the beginning of the rapid increase in the overall pigment concentration around 1959 CE (±3 years). b) Abundance plot of cyanobacterial pigments and cyanobacterial sequence reads over sediment depth. Both sedimentary pigments (yellow) and sequencing (green) datasets reveal an abrupt increase in the cyanobacterial abundance at ca. 12 cm depth downcore, which corresponds to 1954 – 1959.
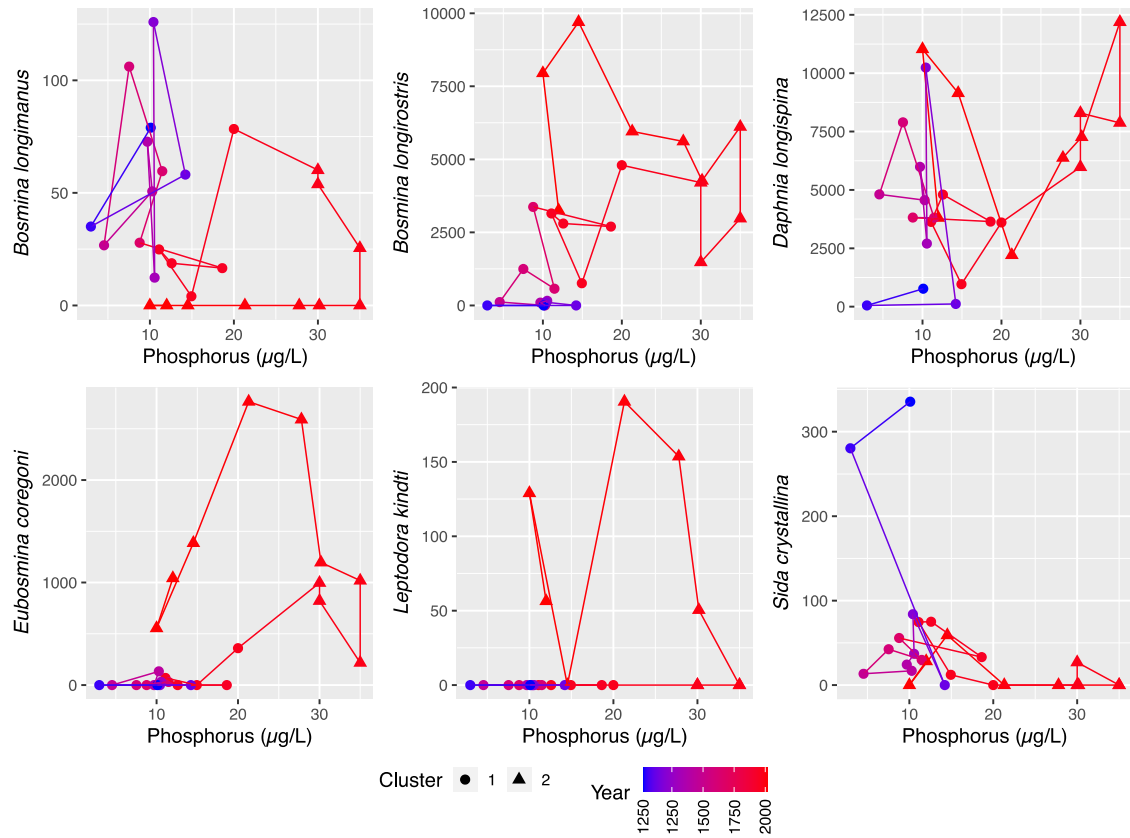
Figure S5. Temporal trajectory of the main Cladoceran taxa (number of individuals) plotted against water total phosphorus concentrations.
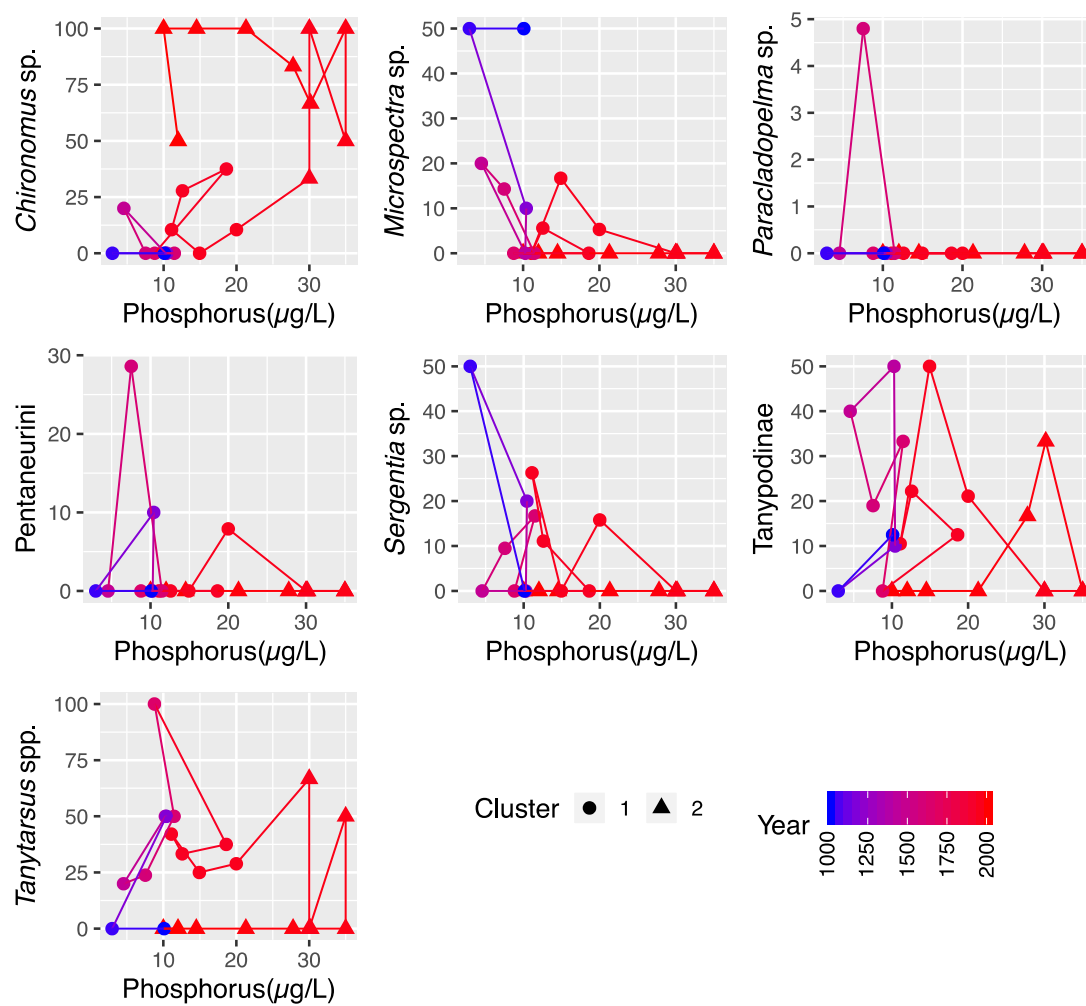
Figure S6. Temporal trajectory of the main Chironomid taxa (percentage over total abundance) plotted against water total phosphorus concentrations.