Homogenization of lake cyanobacterial communities over a century of climate change and eutrophication

Marie-Eve Monchamp^{1,2*} me.monchamp@gmail.com, Piet Spaak^{1,2}, Isabelle Domaizon³,
Nathalie Dubois^{4,5}, Damien Bouffard⁶, Francesco Pomati^{1,2*} francesco.pomati@eawag.ch

¹ Eawag, Swiss Federal Institute of Aquatic Science and Technology, Department of Aquatic Ecology, 8600 Dübendorf, Switzerland;

² Swiss Federal Institute of Technology (ETH) Zürich, Institute of Integrative Biology, 8092 Zürich Switzerland;

³ INRA, Université de Savoie Mont Blanc, UMR CARRETEL, 74200, Thonon-les-bains, France;

⁴ Eawag, Swiss Federal Institute of Aquatic Science and Technology, Department of Surface Waters Research & Management, 8600 Dübendorf, Switzerland;

⁵ Swiss Federal Institute of Technology (ETH) Zürich, Department of Earth Sciences, 8092 Zürich, Switzerland;

⁶ Eawag, Swiss Federal Institute of Aquatic Science and Technology, Department of Surface Waters Research & Management, 6047 Kastanienbaum, Switzerland

* Corresponding authors:

Mailing address: Eawag, Department of Aquatic Ecology, Überlandstrasse 133, 8600 Dübendorf, Switzerland.

Phone: +41 58 765 5410

This document is the accepted manuscript version of the following article:
Monchamp, M. E., Spaak, P., Domaizon, I., Dubois, N., Bouffard, D., & Pomati, F.
(2018). Homogenization of lake cyanobacterial communities over a century of
climate change and eutrophication. *Nature Ecology & Evolution*, 2, 317-324.
<https://doi.org/10.1038/s41559-017-0407-0>

24 **Abstract**

25 Human impacts on biodiversity are well recognized, but uncertainties remain regarding
26 patterns of diversity change at different spatial and temporal scales. Changes in microbial
27 assemblages are, in particular, not well understood, partly due to the lack of community
28 composition data over relevant scales of space and time. Here, we investigated biodiversity
29 patterns in cyanobacterial assemblages over a century of eutrophication and climate change
30 by sequencing DNA preserved in the sediments of 10 European peri-Alpine lakes. We found
31 species losses and gains at the lake scale, while species richness increased at the regional
32 scale over the past ~100 years. Our data show a clear signal for beta diversity loss, with the
33 composition and phylogenetic structure of assemblages becoming more similar across sites in
34 the most recent decades, as have the general environmental conditions in and around the
35 lakes. We attribute patterns of change in community composition to raised temperatures
36 affecting the strength of the thermal stratification and, as a consequence, nutrient fluctuations,
37 which favoured cyanobacterial taxa able to regulate buoyancy. Our results reinforce previous
38 reports of human-induced homogenization of natural communities, and reveal how potentially
39 toxic and bloom-forming cyanobacteria have widened their geographic distribution in the
40 European temperate region.

41

42 **Main text**

43 Anthropogenic-induced alterations of ecosystems have contributed to reshaping biodiversity
44 of natural communities globally ^{1,2}. Patterns of responses in local and regional biodiversity are
45 regulated by mechanisms acting at different spatial and temporal scales ³. Recent controversial
46 debates on the accuracy of meta-analyses reporting biodiversity patterns have highlighted the
47 sensitivity of the choice of the spatial and temporal scales, or geographical focus, for the final

assessment of biodiversity loss or gain ⁴⁻⁶. However, beta diversity loss over space or time (i.e. previously differentiated assemblages increasingly resembling one another) has been consistently reported ^{4,7,8}, supporting the hypothesis that human impacts can reduce environmental heterogeneity and thus increase homogenization of natural communities ^{8,9}, with potential impacts on ecosystems functioning and services ¹⁰. Yet, this hypothesis remains untested in aquatic microbial assemblages, particularly photosynthetic microorganisms, whose biodiversity patterns are mostly unknown at large spatial and temporal scales.

Aquatic ecosystems are among the most sensitive to anthropogenic activities and biodiversity loss ¹¹. In lakes, combined warming and eutrophication (i.e. pollution caused by excessive discharge of nutrients) have favoured the dominance of cyanobacteria over eukaryotic phytoplankton ^{12,13} due to eco-physiological adaptations, such as buoyancy regulation, fast growth rate, and adaptation to low light levels ^{14,15}. Cyanobacterial blooms create unfavourable conditions for other phytoplankton (e.g. low light conditions), as well as for organisms of higher trophic levels such as zooplankton and fish (e.g. anoxia) ^{12,16}. Over the past few decades, the frequency and severity of cyanobacteria-dominated communities have increased in lakes and reservoirs worldwide ¹⁷ despite remediation measures applied at the regional and international scale ^{14,18}. Cyanobacterial blooms are often dominated by toxic species, and there is a global concern that climate change is promoting the geographic expansion of some potentially harmful taxa ¹⁹⁻²¹ enhancing their dominance due to a combined effect of increasing nutrient load ^{14,15}.

The lack of long-term historical data (both biotic and abiotic), however, limits our understanding on how anthropogenic-induced changes have affected lake cyanobacterial taxa distribution and community composition over broad spatial and temporal scales. Studies in European peri-Alpine lakes have reported increasing occurrence of potentially harmful cyanobacteria such as *Planktothrix rubescens* ²² and *Dolichospermum lemmermannii* ²¹ over

the last few decades that could be attributed to changes in local nutrient concentrations and a warmer climate. Yet, the relative influence of these environmental factors on the structure (diversity and composition) of cyanobacterial communities remains unclear.

