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Ancient DNA reveals potentially toxic cyanobacteria increasing with climate change

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ABSTRACT

Cyanobacterial blooms in freshwater systems are a global threat to human and aquatic ecosystem health, exhibiting particularly harmful effects when toxin-producing taxa are present. While climatic change and nutrient over-enrichment control the global expansion of total cyanobacterial blooms, it remains unknown to what extent this expansion reflected cyanobacterial assemblage due to the scarcity of long-term monitoring data. Here we use high-throughput sequencing of sedimentary DNA to track ~100 years of changes in cyanobacterial community in hyper-eutrophic Lake Taihu, China's third largest freshwater lake and the key water source for ~30 million people. A steady increase in the abundance of *Microcystis* (as potential toxin producers) during the past thirty years was correlated with increasing temperatures and declining wind speeds, but not with temporal trends in lakewater nutrient concentrations, highlighting recent climate effects on potentially increasing toxin-producing taxa. The socio-environmental repercussions of these findings are worrisome as continued anthropogenic climate change may counteract nutrient amelioration efforts in this critical freshwater resource.

1. Introduction

The worldwide expansion of freshwater cyanobacterial blooms has severely threatened water quality, food-webs, habitat stability and human health (Huisman et al., 2018; O'neil et al., 2012; Paerl and Barnard, 2020; Paerl and Huisman, 2009; Paerl and Otten, 2013; Paerl and Paul, 2012; Taranu et al., 2015). The effects of these blooms are particularly adverse when toxigenic taxa are present (Komárek et al., 2014; Merel et al., 2013). Over recent decades, the magnitude, areal extent, and phenology of cyanobacterial blooms and their potential drivers have been widely documented through monitoring, satellite remote sensing and modeling (Ho et al., 2019; Shi et al., 2017, 2019). These studies generally agree that human-induced nutrient (phosphorus and nitrogen) inputs and climatic change were the main contributors to the worldwide expansion of cyanobacterial blooms (Paerl and Huisman, 2008; Paerl and Otten, 2013; Paerl et al., 2015; Posch et al., 2012; Rigosi et al., 2014; Wagner and Adrian, 2009; Xu et al., 2021). However, the lack of reliable long-term monitoring data that include pre-impact conditions limits our understanding of whether potentially toxic cyanobacteria are following this same trend and, if so, which environmental triggers lead to their blooms.

To compensate for the lack of temporally appropriate direct monitoring data, palaeolimnologists can reconstruct the influence of long-term environmental stressors on aquatic ecosystems using various physical, chemical, and biological proxies preserved in lake sediment records (Liu et al., 2017; Smol, 2010). The composition and structure of

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cyanobacteria in sediment records are rarely determined using traditional microscopic techniques, as these taxa are typically not well preserved as microfossils. Pigment analyses have been used to reconstruct historical changes in the abundance of several divisions of phytoplankton (e.g., cyanobacteria, diatoms, chlorophytes and cryptophytes), while different taxa of cyanobacteria are difficult to be distinguished by this method (McGowan et al., 2005; Pal et al., 2015). On the other hand, newly developed sedimentary ancient DNA (sedaDNA) techniques offer a novel window into the long-term dynamics of cyanobacterial succession and its linkages to environmental forcings (Cao et al., 2020; Domaizon et al., 2013; Li et al., 2019; Monchamp et al., 2016, 2018; Tse et al., 2018; Yan et al., 2019, 2020; Zhang et al., 2021a, 2021b).

Paleoclimate records have documented that past warm periods in China were typically accompanied by a sharper land/ocean thermal contrast resulting in the development of strong Asian summer monsoons with greater wind intensity (Liu et al., 2013, 2017). Unlike major warming periods in the past, recent climatic changes are further complicated by interactions with multiple anthropogenic forcings, including greenhouse gasses, anthropogenic aerosols, and land-use changes. For example, increases in anthropogenic aerosols over the past few decades have weakened the Asian summer monsoon wind intensity (Menon et al., 2002), resulting in unparalleled changes to important freshwater ecosystems (Liu et al., 2017). However, it remains unknown to how these anthropogenic changes affect the cyanobacterial community.

Lake Taihu (meaning “large lake” in Mandarin) is China’s third largest freshwater lake with an area of 2338 km^2, located in the country’s most economically developed region (Fig. 1). Immense cyanobacterial blooms on this large lake have threatened this vital water supply to approximately 30 million people (Qin et al., 2010; Shi et al., 2017). For example, in May 2007, massive toxic blooms of *Microcystis* led to a highly publicized drinking crisis in the city of Wuxi, leaving ~2 million inhabitants without drinking water for more than one week (Guo, 2007; Qin et al., 2010). These large-scale threats have resulted in the investment of more than 200 billion RMB (> US$30 billion) to fund thousands of projects aimed at clean-up efforts in Lake Taihu (Cai, 2008). During this period, the Chinese government also closed more than 6000 chemical industries linked to Lake Taihu water pollution. Within the next ten years, the Chinese government plans to invest in many additional projects to further control cyanobacterial blooms and related environmental problems in Lake Taihu.

Although important for tracking the effects of environmental pollution, direct water quality monitoring data for Lake Taihu only extend back approximately three decades, post-dating the onset of intense industrialization in the catchment and accelerated climatic change. Moreover, the current monitoring programs can over or underestimate

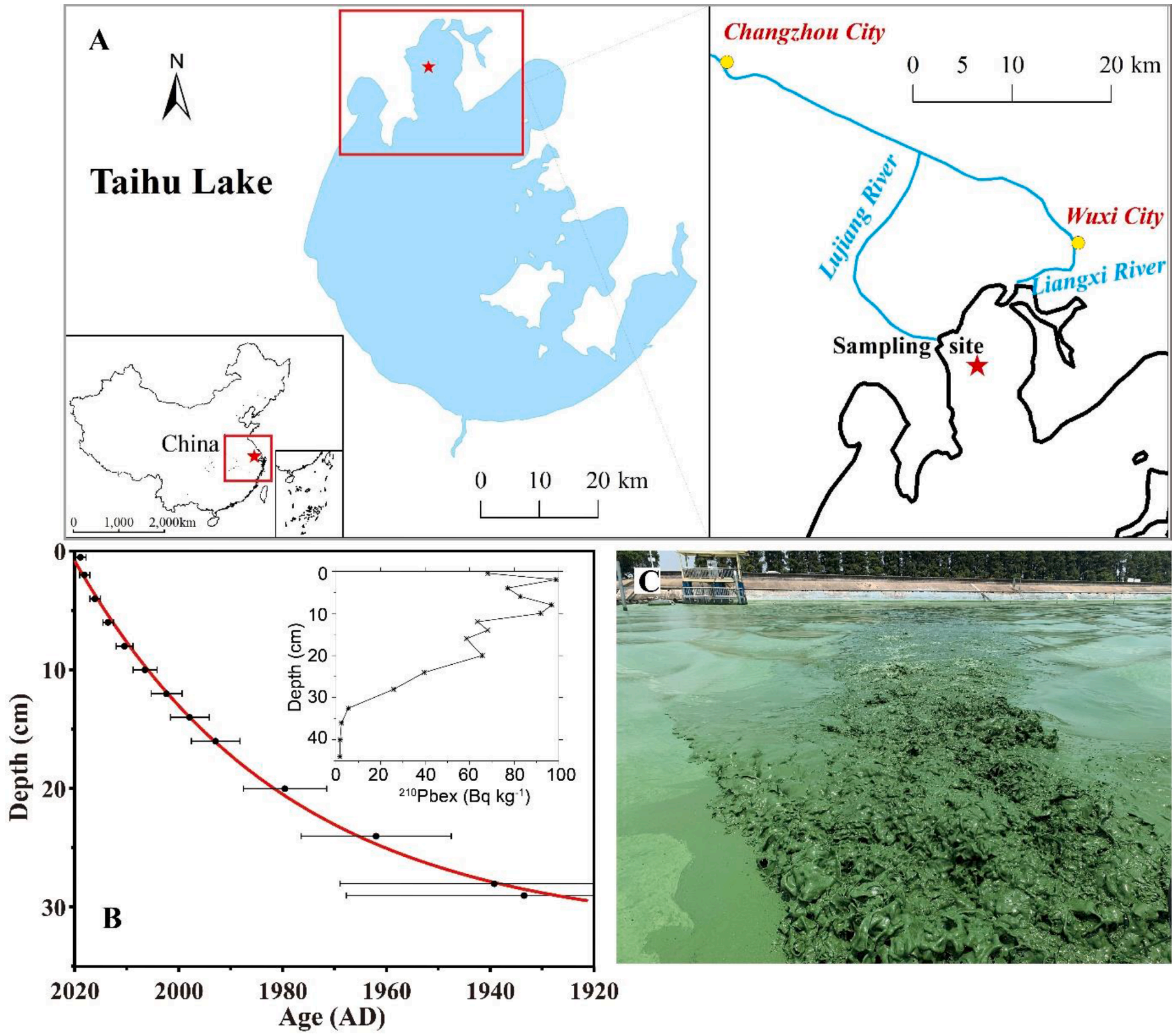

Fig. 1. Location and setting. (**A**) Location of Lake Taihu, with the red star identifying the coring site for core TH19B. (**B**) The age model with uncertainty for core TH19B using ^{210}Pb chronology with the inserted figure showing the decay of ^{210}Pb with depth. (**C**) Image of a bloom event in Lake Taihu on July 2, 2021.

