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ORIGINAL ARTICLE

Sedimentary DNA and pigments show increasing abundance and toxicity of cyanoHABs during the Anthropocene

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Abstract

1. Cyanobacterial harmful algal blooms (cyanoHABs) are assumed to be increasing in abundance and toxicity, but comprehensive analysis of change through time is limited, in part, because some key taxa (e.g., *Microcystis*) leave ambiguous evidence of historical abundance and toxicity. Sedimentary DNA (*sed*DNA) can allow the reconstruction of the cyanobacteria community as well as the frequency of genes specific to cyanotoxin production, enabling us to determine which taxa are present and their potential for toxin-production.
2. Using a combination of droplet digital polymerase chain reaction (ddPCR) and high-throughput sequencing (HTS), we quantified the abundance of cyanobacterial genes of known function and changes in cyanobacteria taxa from *sed*DNA over the last century in nine lakes along a gradient of lake size, depth and trophic state in Minnesota, U.S.A. Using ddPCR, we quantified genes associated with microcystin toxin-producing potential (*mcyE*), total cyanobacteria (CYA, 16S rRNA) and the genus *Microcystis* (MICR, 16S rRNA). Using HTS on a subset of lakes, we investigated how the abundance of this toxin-producing gene covaried with the cyanobacteria community composition. We also compared ddPCR and HTS data to fossil pigments, a well-established palaeolimnological method used to track changes in primary producers over time.
3. Our results showed a significant correlation between MICR and the quantity of *mcyE* gene and cyanobacterial taxa with known toxin-production potential. The abundance of both genes likewise increased concomitantly through time.
4. Community analyses of HTS data showed significant change in cyanobacterial communities commencing c. 1950 when major land-use change in this region led to increased lake productivity, and c. 1990 when *Dolichospermum* and *Microcystis* genera increased in abundance, and the subtropical exotic cyanobacteria *Raphidiopsis raciborskii* and *Sphaerospermopsis aphanizomenoides* became abundant. Cyanobacteria pigment data reflected these changes only in deeper lakes, suggesting issues related to benthic production or biomarker preservation in shallower systems.

5. This study provides evidence for historical development of increasingly toxic cyanoHABs across a diverse set of lakes and illustrates how *sed*DNA may help link changes in the cyanobacteria community to the expression of potentially toxic genes.

KEYWORDS
cyanobacteria, cyanotoxin, fossil pigments, microcystin, *sed*DNA

1 | INTRODUCTION

Cyanobacterial harmful algal blooms (cyanoHABs) are one of the most pressing and prevalent water-quality problems on the planet and are thought to arise from the combined effects of nutrient pollution and climate change (Paerl & Paul, 2012). As cyanobacteria predominate in phytoplankton assemblages, they alter ecosystem structure, favouring deepwater anoxia and disrupting foodwebs (Filstrup et al., 2014, 2016; Heathcote et al., 2016), and can produce toxins harmful to humans and wildlife (Otten & Paerl, 2015). Although increased temperature and elevated phosphorus concentrations result in elevated cyanobacterial biomass (Paerl et al., 2016; Paerl & Scott, 2010; Taranu et al., 2015), factors regulating the production of cyanobacterial toxins (cyanotoxins) apparently are more complex (Boopathi & Ki, 2014), and require additional research to understand the relationship between cyanobacterial biomass and toxicity in ecosystem-scale investigations.

One of the primary difficulties in elucidating the relationship between cyanobacterial abundance and toxicity in whole-lake studies is the absence of the long-term data needed to define how the patterns of toxin production may vary through time. Although cyanobacteria densities have been measured at select sites over several decades (Pomati et al., 2015; Swarbrick et al., 2019), there exist very few measurements of cyanotoxin concentrations that span more than a decade in duration (Hayes et al., 2020; Hobbs et al., 2021; Konkel et al., 2020). Annual- to decadal-scale studies confirm the importance of nutrients and increasing temperatures (Filstrup et al., 2016; Paerl & Paul, 2012), yet these studies usually have focused on fully eutrophied systems, and little is known of how toxicity varies in the early stages of cyanobacterial development or bloom formation. Consequently, there is a growing need to determine whether the toxicity of cyanoHABs is changing through time or whether there is simply an increased acknowledgement of the problems as a consequence of improved toxin monitoring and public awareness.