In this study, we reconstructed trends in cyanobacterial community structure across 10 lakes of the European peri-Alpine region (see the map in Supplementary Fig. 1), and related diversity and compositional patterns to known long-term environmental drivers such as temperature and nutrients (Fig. 1). The average annual air temperature in the region investigated has increased over the past ~150 years (Supplementary Fig. 2a), with an intensification of the trend since the 1980s. Increased air temperature raised surface lake water temperatures and led to a stronger (Fig. 1a, R^2 adj. = 0.1683, $p = 2.568 \times 10^{-14}$) and longer (Supplementary Fig. 2b) thermal stratification. Over the twentieth century, peri-Alpine lakes also underwent evident shifts in phosphorus and nitrogen levels (Fig. 1b-c). The severity of change in nutrient loads varied from lake to lake, but the main shifts occurred roughly simultaneously across the whole peri-Alpine region. Total phosphorus (TP) increased rapidly around the 1950s to reach a maximum in the late 1960s – early 1970s (Fig. 1b). Mitigation of phosphorus discharges into lakes facilitated a rapid decrease of this nutrient, leading to the return of most lakes to TP concentrations that are similar to pre-eutrophication. On the contrary, nitrogen inputs have not been controlled and the concentration of this nutrient has increased over the twentieth century^{23,24} (Fig. 1c).

Results & Discussion

We found that, along with climate change, eutrophication, and re-oligotrophication, the general environmental conditions across the study lakes have homogenized. The difference in mean annual air temperatures between the northern and the southern sides of the Alps decreased significantly over the last century (Fig. 1d). The annual mean TP concentrations across the lakes also became more uniform in the two most recent decades compared to the

range of concentrations measured in earlier times (i.e. between 1950 and 1999; Fig. 1e). TP concentrations at present are equivalent to mesotrophic to eutrophic conditions in most lakes (Supplementary Table 1, Fig. 1b and 1e). Conversely, the lakes display a wide range of NO₃-N concentrations, which have remained relatively constant since the maximum of eutrophication (Fig. 1f).

To study compositional and phylogenetic changes of cyanobacterial communities across lakes and over time, we used DNA extracted from sediment cores (sedDNA) collected in the 10 lakes (Methods). SedDNA has been successfully used to elucidate changes in freshwater pelagic community composition of various plankton forms²⁵, including cyanobacteria^{26–28}. We reconstructed patterns in richness and community similarity by amplifying and sequencing part of the cyanobacterial 16S rDNA from dated sediment layers, and studied the dynamics of operational taxonomic units (OTUs), which represent groups of sequences with a minimum similarity of 97%, within and between lakes (Methods). We have demonstrated previously that our reconstruction method in sedimentary archives provides an accurate description of cyanobacterial community composition, which is highly correlated to patterns obtained by historical microscopic observations of water samples²⁸.

We found that the cyanobacterial community composition varied over time and across lakes (ranging from 9 to 52 OTUs, average = 25 ± 10.5 OTUs per lake) (Fig. 2a). Individual lake change in OTU richness over time was generally insignificant (at the $p = 0.05$ level), with three exceptions: lakes Zurich, Lugano, and Hallwilersee that showed a significant increase. Overall, there was a significant temporal trend towards a slight increasing OTU richness across all lakes ($R^2 = 0.18$, $p = 0.000132$; $n = 76$; Fig. 2a). Species accumulation curves confirmed this trend across all lakes (Fig. 2b), suggesting a generalized gain of species at the peri-Alpine regional scale over the last century. A similar increase in taxa richness has been observed in meta-analysis studies across sites for communities that have been affected

by anthropogenic disturbance, particularly post-disturbance successional changes in community composition^{6,7,29}.

While reports of successful reconstructions of lake plankton community richness and composition based on relatively long DNA markers exist (e.g.^{30,31}), possible biases due to DNA degradation with sediment age are of concern in palaeo-genetic studies³². We carefully inspected the dataset for the identification of patterns suggesting preferential DNA degradation (e.g. disappearance of OTUs in specific clades with sediment age), and we did not detect any significant bias. For example, cyanobacteria forming dormant cells (akinetes) were not relatively more represented or more diverse in older layers compared to more recent sediments. Our reconstructed data compare well with historical records of several of these well-studied lakes²⁸, reporting, for example, the presence of the cyanobacterium *P. rubescens* in Lake Zurich, Baldeggersee, and Hallwilersee over the last two centuries^{33,34}. In Lake Zurich, Pomati *et al.* have also shown an increase in phytoplankton richness over the past 30 years possibly due to reduction in phosphorus inputs combined with climate warming-mediated enhancement of resource heterogeneity over the water column³⁵.

Temperature can have a direct metabolic effect on phytoplankton, but also indirect effects mediated by changes in the physical (strong thermal stratification leading to increased stability of the water column) and chemical lake state (nutrient recirculation due to mixing events). For example, the strength of thermal stratification in Lake Zurich has increased by ~20% over the last three decades due to warming, and this impaired the frequency and magnitude of lake annual mixing^{36,37}. Weaker mixing contributed to a reduction in the annual phytoplankton turnover in the latter lake³⁸ due to limited winter resetting of the phytoplankton community, and favoured the dominance of the harmful cyanobacterium *Planktothrix rubescens* (order Oscillatoriales)³⁶. We used linear mixed-effect models to study the potential environmental drivers of cyanobacterial OTU richness change in our dataset

(Methods). The normalized maximal annual Schmidt Stability Index (SSI) and average annual TP concentrations were the only two significant and interacting variables ($p = 0.002$, $n = 30$) predicting natural log-transformed OTU richness (marginal $R^2 = 0.614$; Fig. 2c). The fixed (SSI and TP) effects explained 61.4% of the variation in OTU richness, with no detectable additional effect of the random factor (lake). The remaining explanatory variables ($\text{NO}_3\text{-N}$ and pairwise interactions) were not statistically significant ($p > 0.2$). Human activities in and around lakes, such as recreational boating and fish stocking, have intensified over the last century and might have contributed to the dispersal of cyanobacterial taxa across lakes of the same region. However, the successful establishment of an invading population is obviously strongly dependent on environmental conditions^{39,40}.

The observed change in community structure co-occurred with an increase in the average similarity between lake communities (estimated at each decade based on unweighted Unifrac distance matrices – Methods) (Fig. 2d). This suggests a general homogenization of the phylogenetic community structure across lakes (i.e. loss of beta diversity), particularly over the few most recent decades. PERMANOVA (Methods) confirmed that the groups of communities sampled at each period have significantly different centroids ($p = 0.01$).

The proportion of weakly spatially distributed OTUs among lakes (“rare”; found in <25% of lakes at a given period) decreased over the last century. Conversely, the proportion of OTUs common in multiple lakes (found in >75% of lakes at a given period) increased about 4-fold between the 1950s (6%) and the 1990s (24%), after having remained relatively stable until the onset of lake re-oligotrophication in the late 1970s (Fig. 3). Our data suggest that changes in lake chemistry and physics might have promoted the dispersion of taxa that best fit the generalized contemporary environmental conditions.