the relative contributions of some cyanobacterial taxa because the extracted cyanobacteria from water samples tend to exclude smaller cells (<2 μm) that may not be captured on the filters used in water sample monitoring (Kolmonen et al., 2004; Ye et al., 2011). Paleolimnological approaches and high-throughput sequencing of sedaDNA are therefore needed to provide important pre-impact environmental conditions, determine the long-term trajectories of potentially toxin-producing cyanobacteria and help disentangle the effects of potential stressors.

Here, we examined the paleo-record from Lake Taihu to track cyanobacteria responses to environmental changes within the context of the past ~100 years. Specifically, we reconstructed changes in the cyanobacterial community structure using high-throughput sequencing of sedimentary ancient DNA archived in a highly-resolved sediment record (TH19B) with chronology established using ^{210}Pb gamma spectroscopy (Fig. 1). We aimed to (i) reconstruct the temporal changes of cyanobacterial community, (ii) identify the timing and tipping point of cyanobacterial community shifts and analyze the community characteristics of the corresponding stages, and (iii) assess the relationships of cyanobacterial taxa, especially the potential toxin producers, with climate and eutrophic stressors.

2. Materials and methods

2.1. Sediment archives

In October 2019, a 81.5-cm long gravity core (TH19B) was taken at a water depth of 2.5 m from Meiliang Bay (31.48°N, 120.17° E) (Fig. 1), a highly nutrient-enriched embayment (Chen et al., 2003) that supports some of Lake Taihu's densest cyanobacterial blooms (Shi et al., 2017, 2019). Meiliang Bay is located in the northwestern part of this lake and is connected to two main rivers (the Liangxi and the Lujiang), which discharge effluents from the nearby cities of Wuxi and Changzhou. The nutrient-rich wastewater inputs to the lake mainly originate from these sources in the northern and northwestern parts of the watershed, resulting in the most severe cyanobacterial blooms of the lake. The TH19B core was sliced into 0.5 cm contiguous segments for the length of the core. A sediment aliquot was carefully subsampled in a sterilized room and then frozen at −80 °C until DNA extraction was undertaken. The remaining sediments were freeze-dried for ^{210}Pb dating and physicochemical analyses.

The uppermost 45 cm of freeze-dried sediment were analysed for ^{210}Pb activities using a High-Purity Germanium (HPGe) radiation detection system (GWL-120–15, AMETEK, USA). The ages and sedimentation rates of core TH119B were calculated using the constant rate of supply (CRS) model (Appleby, 2001). It is well recognized that sediment TN and TP concentrations are often unreliable as proxies of past nutrient concentrations and commonly do not reflect historical changes in lake trophic conditions (Ginn et al., 2012). Thus, we only used lakewater quality monitoring data for TN and TP, rather than from sediment geochemistry, to track long-term trends more reliably in nutrient conditions, and for conducting statistical analysis with the DNA data. Lakewater measurements of TN (mg/L) and TP (mg/L) that were collected from the northern part of Lake Taihu between 1987 and 2018 were synthesized from two studies (Dai et al., 2016; Wu et al., 2019) (Fig. 2). We used the composite nutrient curve for the northern part of the lake because the data for a specific point were not provided by the original literature (Dai et al., 2016). The selected monitoring sites were mainly located in Meiliang Bay.

2.2. Meteorological data

Meteorological data collected from 1956 to 2019, including wind speed (m/s), rainfall (mm) and temperature (°C), from the nearby (3 km from Lake Taihu) Dongshan meteorological station (31°4′N, 120°26′E) were used for examining potential climatic drivers of cyanobacterial population changes. These data were downloaded from the China Meteorological Data Sharing Service System (https://data.cma.cn/). Additionally, mean annual temperature data for the Northern Hemisphere (Morice et al., 2021) from 1933 to 2019 were included for climatic drivers' analysis.

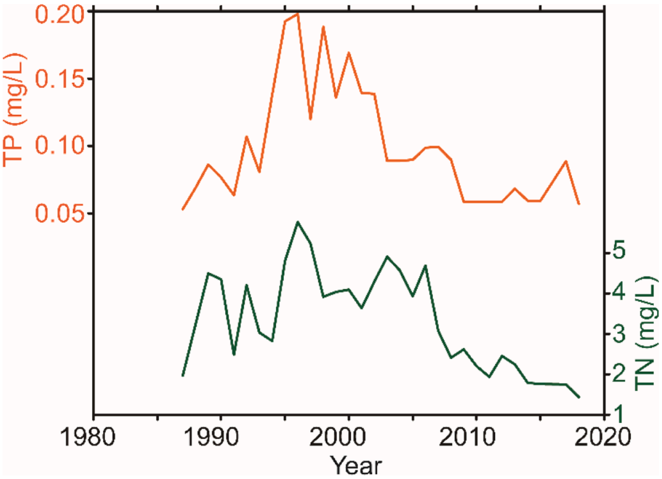

Fig. 2. Monitored lake water nutrient trends in northern Lake Taihu for 1987 to 2018 (Dai et al., 2016; Wu et al., 2019). (A) lakewater total nitrogen (TN). (**B**) lakewater total phosphorus (TP).

2.3. DNA extraction, amplification, and high-throughput sequence analysis

For each interval in the upper 29 cm of core TH19B, lake sediment DNA was extracted from 0.25 g wet sediment, using a Power Soil DNA Isolation kit (Qiagen, Germany). The extracted material was subsequently amplified using indexed algae-specific primer pair p23SrV-f1/p23SrV_r1 (Sherwood and Presting, 2007). This primer set could provide better coverage of cyanobacterial community diversity than the 16S rDNA primer set (Marcelino and Verbruggen, 2016). The domain V of the 23S rRNA gene own universality which could cover most photosynthetic microbial groups (Presting, 2006; Sherwood and Presting, 2007). This primer set has also been widely used in previous studies related to cyanobacterial community (e.g., Hou et al., 2014; Marcelino and Verbruggen, 2016; Presting, 2006; Sherwood and Presting, 2007; Steven et al., 2012; Yan et al., 2019; Zhang et al., 2021). One negative control was added in each batch (15 samples) to prevent contamination during the DNA extraction and during the polymerase chain reaction (PCR) amplification. The PCR thermal profile was as follows: 95 °C, 2 min; 17 cycles with 0.5 °C step-down cycles from 66 to 58 °C for 30 s, and then annealing at 58 °C for 30 s, extension at 72 °C for 30 s; 18 cycles (94 °C, 30 s; 58 °C, 30 s; 72 °C, 30 s), and final extension at 72 °C for 5 min (Sherwood et al., 2008). Purified DNA was pooled to be sequenced on the Illumina HiSeq platform (Illumina Inc., USA) at the Magigene Company.

The raw sequencing data obtained were analyzed by a combination of USEARCH10 and R software, providing effective sequences for clustering of amplicon sequence variants (ASVs) based on UNOISE3 algorithm (Edgar, 2016) and an abundant frequency threshold of 10. The taxonomic identification of representative ASVs was done manually using the Basic Local Alignment Search Tool (BLAST) with a similarity threshold of higher than 0.9 to compare with the cultured species in the NCBI database (https://blast.ncbi.nlm.nih.gov/Blast.cgi). ASVs that were assigned to phytoplankton were kept for subsequent analysis.