The increase in similarity among cyanobacterial communities across lakes was in fact only driven by certain phylogenetic and taxonomic groups. Only three out of five

173 cyanobacterial orders showed an increase in richness over the past century: Chroococcales,
174 Nostocales, and Oscillatoriales (Fig. 4). This result indicates a higher gain in filamentous and
175 colonial taxa able to regulate buoyancy relative to other cyanobacteria across peri-Alpine
176 lakes. This observation is consistent with previous studies in lakes of the same region
177 reporting phytoplankton compositional shifts in favour of colony-forming taxa due to climate
178 warming and changes in nutrient concentrations^{27,36,41–43}. The ability to regulate cells
179 buoyancy allows Chroococcales, Nostocales and Oscillatoriales to float and sink periodically
180 to access light (at the surface) and nutrients (below the photic zone) during strong and
181 extended water stratification^{14,44}.

182 Taxa from the above orders, which comprise bloom forming and toxic organisms,
183 particularly species of the genus *Microcystis* (Chroococcales), *D. lemmermannii* (Nostocales),
184 and *P. rubescens* (Oscillatoriales), increased in prevalence and geographic range across all
185 lakes and over time (Fig. 5). Hierarchical clustering based on Euclidean distances revealed
186 five clusters of taxa with different temporal patterns. Cluster I comprises five OTUs assigned
187 to unicellular and non-bloom forming *Synechococcus* spp. (order Synechococcales) that were
188 highly prevalent over all the studied time period. Cluster II contains taxa that were relatively
189 rare (i.e. isolated in some lakes) until the 1990s when they became common in most lakes
190 investigated Cluster II includes genera from Chroococcales (such as *Microcystis*) and
191 Oscillatoriales (such as OTU #344, 100% similarity to *P. rubescens* previously isolated from
192 Lake Zurich⁴⁵). Cluster III, which contains about 43% of all OTUs identified in this study, is
193 characterized by rare OTUs that were never highly prevalent across the study lakes. Some of
194 these taxa were detected in a few lakes prior to the 1970s, and were not found in recent
195 decades. Cluster IV is a group of taxa with relatively low prevalence throughout the twentieth
196 century, but that are more common during the eutrophication peak. Finally, cluster V contains
197 OTUs that mostly increased in prevalence after the period of maximal eutrophication between

1950 and 1974, and that remained common thereafter. This cluster was characterized mostly by the presence of Chroococcales, Nostocales, and Oscillatoriales, including the genera *Microcystis* and the known invasive *Dolichospermum* (OTU #614; 99% similarity to *D. lemmermannii* LN871456) previously isolated from a southern peri-Alpine lake²¹. Overall, these results revealed long-term changes in prevalence of cyanobacterial taxa that were unknown to date across the region, and broadened our knowledge about dynamics of colony-forming cyanobacterial populations over recent decades^{20,21,46}.

Earlier studies have reported the increasing occurrence of blooms of *P. rubescens* in recently re-oligotrophied lakes around the European Alps²². The competitive advantage of this cyanobacterium is thought to rest in its adaptation to low light conditions, and its ability to migrate vertically over the water column to harvest nutrients⁴⁷. The invasion by *D. lemmermannii*, in several deep southern subalpine lakes was also recently reported^{20,21}. In our study, we found two *Dolichospermum* spp. in the sediments of several northern peri-Alpine lakes in the 1800s and during the twentieth century (Fig. 5). In the southern lakes, however, *D. spp.* were only detected after the 1980s. Interestingly, these OTUs were found in sediments of all lakes, except in the oligotrophic Lake Annecy, suggesting that *D. spp.* are able to colonize a wide range of lakes where N and P are not limiting.

The interaction between climate warming and nutrient fluctuations likely played an important role in our study system. It appears to have favoured taxa able to thrive in environments with a more stable water column and where limiting nutrients were on a declining trend (after they had been enriched). For instance, colony-forming and buoyancy-regulating species are favoured under such conditions¹⁵. Evidence in the literature supports the hypothesis that reduction in spatial environmental heterogeneity filters species traits and determines homogenization in the composition of natural communities across sites^{8,9}. Coloniality, by increasing the species realized size, is also a grazing-defence trait⁴⁸. We

cannot exclude that top-down control from changing zooplankton communities might have played a role in our detected trends in cyanobacterial community composition. Recent experimental evidence suggests that grazers (i.e. *Daphnia*) can significantly affect the local and regional diversity and community composition of phytoplankton⁴⁹. We have, however, no direct evidence from our data to test this hypothesis.

Our results reveal previously unreported patterns of taxa richness and community composition in cyanobacterial assemblages over spatial and temporal scales that are relevant to both lake ecology and environmental policy. We show prime evidence based on sedDNA that homogenization of natural communities has occurred in lake cyanobacterial assemblages during decades of climate change and eutrophication, and this process coincided with the spread of bloom forming and toxic cyanobacteria in the European peri-Alpine region^{21,22,36,46}. Our findings are relevant for a broad range of ecosystems under human-induced environmental pressure, as patterns of biotic homogenization similar to the ones reported here have been shown in marine fish communities, microbial decomposers, plants, and animals in grassland communities, with implications for local ecosystem services^{8,50}. The results presented here are spatial- and temporal-scale dependent, and more knowledge is necessary about the state and history of cyanobacterial diversity and community composition on a European or continental scale. Loss of spatial beta diversity alongside regional changes in climate and nutrient loading signals a general problem in freshwater ecosystems that might not be reversible by applying traditional lake management schemes focused solely on phosphorus reduction.

Methods

Long-term physico-chemical characteristics of the lakes and environmental change.