2.4. Statistical analysis

Nonmetric multi-dimensional scaling (NMDS) analysis was based on the Bray-Curtis dissimilarity matrix, using the vegan package for R. "Change-point Analyzer" (Taylor, 2000) was used to identify periods of significant change in cyanobacterial community shifts. The significance ($p<0.05$) of the change points was tested by a combination of cumulative sum charts and bootstrapping techniques (1000 repetitions). Co-occurrence networks have proved to be an effective approach for revealing the structure and function of phytoplankton communities (Cao et al., 2020; Escalas et al., 2019; Tse et al., 2018). The co-occurrence network analysis was carried out for the stages defined by the change points with the Gephi v 0.9.2 (Bastian et al., 2009). The nodes in the network plot represent the cyanobacterial taxa. A Spearman's correlation matrix was calculated and significant correlations ($|r| > 0.6$ and $p < 0.05$) were considered to be valid for the co-occurrence network and were shown as the edges between nodes in the network plot. The Fruchterman-Reingold placement algorithm was used for module detection and network visualization (Bastian et al., 2009).

3. Results and discussion

3.1. Chronology and temporal changes in cyanobacteria community

Age-depth model for the sediment core TH19B based on the CRS model indicated the upper 29 cm ranged from the ~1933 to 2019 with an average sedimentation rate of ~2.97 cm/y (Fig. 1). Calculated ages beyond 29 cm would have large age uncertainties (more than 34 years) and were considered to be meaningless. The ^{137}Cs activities of core TH19B did not display a peak and the concentrations most samples were <5 Bq kg^{-1}. Thus, we considered the ^{137}Cs dates unreliable. Using high-throughput sequencing of sedaDNA, a total of 257 operational taxonomic units (ASVs) associated with cyanobacteria were recovered from the dated sediment core, and then grouped into 29 genera (Fig. 3). We identified a clear pattern whereby the abundance of a potentially toxic cyanobacteria taxa, *Microcystis*, progressively increased since the 1990s, with a corresponding significant decrease of the previously dominant *Synechococcus* (Fig. 3). Specifically, the proportion of *Microcystis* averaged approximately 7% of the total population until 1991, then from 1992 – 2009, increased rapidly to a mean relative abundance of approximately 19%. Following 2009, the proportion of toxin-producing cyanobacteria fluctuated, but overall remained high with a mean relative abundance of approximately 69%.

Shifts in the entire cyanobacterial community were assessed using axis 1 scores of a nonmetric multi-dimensional scaling (NMDS) analysis, which helped to identify points of change over time. Significant change points (with a 95% confidence level) were detected in ~1991 (1989–1995) and ~2009 (2009–2010), dividing the community-level changes into three stages of cyanobacterial composition (Fig. 4). The NMDS plot, based on the dissimilarities of cyanobacterial ASVs, clearly indicated strong differences among the three stages (Fig. 4). Stage I (ca. 1933–1991) is dominated by *Synechococcus* (49–84% with a median value of 69%), followed by *Dolichospermum* (formerly *Anabaena*) (1–21% with a median value of 3.9%), *Nostoc* (0.4–13% with a median value of 2.5%), *Cyanobium* (4–11% with a median value of 7%), and

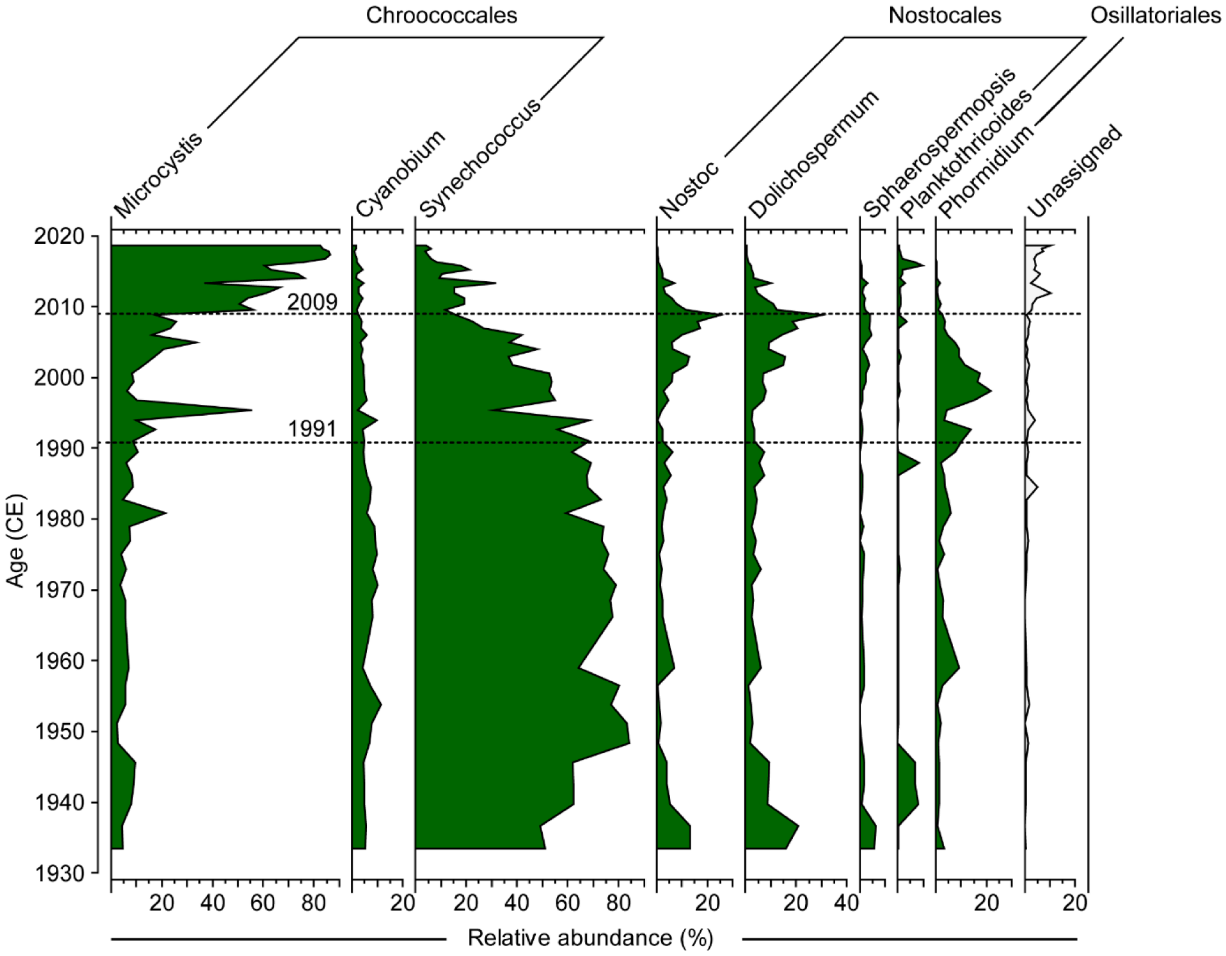

Fig. 3. Meiliang Bay cyanobacteria community changes over the past century based on ancient DNA recovered from sedimentary archives. The relative abundances of the most common cyanobacteria recovered in a dated sedimentary record using high-throughput sequencing of sedaDNA. Ages of the sediment record were estimated using ^{210}Pb gamma spectrometry. Dark dashed lines correspond to significant compositional shifts (~1991 and ~2009) identified by change-point analysis.

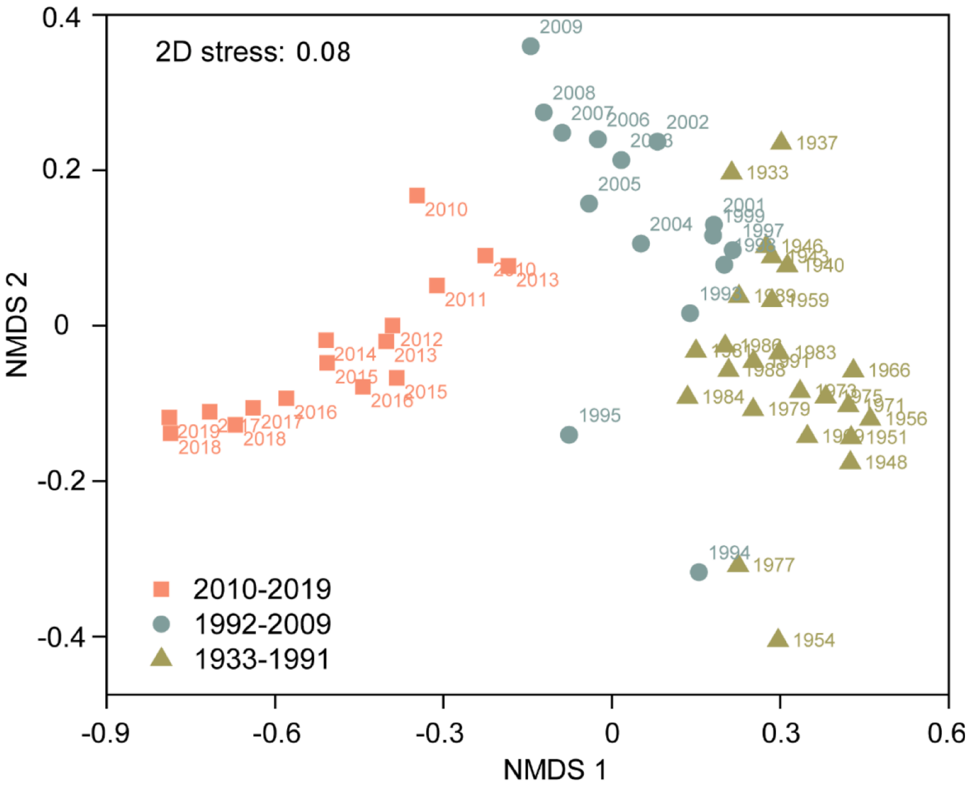

Fig. 4. Non-metric multi-dimensional scaling (NMDS) plot showing differences in cyanobacterial ASVs.

Phormidium (0.6–14% with a median value of 2.8%). Stage II (ca. 1992–2009) is a transition stage where the relative abundance of *Synechococcus* gradually decreases (16–69% with a median value of 42%), with concurrent increases in *Phormidium* (2.6–22% with a median value of 9.1%), *Nostoc* (0.5–25% with a median value of 6.2%), *Dolichospermum* (2.4–30% with a median value of 9.0%) and the first notable occurrence of *Microcystis* (6.5–55% with a median value of 6.6%). Stage III (ca. 2010–2019) is marked by a sharp increase to dominance of *Microcystis* (37–87% with a median value of 70%), and concurrent decreases in *Synechococcus* (4–32% with a median value of 10.9%), *Nostoc* (0.2–11.7% with a median value of 2.1%) and *Dolichospermum* (0.4–12.2% with a median value of 2.8%).

Co-occurrence network analysis was also used to reveal the differences between the three stages defined by the two change points in Tahu (Table 1 and Fig. 5). The number of nodes of the resulting network was highest (29) during 1933–1991, which is consistent with the higher cyanobacterial diversity than the other stages. Meanwhile, the average path length (2.25) during 1992–2009 was higher than those of the other two stages (1.66 and 1), implying more interactions between different species. Although with fewer nodes (24), the period during 2010–2019 displayed the highest network density (0.20), the highest number of edges (54) and the highest average node degree (4.50), suggesting that this stage was more complex than the others. For the three stages, significantly difference also existed in the keystone taxa (Fig. 5), demonstrating remarkable changes in cyanobacterial structure and functioning. Furthermore, the networks of the three stages differed in terms of proportion of negative links and the stage during 2010–2019 exhibited a higher value (26%) than the other two stages (13% and 9%).

Table 1
Characteristics of co-occurrence networks.

	1933–1991	1992–2009	2010–2019
Network characteristics			
Number of nodes	29	25	24
Number of edges	10	23	54
Negative links (%)	9	13	26
Density	0.03	0.08	0.20
Node characteristics			
Average degree	0.69	1.84	4.50
Edge characteristics			
Average path length	1	2.25	1.66

3.2. *Reconstructing long-term cyanobacterial community changes using high-throughput sequencing of sedaDNA*

High-throughput sequencing of sedaDNA offered a taxonomically-sensitive way to assess long-term changes in cyanobacterial communities, allowing us to extend the time span of cyanobacterial community changes from about thirty years (based on identification of taxa in lakewater monitoring samples using traditional microscopy methods) to about one century. Although post-depositional DNA preservation may possibly result in some biases in sedaDNA studies, no obvious decreases in operational taxonomic units (ASVs) of specific genera with increasing sediment age were detected, suggesting that there was no apparent preferential DNA degradation. Our sedaDNA results identified several cyanobacterial genera (e.g., *Microcystis and Dolichospermum*), which were also found in previous microscopic assessments of water quality monitoring data from Lake Taihu, as well as some microalgae (e.g., *Synechococcus*) that may not have been detected microscopically, likely due to their small size (Chen et al., 2003; Deng et al., 2019). This apparent discrepancy may be related to a bias against microalgae using the traditional light microscopic analysis (Monchamp et al., 2016). In addition, some biases may be generated with microscopic monitoring, because the plankton identification can differ greatly from one investigator to another. Overall, our results highlight the important role of high-throughput sequencing of sedaDNA to obtain long-term and high-resolution information on cyanobacterial community structure and function.

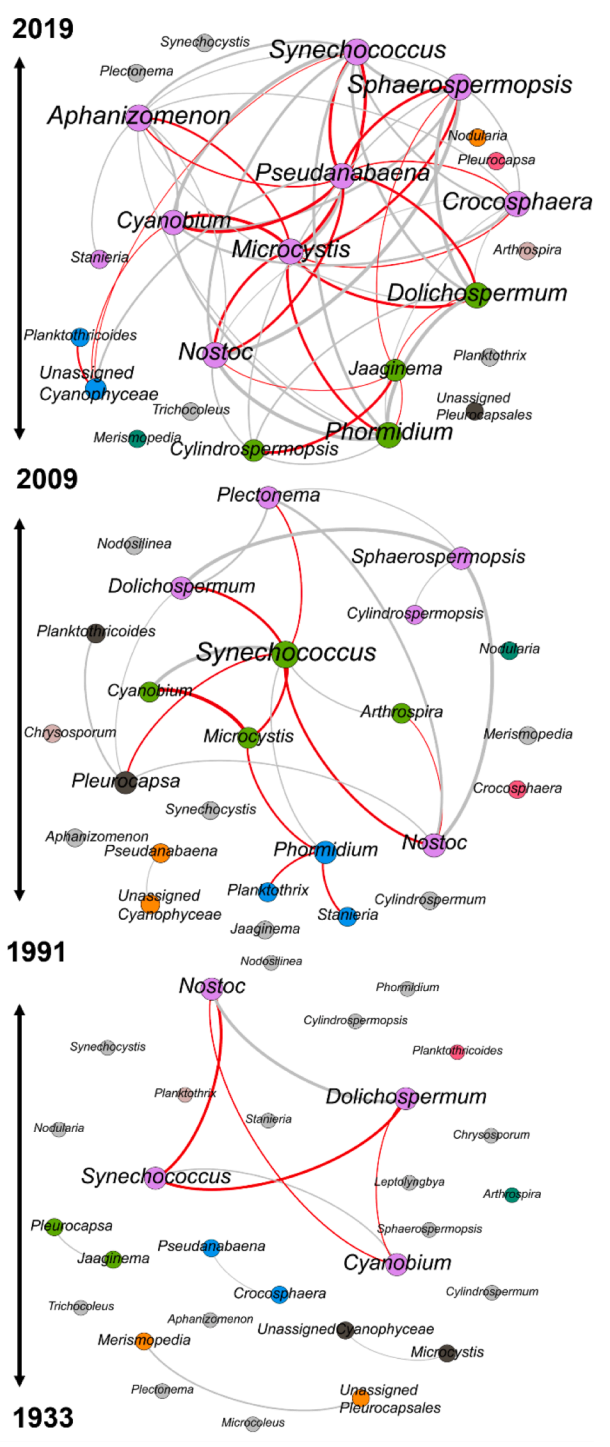

Fig. 5. Co-occurrence network analysis comparing the three stages of Meiliang Bay cyanobacterial communities recovered by sediment analyses. Each edge represents a significant correlation (Spearman's correlation coefficients $r > 0.6$, $p < 0.05$), with red edges standing for a negative relationship and gray edges for a positive relationship. Different colors of the nodes are representative of different modularity classes. The node and font size are proportional to the node degree which are calculated to characterize the topology of resulting networks.