Seven lakes north, and three lakes south of the European Alps were cored between 2009 and 2014 (see the map in Supplementary Fig. 1). The lakes were selected on the basis of their geographical location, their eutrophication history, and because they cover a wide gradient of trophic levels and morphological characteristics (Supplementary Table 1). Water samples from these lakes have been collected and analysed as part of multiple sampling programmes by various governmental and institutional instances over the last two to six decades (See Supplementary Table 2 for details about sampling and source of monitoring data). Total phosphorus (TP) and nitrate ($\text{NO}_3\text{-N}$) concentrations were generally determined on a monthly basis. From these time series, we averaged TP and $\text{NO}_3\text{-N}$ integrated over the 20 uppermost meters, or over whole water when the depth-resolved data was not available (Lake Pusiano). We used publicly available homogenized monthly air temperature data⁵¹ recorded at the three following meteorological stations (see map in Supplementary Fig. 1): Zurich/Fluntern (N; Alpine north side – eastern Plateau), Lugano (S; Alpine south side), and Geneva (NW; Alpine north side – western Plateau) to show the increase in air temperature between the years 1860 and 2015.

Water stability index. We used the Schmidt Stability Index (SSI; as defined by⁵²) to calculate the strength of the water column thermal stratification in all the lakes, with the exception of Lake Pusiano where no depth-resolved temperature profiles were available. The maximal annual index values were obtained for each lake in years during which a minimum of three profiles had been collected in summer (between July and September; $n = 312$). To account for possible biases related to an uneven number of temperature profiles sampled per year, the depth-resolved water stability profiles were bootstrapped 1,000 times by choosing 3 random points during the summer period, and were compared to hourly-resolved one-

dimensional hydrodynamic model recently published on Lake Geneva⁵³. The estimated error in the maximal SSI calculation was around 8% over a period of 30 years.

Sediment core sampling and dating. Two types of gravity corer (UWITEC-63 or Eawag-63/S corer) were used to collect sediment cores of approximately 1 m in length and 63 mm in diameter in 10 peri-Alpine lakes between 2009 and 2015. The cores from Greifensee, Hallwilersee, Baldeggersee, Lake Zurich, Lake Constance, Lake Lugano, Lake Maggiore and Lake Pusiano were transported to Eawag facilities in Dübendorf where they were stored in upright position in the dark at 4°C until analysis. The cores from Lake Geneva and Lake Annecy were preserved and processed similarly at INRA (Thonon-Les-Bains, France).

The cores collected in Greifensee and Lake Zurich had been previously dated based on varve counting, in addition to radiometric methods for the Greifensee core (²¹⁰Pb, ²²⁶Ra, ¹³⁷Cs)²⁸. The sediments in all other cores were dated based on both varve counting (when applicable) and radiometric methods (Supplementary Fig. 4). A core from each lake was opened longitudinally, photographed, and described. A half core from each lake was subsampled at 1-cm intervals for radionuclide measurements on a high-purity germanium (HPGe) well detector (gamma spectrometer, Canberra Industries Inc.) at the Eawag (Switzerland) for lakes Hallwilersee, Baldeggersee, Constance, Lugano, Maggiore, and Pusiano) and at the Modane underground laboratory (LSM environmental radioactivity facility, France) for lakes Annecy and Geneva.

Molecular analysis. The sediment samples dedicated to sedimentary DNA (sedDNA) analysis were collected either from a half of the reference core, or from a second half-core that was correlated to the reference core based on lithological tie points and lamina counting performed on the reference and working cores. All sediment cores were opened and processed in a room where no genetic or molecular work had been done before, following procedures to

reduce contamination with foreign DNA, as detailed in ²⁸. The sediment samples were collected in sterile tubes and transferred to a clean laboratory facility, where no PCR work has been done before and where strict clean laboratory procedures are followed. Briefly, disposable gloves, shoe covers, and a head net were worn, and a clean laboratory coat was used each time. The DNA extraction and PCR preparation steps were carried out inside a laminar flow hood cleaned with bleach and sterilized under UV light for at least 20 minutes prior to use. The PCRs and post-PCR steps were carried out in a regular molecular genetic laboratory, and PCR products were never in contact with the sediment or the DNA samples. DNA was extracted from approximately 1g of sediments using the PowerSoil DNA Isolation kit (MoBio Laboratories, Inc., CA, USA) for all lakes except Geneva and Annecy, for which the UltraClean Soil DNA Isolation Kit from the same company was used, according to the manufacturer's instructions. DNA extractions were performed in batches of seven samples, with the addition of one extraction negative control each time. Each sediment layer was used for two independent extractions performed at different times, and the two extracts were subsequently combined to reduce heterogeneity.

The sequencing library preparation was done following the MetaFast protocol developed by Fasteris (Geneva, Switzerland) using cyanobacteria-specific primers ⁵⁴ that included a 10-12 nt-long tag for identifying the samples and for creating size variation in the amplicon (Supplementary Table 3). PCR reactions in a final volume of 40 μ L contained 1 $\times$ AmpliTaq Gold Buffer I (ThermoFisher Scientific), 0.2 mM of each dNTP, 0.5 $\times$ MgCl₂, 1.25U AmpliTaq Gold polymerase, 0.2 μ g/ μ L BSA, and 0.2 μ M each primer (Microsynth, Balgach, Switzerland) and 3 μ L template sedDNA at a concentration of 4 ng/ μ L (slightly less in the 10 samples with a lower sedDNA concentration). The PCR program on the thermal cycler included a first denaturation step at 95°C 10 min, 35 cycles of 95°C for 15 s, 60°C for 30 s and 72°C for 45 s, followed by a last extension step at 72°C 5 min. Each PCR plate contained

318 between 30 to 96 samples (including negative controls at a ratio of 1 per 8-11 samples). PCR
319 was replicated three times in independent PCR runs, and the products of each sample were
320 pooled. These pooled PCR products were then purified using the Illustra GFX PCR DNA and
321 gel band purification kit (GE Healthcare, Chicago, Illinois, USA) and eluted in 30 μ L of TE
322 buffer (10 mM). Purified PCR products were finally quantified with a Qubit Fluorometer, and
323 a total of 111 samples were pooled in equimolar concentration into one multiplex library.
324 Negative PCR controls (i.e. PCR reactions not containing DNA template) were added to the
325 sequencing run to control for contamination. The final library was sent to the Fasteris
326 sequencing company (Geneva, Switzerland) for the adapter ligation and sequencing on a
327 MiSeq Illumina platform.