3.3. *Climate change as the driver of the increasing trend in potentially toxic cyanobacteria*

Rapid economic development in Asia over recent decades has led to a pronounced increase in anthropogenic aerosol emissions (e.g., black carbon), reducing incoming solar radiation through increased cloud albedo, thereby attenuating the sea-land thermal contrast and the intensity of monsoonal winds (Menon et al., 2002). Lake Taihu, located in the most economically developed region of China, is experiencing both significant increases in regional air temperatures and reduced wind speeds. Meteorological observations recorded at the Dongshan climate station near Lake Taihu since 1970 show that the mean annual wind speed has weakened by about 1.55 m/s with a linear decreasing rate of 0.29 m/s per decade ($R^2 = 0.67$, $p < 0.001$), while the mean annual air temperature has increased by about 2.17 °C with a linear increasing rate of 0.40 °C per decade ($R^2 = 0.89$, $p < 0.001$).

Although many cyanobacterial species have the potential to produce toxins (Huisman et al., 2018), the toxic cyanobacterial blooms were mainly dominated by *Microcystis* spp. in Lake Taihu (Otten and Paerl, 2011; Qin et al., 2010). In our study, the *Microcystis* we recovered from sediment can be highly potential toxin producers. Significant positive correlations were identified between the increase in abundance of *Microcystis* and increases in the mean annual air temperature of the Dongshan meteorological station near Lake Taihu ($r = 0.65$, $p < 0.001$; 1956–2019) (Fig. 6A). This upward warming trend over recent decades (Fig. 7F) coincided with the increase in the proportion of *Microcystis* and a corresponding decrease in picocyanobacteria including *Synechococcus* (Fig. 7B) and *Cyanobium* (Fig. 3). The increasing proportion of *Microcystis* (Fig. 7B) and declines in mean annual wind speed (Fig. 7E) were negatively correlated ($r = -0.89$, $p < 0.001$; 1956–2019) (Fig. 6C). In contrast, no significant correlation was found between the abundance of *Microcystis* and mean annual precipitation (Fig. 6B), nor with lakewater total nitrogen (TN) and total phosphorus (TP) concentrations (Fig. 6D). These trends likely indicate that N and P are in excess of algal demands throughout much of the year (Xu et al., 2021). Our results show that, given the current high nutrient hypertrophic state of Lake Taihu, climate change (warming and weakened wind speed) is now the key driver of the observed increasing abundance of *Microcystis*.

Elevated temperatures may favor *Microcystis* in several ways. Increasing regional air temperatures, accompanied by low wind speeds, can encourage stable stratification of the water column and weaken vertical mixing, promoting ideal conditions for the growth of highly buoyant, surface bloom-forming *Microcystis* (Huisman et al., 2004; Jöhnk et al., 2008; Walsby, 1994). Although the growth rates of many cyanobacteria increase with higher temperatures, *Microcystis* is especially sensitive to temperature increases when compared to other cyanobacterial genera (Rigosi et al., 2014), such as *Synechococcus*. In addition, dense surface blooms are capable of strong light absorption that can increase the temperature of bloom areas by approximately 1.5–3 °C compared to the surrounding surface waters (Kahru et al., 1993). This positive feedback mechanism may further facilitate the dominance of *Microcystis*. These surface blooms can then shade non-buoyant phytoplankton species, thus suppressing their photosynthetic growth (Jöhnk et al., 2008). The *Synechococcus*, as fast-growing "r-strategy'' picocyanobacteria, are sensitive to nutrient pulses and have been found to be more abundant in nutrient-rich conditions (Scanlan and West, 2002; Zhang et al., 2021). The growth of *Synechococcus* exhibited larger sensitivity to nutrients than *Microcystis* (Rigosi et al., 2014). Interestingly, when the nutrients increased fast since 1980s in northern Lake Taihu, the abundance of *Synechococcus* decreased, accompanied by an increase in *Microcystis*. This phenomenon suggests that, even if the nutrient conditions are suitable for the growth of *Synechococcus*, the lack of buoyancy advantage in the competition with *Microcystis* may lead to the decrease of the abundance of picocyanobacteria.

Although lakewater TN and TP concentrations in the northern Lake Taihu were not correlated significantly with the abundance of *Microcystis* during 1987–2018 (Fig. 6D), their pronounced increase in the 1990s was accompanied by accelerated eutrophication. This suggests that, in the early stages of *Microcystis* expansion, the impact of nutrient enrichment on cyanobacterial biomass and composition (Chen et al.,

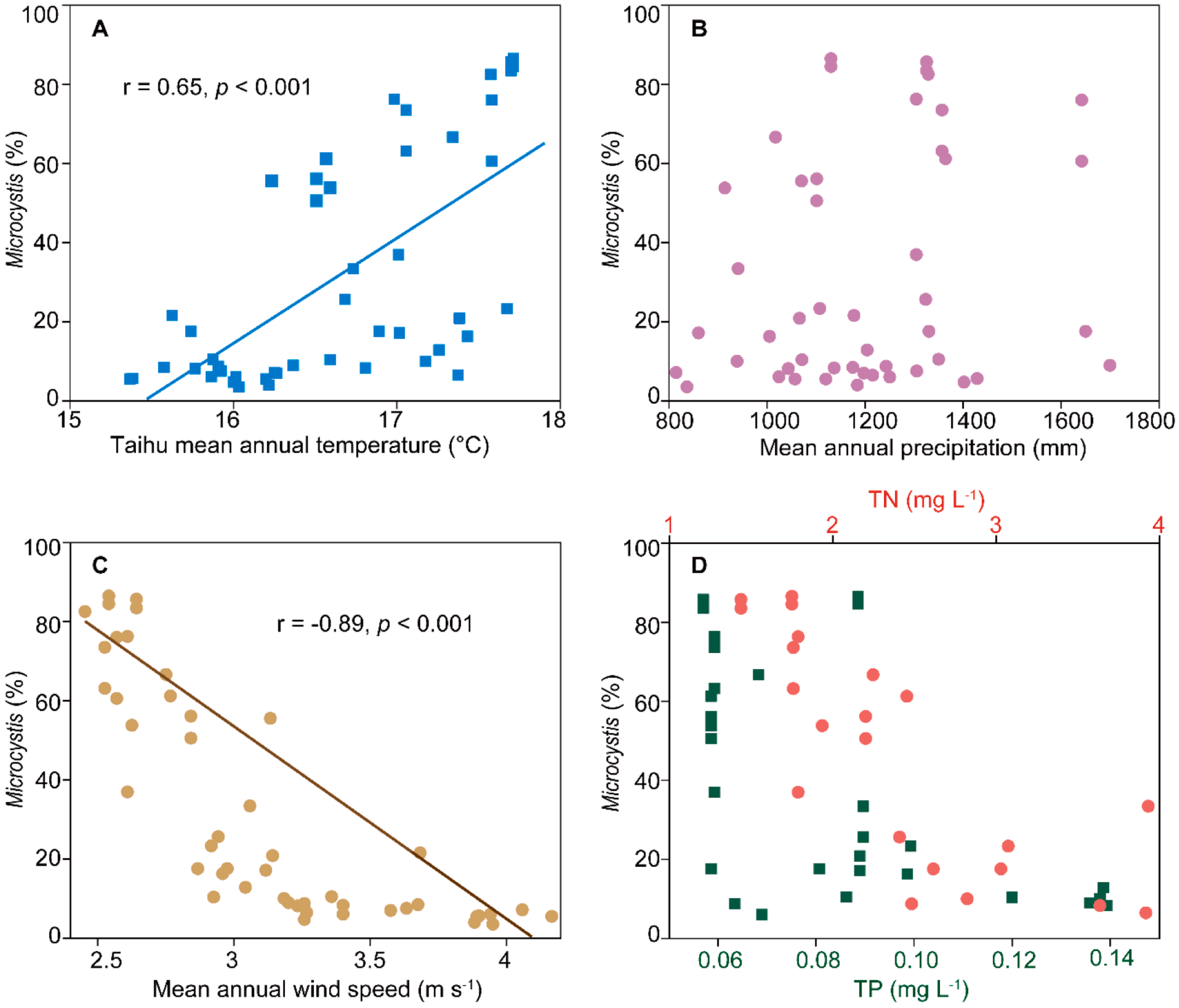

Fig. 6. Relationships between the abundance of *Microcystis* in Meiliang Bay sediment record and environmental variables. (**A**) Significant and positive correlations were observed between the abundance of *Microcystis* and the mean annual temperature at Lake Taihu between ~1933 and ~2019. (**B**) The abundance of *Microcystis* was not significantly correlated with annual rainfall observed at Lake Taihu between ~1956 and 2019. (**C**) Significant correlations were observed between the abundance of *Microcystis* and the wind speed recorded at Lake Taihu from ~1956 to ~2019. (**D**) Monitored lakewater total nitrogen (TN) and total phosphorus (TP) in northern Lake Taihu (Dai et al., 2016; Wu et al., 2019) from ~1987 to ~2018 were not significantly correlated with the *Microcystis* abundances.

2003) are important. However, over the last thirty years, climatic factors, rather than nutrient conditions, are playing an increasingly important role in modulating changes in the cyanobacterial community in northern Lake Taihu. For example, after 1996, despite the implementation of a series of pollution management strategies that effectively reduced lakewater TN and TP concentrations (Fig. 7D), *Microcystis* abundance continued to increase. During this period of nutrient reductions, the striking shift in cyanobacterial community composition was also reflected by an equally pronounced rise in chlorophyll *a* concentrations (Qin et al., 2019) and an earlier start to the bloom period by up to 29.9 days since 2003 (Fig. 7C) (Shi et al., 2019). These phytoplankton community trends, including the clear disconnect between declining nutrients and increasing algal biomass, agree with a response to regional warming and weakening wind speeds.

3.4. The underestimation of climate change effects on potentially toxic cyanobacteria

Our results revealed that the increase of potentially toxic *Microcystis*, which we link mainly to recent climate change, may weaken the stability of cyanobacterial communities, and thus negatively impact the aquatic ecosystem. Based on co-occurrence network analysis (Table 1 and Fig. 5) Stage III of Lake Taihu cyanobacteria composition (ca. 2010 – 2019) was characterized by the highest negative link values of the record, indicating weak community stability and an increase in mutual exclusion between cyanobacterial taxa (Cao et al., 2020; Escalas et al., 2019; Lawes et al., 2017). The majority of these negative links were correlated to increases in *Microcystis*, suggesting that the potentially toxic cyanobacteria are the main contributors to the community structure changes.

While previous studies have confirmed that climate change can lead to cyanobacterial bloom expansion, our study demonstrates that warming and declining wind speeds can also increase the proportion of potentially toxin-producing taxa. In Lake Taihu, the relative contribution of potentially toxic cyanobacteria to blooms after ca. 2009 was approximately four times higher than before ca. 1991. This suggests that recent climate changes have been significantly underestimated as drivers of the accelerated rate of potentially toxic cyanobacteria bloom formation. Whilst 30 years of water quality monitoring would be considered an exceptionally long data set by any international standard (Smol, 2019), these data could not effectively distinguish the proportion of potentially toxin-producing cyanobacterial taxa. Furthermore, the initiation of the monitoring program post-dates the onset of accelerated warming and industrialization and was therefore not of sufficient length to identify the relative roles of eutrophication and climate-related

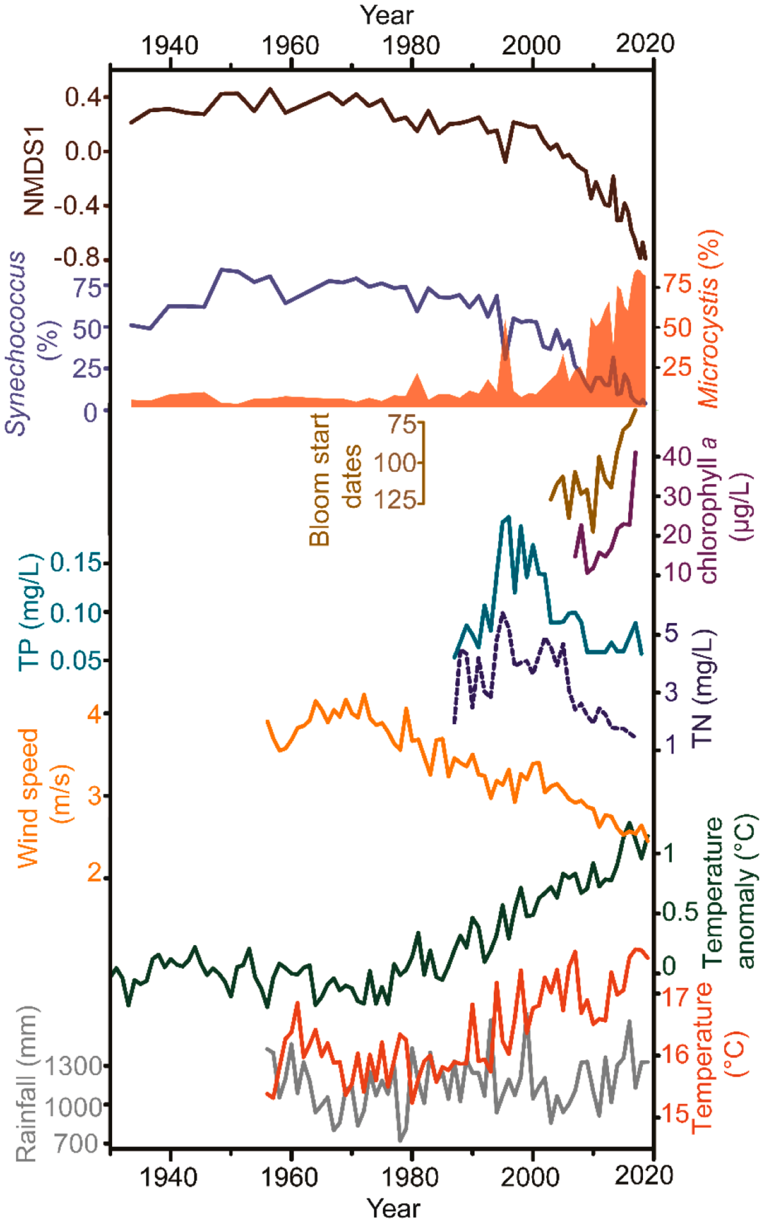

Fig. 7. Comparison of long-term trends in Meiliang Bay potentially toxic cyanobacteria with regional climate and lake water nutrient conditions. (**A**) Non-metric multi-dimensional scaling axis 1 scores (NMDS1) represents the main direction of cyanobacterial community change. (**B**) The percent relative abundance of *Microcystis* (potential toxin producers; orange shading) and *Synechococcus* (light blue line) derived from ancient DNA in the Meiliang Bay sediment record. (**C**) Changes in chlorophyll *a* concentration from lake water quality monitoring data (Qin et al., 2019) and bloom start dates (11) in Lake Taihu. (**D**) Changes in lakewater total nitrogen (TN) and total phosphorus (TP) concentrations from northern Lake Taihu (Dai et al., 2016; Wu et al., 2019). (**E**) Trends in mean annual wind speed recorded at the Dongshang climate station near Lake Taihu. (**F**) Changes in mean annual temperature for the Northern Hemisphere (dark green line) (Morice et al., 2021) and Dongshang station (orange), and Dongshang annual rainfall (light gray line). Vertical gray lines represent significant cyanobacterial compositional shifts identified by change-point analysis (~1991 and ~2009).

variables. SedaDNA affords the unique advantage of providing the requisite temporal context (ca. 100 years) to distinguish between the relative contributions of nutrients and climate change to cyanobacterial bloom history. As climate warming continues, and wind speeds decline further, the intensity and frequency of toxic algal blooms in Lake Taihu and elsewhere will likely increase, despite effective nutrient abatement programs. These findings further highlight that anthropogenic climate change is the new “threat multiplier” (Smol, 2010), making it even more critical for lake managers to continue implementing effective nutrient control strategies, particularly in lakes that continue to be affected by cultural eutrophication

4. Conclusion

We firstly reconstructed changes in cyanobacterial community based on sedaDNA data over the last century at Lake Taihu, the third largest freshwater lake in China. Abrupt change of cyanobacterial communities occurred around two change points around 1991 and 2009. After each point, cyanobacterial communities were progressively getting more complex and unstable evidenced by correlation-based network analysis. This cyanobacterial community shift may be linked to the progressively increasing of potentially toxic cyanobacteria taxa (*Microcystis* spp.) abundance since 1980s. Correlation analysis reveals that temperature and wind speed may be the main drivers of cyanobacterial community changes. The general trend of warming and weakening winds in recent decades in Lake Taihu facilitated the growth of potentially toxic cyanobacterial taxa, which could have counteracted the efforts of pollution control measures. Climate change contributed to the acceleration of potentially toxic cyanobacteria dominance in Lake Taihu during recent global warming. Toxic cyanobacteria blooms in eutrophic lakes may be more severe in the context of future warming, seriously threating aquatic ecosystems and drinking water safety.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare no competing interests.