328 **Sequence data processing.** For the quality control and processing of the sequencing data, we
329 followed an in-house workflow developed at the Genomic Diversity Centre (GDC; ETH
330 Zürich, adapted from²⁸). A total of 10,609,311 quality-checked, primer-trimmed and cleaned
331 sequences from 111 samples were clustered in 2,826 operational taxonomic units (OTUs)
332 using the UPARSE workflow⁵⁵ at a minimum sequence similarity of 97% and a read
333 abundance threshold of 5. Four samples had fewer than 10,000 reads each, and were therefore
334 removed from the dataset. Finally, we filtered out OTUs that were not assigned to
335 cyanobacteria (confidence threshold of 0.89), and removed 13 more OTUs that were found in
336 less than three samples over the whole dataset. This was done to reduce biases introduced by
337 very rare taxa or possible sequencing errors. The seven negative controls that were included in
338 the sequencing run each comprised less than 1% of the reads compared to the samples
339 average, and were therefore removed. For the OTU richness estimation, the OTU counts were
340 rarefied to account for differences in sequencing depth. In total, the filtered dataset comprised
341 143 OTUs assigned to photosynthetic cyanobacteria distributed across 108 samples. After the
342 rarefaction step, 133 OTUs in 76 samples were left.

The taxonomic assignment of the OTUs was based upon a GreenGenes database⁵⁶ supplemented with few decoy sequences belonging to non-cyanobacterial prokaryotes (see²⁸). Of the 143 photosynthetic cyanobacteria OTUs found in this study, 139 were assigned to order level, 125 were assigned to family level, 65 were assigned to genus level, and none were assigned a species name with high confidence (based on a confidence threshold of 0.89).

Phylogenetic diversity estimation. The reference sequences for each cyanobacterial OTU were aligned with PyNAST⁵⁷ and used to infer a phylogeny based on maximum likelihood in FastTree⁵⁸. All diversity analyses were performed with the software R v3.3.2⁵⁹. The phyloseq package in Bioconductor⁶⁰ was used to import, visualize, and subset sequence data, as well as for estimating richness and community similarity, which we respectively defined as the number of OTUs in a lake at a given point in time, and the compositional dissimilarity between sites. Community dissimilarity was estimated using both taxonomic-based (Jaccard)⁶¹ and phylogenetic-based (Unifrac) distances⁶². The patterns were comparable between the Jaccard and Unifrac indices; therefore we present and discuss only the analyses based on phylogenetic distances. We estimated OTU richness in the meta-community formed by all lakes at each given point in time using randomization in the function ‘specaccum’ in the vegan⁶³ package for R. The rarefied OTU counts were used to estimate richness and community dissimilarity.

Compositional turnover. We compared the phylogenetic dissimilarity among communities of lakes using the function ‘adonis’ in the vegan package by permutational multivariate analysis of variance using distance matrices (PERMANOVA). The samples were split in different time periods to cover the timespan of the study. The number of samples prior to the 1940s was insufficient for calculating pairwise dissimilarity across communities, and therefore these samples were excluded from the analysis. To understand changes in cyanobacterial community composition across sites, we studied the prevalence and

distribution of shared and specific OTUs across the 10 lakes over time. We calculated the proportion of lakes where each OTU was found at each given time period (prevalence). The colour-coded image map of taxa prevalence was produced using CIMminer⁶⁴ and taxa were clustered for pattern detection by calculating Euclidean distances based on prevalence and the dendrogram constructed by average linkage method. Rare OTUs were defined as the OTUs found in less than 25% of the lakes at a given time period, and the common OTUs were defined as the ones present in at least 75% of the lakes at a given time period.

Statistical models. Our objective was to compare and quantify the strength of the relationship between cyanobacteria and environmental drivers (strength of water thermal stratification, TP, and NO₃-N) over the last century in the 10 peri-Alpine lakes. The annual average values of NO₃-N and TP of the year before, the year, and the year after the approximate year corresponding to a sedDNA sample was calculated for each observation (i.e. for a sample dated to year 1990, the mean nutrient values of years 1989, 1990, and 1991 were averaged and used in the model). This averaging over a 3-year period was done to account for uncertainties related to the sediments dating. The abiotic variables were weakly cross-correlated ($r < 0.55$). OTU richness was used as response variables in individual models, and a lake random effect was evaluated. We applied a natural logarithm transformation to the response variable, and verified that the residuals were normally distributed. We used linear mixed-effect models (LMMs) from the ‘lme4’ package for R based on the 30 observations for which OTU richness, depth-resolved water temperature, TP, and NO₃-N data were available (Lake Annecy and Lake Pusiano were therefore excluded from the model). The best model was selected based on the comparison of the nested models using parametric bootstrap methods in the pbkrtest package for R.

For calculating community similarities, and for reporting the richness and prevalence of OTUs over time across all sites, the sedDNA samples were grouped by time periods of either

ten or twenty-five years, depending on data availability. The lakes chemical data were pooled in a similar way (twenty-five-year periods) to reflect temporal changes in the overall nutrient concentrations across lakes (Fig. 1e-f).

Data availability statement

The Illumina sequence data that support the findings of this study have been deposited in the European Nucleotide Archive (ENA) with the project number PRJEB21329.

Acknowledgements

High-throughput sequencing data was produced at Fasteris (Geneva). We thank J-C. Walser (GDC; ETH Zürich) for bioinformatics support, and B. Müller for helping with lake chemical data acquisition. We thank M. Thali, A. Lück, A. Zwyssig, C. Chardon, A. Lami, S. Gerli, H. Penson, and C. Ouellet-Plamondon for technical assistance, as well as M. Lavrieux for help with the sediment dating. We are grateful to I. Gregory Eaves, H. Hartikainen, R. Ptacnik, K. Räsänen, C. Tellenbach, and M.K. Thomas for intellectual feedback and fruitful discussions. Coring, dating and DNA extractions for lakes Annecy and Geneva were performed in the context of the “REPLAY” program funded by EC2CO INSU, and the Iper Retro program funded by ANR- VULNS-005 (France). Physical-chemical data of Greifensee was produced by AWEL (Canton Zurich); Wasserversorgung Zürich (WVZ) for Lake Zurich; Lake Constance Water Supply for Lake Constance; Abteilung für Umwelt (AfU) Kanton Aargau (A. Stöckli) for Hallwilersee; Eawag/Kanton Luzern for Baldeggersee; F. Lepori, the Swiss Federal Office for the Environment (FOEN) and CIPAIS for Lake Lugano; M. Manca (CNR Institute of Ecosystem Study, Italy) for Lake Maggiore; the Observatory on Alpine Lakes (SOERE OLA), CIPEL, SILA, and OLA-IS developed by Eco-Informatics ORE INRA for lakes Annecy and Geneva; and G. Tartari (IRSA-CNR Brugherio, Italy) for lake Pusiano. This work was supported by the Swiss Enlargement Contribution, project IZERZO – 142165, “CyanoArchive” to Piet Spaak, in the framework of the Romanian-Swiss Research Programme.

421

422 **Author contributions**

423 M-E.M., P.S. and F.P. designed the study. I.D. and N.D. contributed materials/analysis
424 tools/samples. M-E.M. and I.D. collected data, and all the analyses were carried out by M-
425 E.M. and D.B.. M-E.M. and F.P. wrote the manuscript, which was revised and edited by all
426 authors.

427

428 **Declaration of financial competing interests**

429 The authors declare no financial competing interests.

430

431

References

- 432 1. Vitousek, P. M., Mooney, H. A., Lubchenco, J. & Melillo, J. M. Human Domination of
433 Earth's Ecosystems. *Science*. **277**, 494–499 (1997).
- 434 2. Newbold, T. *et al.* Global effects of land use on local terrestrial biodiversity. *Nature*
435 **520**, 45–50 (2015).
- 436 3. Vellend, M. Conceptual Synthesis in Community Ecology. *Q. Rev. Biol.* **85**, 183–206
437 (2010).
- 438 4. McGill, B. J., Dornelas, M., Gotelli, N. J. & Magurran, A. E. Fifteen forms of
439 biodiversity trend in the anthropocene. *Trends Ecol. Evol.* **30**, 104 (2015).
- 440 5. González-Orozco, C. E. *et al.* Phylogenetic approaches reveal biodiversity threats
441 under climate change. *Nat. Clim. Chang.* **6**, 1110–1114 (2016).
- 442 6. Vellend, M. *et al.* Estimates of local biodiversity change over time stand up to scrutiny.
443 *bioRxiv* **0**, 62133 (2016).
- 444 7. Dornelas, M. *et al.* Assemblage Time Series Reveal Biodiversity Change but Not
445 Systematic Loss. *Science*. **344**, 296–299 (2014).
- 446 8. Magurran, A. E., Dornelas, M., Moyes, F., Gotelli, N. J. & McGill, B. Rapid biotic
447 homogenization of marine fish assemblages. *Nat. Commun.* **6**, 8405 (2015).
- 448 9. Gámez-Virués, S. *et al.* Landscape simplification filters species traits and drives biotic
449 homogenization. *Nat. Commun.* **6**, 8568 (2015).
- 450 10. Cardinale, B. J. *et al.* Corrigendum: Biodiversity loss and its impact on humanity.
451 *Nature* **486**, 59–67 (2012).
- 452 11. Adrian, R., O'Reilly, C. M., Zagarese, H., Baines, S. B. & Dag, O. Lakes as sentinels
453 of climate change. *Limnol. Oceanogr.* **54**, 2283–2297 (2009).
- 454 12. Sukenik, A., Quesada, A. & Salmaso, N. Global expansion of toxic and non-toxic
455 cyanobacteria: effect on ecosystem functioning. *Biodivers. Conserv.* **24**, 889–908
456 (2015).
- 457 13. Paerl, H. W. & Huisman, J. Climate - Blooms like it hot. *Science*. **320**, 57–58 (2008).
- 458 14. Carey, C. C., Ibelings, B. W., Hoffmann, E. P., Hamilton, D. P. & Brookes, J. D. Eco-
459 physiological adaptations that favour freshwater cyanobacteria in a changing climate.
460 *Water Res.* **46**, 1394–1407 (2012).
- 461 15. Rigosi, A., Carey, C. C., Ibelings, B. W. & Brookes, J. D. The interaction between
462 climate warming and eutrophication to promote cyanobacteria is dependent on trophic
463 state and varies among taxa. *Limnol. Ocean.* **59**, 99–114 (2014).
- 464 16. Vonlanthen, P. *et al.* Eutrophication causes speciation reversal in whitefish adaptive
465 radiations. *Nature* **482**, 357–362 (2012).

- 466 17. Downing, J. A. J., Watson, S. S. B. & McCauley, E. Predicting Cyanobacteria
467 dominance in lakes. *Can. J. Fish. Aquat. Sci.* **58**, 1905–1908 (2001).
- 468 18. Taranu, Z. E. *et al.* Acceleration of cyanobacterial dominance in north temperate-
469 subarctic lakes during the Anthropocene. *Ecol. Lett.* **18**, 375–384 (2015).
- 470 19. Sinha, R. *et al.* Increased incidence of *Cylindrospermopsis raciborskii* in temperate
471 zones - Is climate change responsible? *Water Res.* **46**, 1408–1419 (2012).
- 472 20. Salmaso, N. *et al.* Historical colonization patterns of *Dolichospermum lemmermannii*
473 (Cyanobacteria) in a deep lake south of the Alps. *Adv. Oceanogr. Limnol.* **6**, 1–4
474 (2015).
- 475 21. Salmaso, N., Capelli, C., Shams, S. & Cerasino, L. Expansion of bloom-forming
476 *Dolichospermum lemmermannii* (Nostocales, Cyanobacteria) to the deep lakes south of
477 the Alps: Colonization patterns, driving forces and implications for water use. *Harmful*
478 *Algae* **50**, 76–87 (2015).
- 479 22. Ernst, B., Hoeger, S. J., O'Brien, E. & Dietrich, D. R. Abundance and toxicity of
480 *Planktothrix rubescens* in the pre-alpine Lake Ammersee, Germany. *Harmful Algae* **8**,
481 329–342 (2009).
- 482 23. Otten, T. G., Xu, H., Qin, B., Zhu, G. & Paerl, H. W. Spatiotemporal patterns and
483 ecophysiology of toxigenic microcystis blooms in Lake Taihu, China: implications for
484 water quality management. *Environ. Sci. Technol.* **46**, 3480–8 (2012).
- 485 24. Glibert, P. M., Harrison, J., Heil, C. & Seitzinger, S. Escalating Worldwide use of Urea
486 – A Global Change Contributing to Coastal Eutrophication. *Biogeochemistry* **77**, 441–
487 463 (2006).
- 488 25. Domaizon, I., Winegardner, A., Capo, E., Gauthier, J. & Gregory-Eaves, I. DNA-based
489 methods in paleolimnology: new opportunities for investigating long-term dynamics of
490 lacustrine biodiversity. *J. Paleolimnol.* (2017). doi:10.1007/s10933-017-9958-y
- 491 26. Domaizon, I. *et al.* DNA from lake sediments reveals the long-term dynamics and
492 diversity of *Synechococcus* assemblages. *Biogeosciences Discuss.* **10**, 2515–2564
493 (2013).
- 494 27. Savichtcheva, O. *et al.* Effects of nutrients and warming on *Planktothrix* dynamics and
495 diversity: A palaeolimnological view based on sedimentary DNA and RNA. *Freshw.*
496 *Biol.* **60**, 31–49 (2015).
- 497 28. Monchamp, M.-E., Walser, J.-C., Pomati, F. & Spaak, P. Sedimentary DNA reveals
498 cyanobacterial community diversity over 200 years in two perialpine lakes. *Appl.*
499 *Environ. Microbiol.* **82**, 6472–6482 (2016).
- 500 29. Vellend, M. *et al.* Global meta-analysis reveals no net change in local-scale plant
501 biodiversity over time. *Proc. Natl. Acad. Sci. U. S. A.* **110**, 19456–9 (2013).
- 502 30. Hou, W. *et al.* Identification of photosynthetic plankton communities using