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References

Appleby, P.G., 2001. Chronostratigraphic techniques in recent sediments. In: Last, W.M., Smol, J.P. (Eds.), Tracking Environmental Change Using Lake Sediments. Volume 1. Basin Analysis, Coring, and Chronological Techniques, pp. 171–203.

Bastian, M., Heymann, S., Jacomy, M., 2009. Gephi: an open source software for exploring and manipulating networks. In: Third international AAAI conference on weblogs and social media.

Cai, J., 2008. The Total Cost of the Current Round of Pollution Control in Taihu Lake May Rise to 200 Billion RMB. Caixin Media, Beijing.

Cao, X., Xu, X., Bian, R., Wang, Y., Yu, H., Xu, Y., Duan, G., Bi, L., Chen, P., Gao, S., 2020. Sedimentary ancient DNA metabarcoding delineates the contrastingly temporal change of lake cyanobacterial communities. Water Res. 183, 116077.

Chen, Y., Qin, B., Teubner, K., Dokulil, M.T., 2003. Long-term dynamics of phytoplankton assemblages: microcystis-domination in Lake Taihu, a large shallow lake in. China. J. Plankton Res. 25 (4), 445–453.

Dai, X., Qian, P., Liang, Y.E., Song, T., 2016. Changes in nitrogen and phosphorus concentrations in Lake Taihu,1985-2015. J. Lake Sci. 28, 935–943.

Deng, J., Salmaso, N., Jeppesen, E., Qin, B., Zhang, Y., 2019. The relative importance of weather and nutrients determining phytoplankton assemblages differs between seasons in large Lake Taihu, China. Aquat. Sci. 81 (3), 48.

Domaizon, I., Savichtcheva, O., Debroas, D., Arnaud, F., Villar, C., Pignol, C., Alric, B., Perga, M.E., 2013. DNA from lake sediments reveals the long-term dynamics and diversity of Synechococcus assemblages. Biogeosci. Discuss. 10 (6), 3817–3838.

Edgar, R., 2016. UNOISE2: improved error-correction for Illumina 16S and ITS amplicon sequencing. bioRxiv.

Escalas, A., Catherine, A., Maloufi, S., Cellamare, M., Hamlaoui, S., Yéprémian, C., Louvard, C., Troussellier, M., Bernard, C., 2019. Drivers and ecological consequences of dominance in periurban phytoplankton communities using networks approaches. Water Res. 163, 114893.

Ginn, B.K., Rühland, K.M., Young, J.D., Hawryshyn, J., Quinlan, R., Dillon, P.J., Smol, J. P., 2012. The perils of using sedimentary phosphorus concentrations for inferring long-term changes in lake nutrient levels: comments on Hiriart-Baer et al., 2011. J. Great Lakes Res. 38 (4), 825–829.

Guo, L., 2007. Doing battle with the green monster of Taihu Lake. Science 317 (5842), 1166.

Ho, J.C., Michalak, A.M., Pahlevan, N., 2019. Widespread global increase in intense lake phytoplankton blooms since the 1980s. Nature 574 (7780), 667–670.

Hou, W.G., Dong, H.L., Li, G.Y., Yang, J., Coolen, M.J.L., Liu, X.Q., Wang, S., Jiang, H.C., Wu, X., Xiao, H.Y., Lian, B., Wan, Y.Y., 2014. Identification of photosynthetic plankton communities using sedimentary ancient DNA and their response to late-Holocene climate change on the Tibetan Plateau. Sci. Rep. 4, 6648.

Huisman, J., Codd, G.A., Paerl, H.W., Ibelings, B.W., Verspagen, J.M.H., Visser, P.M., 2018. Cyanobacterial blooms. Nat. Rev. Microbiol. 16 (8), 471–483.

Huisman, J., Sharples, J., Stroom, J.M., Visser, P.M., Kardinaal, W.E.A., Verspagen, J.M. H., Sommeijer, B., 2004. Changes in turbulent mixing shift competition for light between phytoplankton species. Ecology 85 (11), 2960–2970.

Jöhnk, K.D., Huisman, J.E.F., Sharples, J., Sommeijer, B.E.N., Visser, P.M., Stroom, J.M., 2008. Summer heatwaves promote blooms of harmful cyanobacteria. Glob. Change Biol. 14 (3), 495–512.

Kahru, M., Leppanen, J.-.M., Rud, O., 1993. Cyanobacterial blooms cause heating of the sea surface. Mar. Ecol. Prog. Ser. 101 (1/2), 1–7.

Kolmonen, E., Sivonen, K., Rapala, J., Haukka, K., 2004. Diversity of cyanobacteria and heterotrophic bacteria in cyanobacterial blooms in Lake Joutikas, Finland. Aquat. Microb. Ecol. 36, 201–211.

Komárek, J., Kaštovský, J., Mareš, J., Johansen, J.R., 2014. Taxonomic classification of cyanoprokaryotes (cyanobacterial genera) 2014, using a polyphasic approach. Preslia 86 (4), 295–335.

Lawes, J.C., Dafforn, K.A., Clark, G.F., Brown, M.V., Johnston, E.L., 2017. Multiple stressors in sediments impact adjacent hard substrate habitats and across biological domains. Sci. Total Environ. 592, 295–305.

Li, F., Zhang, X., Xie, Y., Wang, J., 2019. Sedimentary DNA reveals over 150 years of ecosystem change by human activities in Lake Chao, China. Environ. Int. 133, 105214.

Liu, J., Rühland, K.M., Chen, J., Xu, Y., Chen, S., Chen, Q., Huang, W., Xu, Q., Chen, F., Smol, J.P., 2017. Aerosol-weakened summer monsoons decrease lake fertilization on the Chinese Loess Plateau. Nat. Clim. Chang. 7 (3), 190–194.

Liu, J., Wang, B., Cane, M.A., Yim, S.-.Y., Lee, J.-.Y., 2013. Divergent global precipitation changes induced by natural versus anthropogenic forcing. Nature 493 (7434), 656–659.

Marcelino, V.R., Verbruggen, H., 2016. Multi-marker metabarcoding of coral skeletons reveals a rich microbiome and diverse evolutionary origins of endolithic algae. Sci. Rep. 6, 31508.

McGowan, S., Leavitt, P.R., Hall, R.I., Anderson, N.J., Jeppesen, E., Odgaard, B.V., 2005. Controls of algal abundance and community composition during ecosystem state change. Ecology 86 (8), 2200–2211.

Menon, S., Hansen, J., Nazarenko, L., Luo, Y., 2002. Climate effects of black carbon aerosols in China and India. Science 297 (5590), 2250.

Merel, S., Walker, D., Chicana, R., Snyder, S., Baurès, E., Thomas, O., 2013. State of knowledge and concerns on cyanobacterial blooms and cyanotoxins. Environ. Int. 59, 303–327.

Monchamp, M.-.E., Walser, J.-.C., Pomati, F., Spaak, P., 2016. Sedimentary DNA Reveals Cyanobacterial Community Diversity over 200 Years in Two Perialpine Lakes. Appl. Environ. Microbiol. 82 (21), 6472.

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. Nat. Ecol. Evol. 2 (2), 317–324.

Morice, C.P., Kennedy, J.J., Rayner, N.A., Winn, J.P., Hogan, E., Killick, R.E., Dunn, R.J. H., Osborn, T.J., Jones, P.D., Simpson, I.R., 2021. An updated assessment of near-surface temperature change from 1850: the HadCRUT5 data set. J. Geophys. Res. Atmos. 126 (3), e2019JD032361.

O'neil, J., Davis, T., Burford, M., Gobler, C., 2012. The rise of harmful cyanobacteria blooms: the potential roles of eutrophication and climate change. Harmf. Algae 14, 313–334.