- 503 sedimentary ancient DNA and their response to late-Holocene climate change on the
504 Tibetan Plateau. *Sci. Rep.* **4**, 6648 (2014).
- 505 31. Coolen, M. J. L. *et al.* Ancient DNA derived from alkenone-biosynthesizing
506 haptophytes and other algae in Holocene sediments from the Black Sea.
507 *Paleoceanography* **21**, 1–17 (2006).
- 508 32. Boere, A. C., Sinninghe Damsté, J. S., Rijpstra, W. I. C., Volkman, J. K. & Coolen, M.
509 J. L. Source-specific variability in post-depositional DNA preservation with potential
510 implications for DNA based paleoecological records. *Org. Geochem.* **42**, 1216–1225
511 (2011).
- 512 33. Züllig, H. Untersuchungen über die Stratigraphie von Carotinoiden im geschichteten
513 Sediment von 10 Schweizer Seen zur Erkundung früherer Phytoplankton-Entfaltungen.
514 *Schweizerische Zeitschrift für Hydrol.* **44**, 1–98 (1982).
- 515 34. Liechti, P. *L'Etat des Lacs en Suisse. Cahier de l'Environnement* **237**, (1994).
- 516 35. Pomati, F., Matthews, B., Jokela, J., Schildknecht, A. & Ibelings, B. W. Effects of re-
517 oligotrophication and climate warming on plankton richness and community stability in
518 a deep mesotrophic lake. *Oikos* **121**, 1317–1327 (2012).
- 519 36. Posch, T., Köster, O., Salcher, M. M. & Pernthaler, J. Harmful filamentous
520 cyanobacteria favoured by reduced water turnover with lake warming. *Nat. Clim.*
521 *Chang.* **2**, 809–813 (2012).
- 522 37. Livingstone, D. M. Thermal Structure of a Large Temperate Central European Lake.
523 *Clim. Change* **57**, 205–225 (2003).
- 524 38. Matthews, B. & Pomati, F. Reversal in the relationship between species richness and
525 turnover in a phytoplankton community. *Ecology* **93**, 2435–2447 (2012).
- 526 39. Sukenik, A. *et al.* Invasion of Nostocales (cyanobacteria) to subtropical and temperate
527 freshwater lakes – physiological, regional, and global driving forces. *Front. Microbiol.*
528 **3**, 1–9 (2012).
- 529 40. Cirés, S., Wörmer, L., Wiedner, C. & Quesada, A. Temperature-Dependent Dispersal
530 Strategies of *Aphanizomenon ovalisporum* (Nostocales, Cyanobacteria): Implications
531 for the Annual Life Cycle. *Microb. Ecol.* **65**, 12–21 (2013).
- 532 41. Gallina, N., Salmaso, N., Morabito, G. & Beniston, M. Phytoplankton configuration in
533 six deep lakes in the peri-Alpine region: are the key drivers related to eutrophication
534 and climate? *Aquat. Ecol.* **47**, 177–193 (2013).
- 535 42. Salmaso, N. Long-term phytoplankton community changes in a deep subalpine lake:
536 responses to nutrient availability and climatic fluctuations. *Freshw. Biol.* **55**, 825–846
537 (2010).
- 538 43. Anneville, O., Souissi, S., Gammeter, S. & Straile, D. Seasonal and inter-annual scales
539 of variability in phytoplankton assemblages: comparison of phytoplankton dynamics in

three peri-alpine lakes over a period of 28 years. *Freshw. Biol.* **49**, 98–115 (2004).

44. Reynolds, C., Oliver, R. & Walsby, A. Cyanobacterial dominance: the role of buoyancy regulation in dynamic lake environments. *New Zeal. J. Mar. Freshw. Res.* **21**, (1987).

45. Beard, S. J., Handley, B. A., Hayes, P. K. & Walsby, A. E. The diversity of gas vesicle genes in *Planktothrix rubescens* from Lake Zurich. *Microbiology* **145**, 2757–2768 (1999).

46. Jacquet, S. *et al.* The proliferation of the toxic cyanobacterium *Planktothrix rubescens* following restoration of the largest natural French lake (Lac du Bourget). *Harmful Algae* **4**, 651–672 (2005).

47. Walsby, A. E. Stratification by cyanobacteria in lakes: A dynamic buoyancy model indicates size limitations met by *Planktothrix rubescens* filaments. *New Phytol.* **168**, 365–376 (2005).

48. Litchman, E. & Klausmeier, C. a. Trait-Based Community Ecology of Phytoplankton. *Annu. Rev. Ecol. Evol. Syst.* **39**, 615–639 (2008).

49. Birtel, J. & Matthews, B. Grazers structure the bacterial and algal diversity of aquatic metacommunities. *Ecology* **97**, 3472–3484 (2016).

50. Gossner, M. M. *et al.* Land-use intensification causes multitrophic homogenization of grassland communities. *Nature* **540**, (2016).

51. Begert, M., Schlegel, T. & Kirchhofer, W. Homogeneous temperature and precipitation series of Switzerland from 1864 to 2000. *Int. J. Climatol.* **25**, 65–80 (2005).

52. Schmidt, W. Über die Temperatur- und Stabilitätsverhältnisse von Seen. *Geogr. Ann.* (1928).

53. Schwefel, R., Gaudard, A., Wüest, A. & Bouffard, D. Effects of climate change on deepwater oxygen and winter mixing in a deep lake (Lake Geneva): Comparing observational findings and modeling. *Water Resour. Res.* 8811–8826 (2016). doi:10.1002/2016WR019194

54. Nübel, U., Garcia-pichel, F., Muyzer, G., Nu, U. & Muyzer, G. PCR primers to amplify 16S rRNA genes from cyanobacteria. *Appl. Environ. Microbiol.* **63**, 3327–3332 (1997).

55. Edgar, R. C. UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat. Methods* **10**, 996–8 (2013).

56. McDonald, D. *et al.* An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. *ISME J.* **6**, 610–618 (2012).

57. Caporaso, J. G. *et al.* PyNAST: A flexible tool for aligning sequences to a template alignment. *Bioinformatics* **26**, 266–267 (2010).

- 576 58. Price, M. N., Dehal, P. S. & Arkin, A. P. FastTree 2 - Approximately maximum-
577 likelihood trees for large alignments. *PLoS One* **5**, e9490 (2010).
- 578 59. R Core Team. *R: A language and environment for statistical computing*. R Foundation
579 for Statistical Computing (2013).
- 580 60. McMurdie, P. J. & Holmes, S. Phyloseq: An R Package for Reproducible Interactive
581 Analysis and Graphics of Microbiome Census Data. *PLoS One* **8**, (2013).
- 582 61. Faith, D. P., Minchin, P. R. & Belbin, L. Compositional dissimilarity as a robust
583 measure of ecological distance. *Vegetatio* **69**, 57–68 (1987).
- 584 62. Lozupone, C. & Knight, R. UniFrac: a new phylogenetic method for comparing
585 microbial communities. *Appl. Environ. Microbiol.* **71**, 8228–35 (2005).
- 586 63. Oksanen, J. *et al.* vegan: Community Ecology Package. R package version 2.0-10.
587 <http://CRAN.R-project.org/package=vegan> (2013).
- 588 64. Weinstein, J. N. *et al.* An information-intensive approach to the molecular
589 pharmacology of cancer. *Science*. **275**, 343–349 (1997).
- 590

Figure legends

Figure 1. History of environmental conditions in the 10 peri-Alpine lakes. (a) Normalized Schmidt Stability Index (SSI) values plotted against time. The dashed line shows the average SSI value over all lakes and time points, and the black line shows the significant linear fit. (b) Annual average total phosphorus concentrations in the water column (full lines), and reconstructed based on Diatoms assemblages in the sediments (dashed lines) plotted on a log scale (TP, $\mu\text{g/L}$). (c) Annual average nitrate concentrations in the water column plotted on a log scale ($\text{NO}_3\text{-N}$, mg/L). (d) Pairwise difference between mean annual air temperatures at three meteorological stations (N; Alpine north side - eastern Plateau, NW; Alpine north side - western Plateau, and S; Alpine south side). The significant linear fit in each time series (NW-S: $\text{adj. } R^2 = 0.156$, $p = 8.08 \times 10^{-12}$; N-S: $\text{adj. } R^2 = 0.0949$, $p = 1.10 \times 10^{-10}$) is indicated with a full line. (e) Violin plots showing the probability density, median, and interquartile range of TP and (f) $\text{NO}_3\text{-N}$ concentrations during the periods of eutrophication (1950-1974; 1975-1999) and post-eutrophication (2000-2015).

Figure 2. Changes in cyanobacterial OTU richness and phylogenetic structure. (a) Temporal plot of rarefied OTU richness. Coloured lines show the lake-specific significant (full lines) or non-significant (dashed lines) relationships and the black line shows the overall fit for all lakes combined. (b) Species accumulation curves showing the average regional OTU richness estimated for a given number of lakes sampled at different periods. The 95% confidence intervals for each curve are shown for reference in Supplementary Fig. 3. (c) Ln-transformed OTU richness relative to normalized annual maximal SSI values and ln-transformed mean annual TP concentrations across all lakes over the last century. (d) Violin plots showing the probability density, median, and interquartile range of pairwise unweighted

615 Unifrac similarities estimated across lake communities at each decade. The horizontal line at
616 0.43 represents the mean value of pairwise similarity across all samples.

617 **Figure 3. Proportion of rare and common OTUs across all lakes.** Samples were grouped
618 either by 10 or 25 years in order to have the highest possible number of lakes at each period
619 without replication of lakes within each group. A conceptual model depicting the intensity of
620 change in temperature (increased warming, red) and phosphorus concentrations
621 (eutrophication, darker green) in lakes is shown above the graphs for interpretation of
622 patterns.

623 **Figure 4. OTU richness change within cyanobacterial orders.** The solid black lines
624 indicate significant fits of the generalized additive model (GAM) at a $p < 0.05$ level, and the
625 shaded areas around the curves show the confidence intervals at 95%.

626 **Figure 5. Prevalence of OTUs in all lakes between the 1900s and 2015.** The gradient of red
627 illustrates the proportion of lakes where each taxon was found at each given period
628 (prevalence), while the absence of an OTU at a given period is depicted by a white box.

Normalized maximal stability index

a

b

Lake

- Lugano
- Pusiano
- Maggiore
- Constance
- Greifensee
- Zurich
- Hallwilersee
- Baldeggersee
- Annecy
- Geneva

a

Difference annual
mean temperature ($^{\circ}\text{C}$)

e

f

b

c

α

n lakes = 10

9

10

5

9

10

7

9

Lake

- Annecy
- Constance
- Greifensee
- Lugano
- Pusiano
- Baldeggersee
- Geneva
- Hallwilersee
- Maggiore
- Zurich