Otten, T.G., Paerl, H.W., 2011. Phylogenetic inference of colony isolates comprising seasonal microcystis blooms in Lake Taihu, China. Microb. Ecol. 62 (4), 907–918.

Paerl, H.W., Barnard, M.A., 2020. Mitigating the global expansion of harmful cyanobacterial blooms: moving targets in a human- and climatically-altered world. Harmf. Algae 96, 101845.

Paerl, H.W., Huisman, J., 2008. Blooms like it hot. Science 320 (5872), 57–58.

Paerl, H.W., Huisman, J., 2009. Climate change: a catalyst for global expansion of harmful cyanobacterial blooms. Environ. Microbiol. Rep. 1 (1), 27–37.

Paerl, H.W., Otten, T.G., 2013. Harmful cyanobacterial blooms: causes, consequences, and controls. Microb. Ecol. 65 (4), 995–1010.

Paerl, H.W., Paul, V.J., 2012. Climate change: links to global expansion of harmful cyanobacteria. Water Res. 46 (5), 1349–1363.

Paerl, H.W., Xu, H., Hall, N.S., Rossignol, K.L., Joyner, A.R., Zhu, G., Qin, B., 2015. Nutrient limitation dynamics examined on a multi-annual scale in Lake Taihu, China: implications for controlling eutrophication and harmful algal blooms. J. Freshwater Ecol. 30 (1), 5–24.

Pal, S., Gregory-Eaves, I., Pick, F.R., 2015. Temporal trends in cyanobacteria revealed through DNA and pigment analyses of temperate lake sediment cores. J. Paleolimnol. 54 (1), 87–101.

Posch, T., Köster, O., Salcher, M.M., Pernthaler, J., 2012. Harmful filamentous cyanobacteria favoured by reduced water turnover with lake warming. Nat. Clim. Chang. 2 (11), 809–813.

Presting, G.G., 2006. Identification of conserved regions in the plastid genome: implications for DNA barcoding and biological function. Can. J. Bot. 84, 1434–1443.

Qin, B., Paerl, H.W., Brookes, J.D., Liu, J., Jeppesen, E., Zhu, G., Zhang, Y., Xu, H., Shi, K., Deng, J., 2019. Why Lake Taihu continues to be plagued with cyanobacterial blooms through 10 years (2007–2017) efforts. Sci. Bull. 64 (6).

Qin, B., Zhu, G., Gao, G., Zhang, Y., Li, W., Paerl, H.W., Carmichael, W.W., 2010. A drinking water crisis in Lake Taihu, China: linkage to climatic variability and lake management. Environ. Manage. 45 (1), 105–112.

Rigosi, A., Carey, C.C., Ibelings, B.W., Brookes, J.D., 2014. The interaction between climate warming and eutrophication to promote cyanobacteria is dependent on trophic state and varies among taxa. Limnol. Oceanogr. 59 (1), 99–114.

Scanlan, D.J., West, N.J., 2002. Molecular ecology of the marine cyanobacterial genera Prochlorococcus and Synechococcus. FEMS Microbiol. Ecol. 40, 1–12.

Sherwood, A.R., Chan, Y.L., Presting, G.G., 2008. Application of universally amplifying plastid primers to environmental sampling of a stream periphyton community. Mol. Ecol. Resour. 8 (5), 1011–1014.

Sherwood, A.R., Presting, G.G., 2007. Universal primers amplify a 23S rDNA plastid marker in eukaryotic algae and cyanobacteria. J. Phycol. 43 (3), 605–608.

Shi, K., Zhang, Y., Zhang, Y., Li, N., Qin, B., Zhu, G., Zhou, Y., 2019. Phenology of phytoplankton blooms in a trophic lake observed from long-term MODIS data. Environ. Sci. Technol. 53 (5), 2324–2331.

Shi, K., Zhang, Y., Zhou, Y., Liu, X., Zhu, G., Qin, B., Gao, G., 2017. Long-term MODIS observations of cyanobacterial dynamics in Lake Taihu: responses to nutrient enrichment and meteorological factors. Sci. Rep. 7 (1), 1–16.

Smol, J.P., 2010. The power of the past: using sediments to track the effects of multiple stressors on lake ecosystems. Freshwater Biol. 55 (s1), 43–59.

Smol, J.P., 2019. Under the radar: long-term perspectives on ecological changes in lakes. Proc. Roy. Soc. B. 286 (1906), 20190834.

Steven, B., et al., 2012. Pyrosequencing of plastid 23S rRNA genes reveals diverse and dynamic cyanobacterial and algal populations in two eutrophic lakes. FEMS Microbiol. Ecol. 82, 607–615.

Taranu, Z.E., Gregory-Eaves, I., Leavitt, P.R., Bunting, L., Buchaca, T., Catalan, J., Domaizon, I., Guilizzoni, P., Lami, A., McGowan, S., 2015. Acceleration of cyanobacterial dominance in north temperate-subarctic lakes during the Anthropocene. Ecol. Lett. 18 (4), 375–384.

Taylor, W.A., 2000. Change-point analysis: a Powerful New Tool For Detecting Changes. Taylor Enterprises, Inc.

Tse, T.J., Doig, L.E., Tang, S., Zhang, X., Sun, W., Wiseman, S.B., Feng, C.X., Liu, H., Giesy, J.P., Hecker, M., Jones, P.D., 2018. Combining High-Throughput Sequencing of sedaDNA and Traditional Paleolimnological Techniques To Infer Historical Trends in Cyanobacterial Communities. Environ. Sci. Technol. 52 (12), 6842–6853.

Wagner, C., Adrian, R., 2009. Cyanobacteria dominance: quantifying the effects of climate change. Limnol. Oceanogr. 54 (6, part2), 2460–2468.

Walsby, A.E., 1994. Gas vesicles. Microbiol. Rev. 58 (1), 94.

Wu, D., Li, T., Dai, J., Zhao, X., Xu, F., Xu, B., Gu, S., Ding, H., Yin, P., Wang, J., Hua, P., Wang, Y., Tian, X., Han, C., Ma, X., 2019. The Health Status Report of Taihu Lake (2018). Taihu Basin Authority of Ministry of Water Resources. December 5, 2019.

Xu, H., McCarthy, M.J., Paerl, H.W., Brookes, J.D., Zhu, G., Hall, N.S., Qin, B., Zhang, Y., Zhu, M., Hampel, J.J., Newell, S.E., Gardner, W.S., 2021. Contributions of external nutrient loading and internal cycling to cyanobacterial bloom dynamics in Lake Taihu, China: implications for nutrient management. Limnol. Oceanogr. 66 (4), 1492–1509.

Yan, D., Xu, H., Yang, M., Lan, J., Hou, W., Wang, F., Zhang, J., Zhou, K., An, Z., Goldsmith, Y., 2019. Responses of cyanobacteria to climate and human activities at Lake Chenghai over the past 100 years. Ecol. Indic. 104, 755–763.

Yan, D., Xu, H., Lan, J., Yang, M., Wang, F., Hou, W., Zhou, K., An, Z., 2020. Warming favors subtropical lake cyanobacterial biomass increasing. Sci. Total Environ. 726, 138606.

Ye, W., Tan, J., Liu, X., Lin, S., Pan, J., Li, D., Yang, H., 2011. Temporal variability of cyanobacterial populations in the water and sediment samples of Lake Taihu as determined by DGGE and real-time PCR. Harmf. Algae 10 (5), 472–479.

Zhang, H., Huo, S., Xiao, Z., He, Z., Yang, J., Yeager, K.M., Li, X., Wu, F., 2021a. Climate and Nutrient-Driven Regime Shifts of Cyanobacterial Communities in Low-Latitude Plateau Lakes. Environ. Sci. Technol. 55 (5), 3408–3418.

Zhang, H., Huo, S., Yeager, K.M., Wu, F., 2021b. Sedimentary DNA record of eukaryotic algal and cyanobacterial communities in a shallow Lake driven by human activities and climate change. Sci. Total Environ. 753, 141985.

Zhang, J., Liu, J., Yuan, Y., Zhou, A., Chen, J., Shen, Z., Chen, S., Zhang, Z., Zhang, K., 2021. Pre-industrial cyanobacterial dominance in Lake Moon (NE China) revealed by sedimentary ancient DNA. Quat. Sci. Rev. 261, 106966.