In principle, palaeolimnological analyses allow reconstruction of both cyanobacterial abundance and toxicity over timescales ranging from years to millennia (Leavitt & Findlay, 1994; Waters et al., 2021). For example, morphological fossils (akinetes, heterocytes) from some colonial cyanobacteria (*Dolichospermum*, *Aphanizomenon*) are well-preserved for centuries to millennia (Bunting et al., 2016; Hillbrand et al., 2014), while lipid-soluble carotenoids and their derivatives allow estimation of total cyanobacterial abundance, as well as several major functional and taxonomic groups (colonial, diazotrophic, Nostocales) (Leavitt & Hodgson, 2001). However, neither technique provides genus- or species-level data, consigning a selective view of community compositions. In particular, it remains unclear how well highly buoyant, small-celled taxa such as *Microcystis*, are preserved in sedimentary deposits as a result of slow sinking and high mineralisation rates. Likewise, although toxins themselves have been recovered from lake sediments spanning millennia (Waters et al., 2021), little is known of the degree to which these compounds are quantitatively preserved in lacustrine deposits. Taken together, it has not yet been possible to evaluate how the toxicity of cyanoHABs may change over decadal to centennial timescales using a retrospective approach.

Sedimentary DNA (*sed*DNA) techniques have emerged as a promising approach to study historical changes in the entire cyanobacterial community and its sources of toxicity (Domaizon et al., 2013; Monchamp et al., 2016, 2018; Savichtcheva et al., 2014). Sediments integrate material from throughout lake ecosystems and should preserve source DNA via physical sorption to particles and the absence of light and oxygen within the sedimentary matrix, including material from all cyanobacteria genera (Capo et al., 2021). As with other labile organic material (Cuddington & Leavitt, 1999; Leavitt & Hodgson, 2001), source DNA is subject to oxidation, photo-oxidation, transformation, dissolution and mineralisation during sinking, with the degree of loss increasing as a function of the time spent in suspension. Extrapolation from pigment-based mass balance studies suggests that *sed*DNA from small, slowly sinking particles, as well as distant sources (e.g., terrestrial material), will be under-represented in the lake sediments (Cuddington & Leavitt, 1999). However, these factors often are relatively constant through time such that the production and deposition of labile biomarkers often are highly correlated in retrospective comparisons of centennial duration (Leavitt & Findlay, 1994). In addition, techniques for collecting, extracting and amplifying *sed*DNA are being refined continually (Capo et al., 2021; Domaizon et al., 2013, 2017; Pal et al., 2015; Tse et al., 2018), such that it is now possible to both reconstruct changes in taxonomic composition (e.g., high-throughput sequencing [HTS]) and quantify the occurrence of genes with known functions (e.g., quantitative polymerase chain reaction [qPCR] or droplet digital PCR [ddPCR]) (Mejbel et al., 2021; Pilon et al., 2019) to estimate both the abundance and function of primary producer populations. When studying cyanoHABs, this dual approach can be used to reconstruct changes in cyanobacterial communities and determine the prevalence of genes responsible for the production of cyanotoxins. This, combined with the use

of fossil pigments, has been suggested as a powerful new approach to reconstructing cyanobacterial abundance and toxicity through time (Picard et al., 2022).

Here, we pair HTS for measuring community data, ddPCR for quantifying target genes associated with cyanobacteria, and the production of the most commonly measured cyanotoxin (microcystin), as well as pigment concentrations for measuring major phototrophic groups, to test the hypothesis that cyanoHAB intensity has increased over the 20th century and to quantify how the inherent toxicity of the blooms may have changed through time. The objectives of this study were to: (a) determine if cyanoHABs across a lake region have been potentially more toxic over time; (b) determine what taxa are most correlated to changes in toxicity; and (c) compare *sed*DNA analyses with well-established palaeolimnological techniques (fossil pigments) to provide an independent proxy for reconstructing changes in cyanoHABs abundance over the last century.

2 | METHODS

2.1 | Core collection and DNA sub-sampling

Sediment cores (~1–2 m in length) were collected from nine study lakes (South Center Lake, Pearl Lake, Madison Lake, St James Lake, Lake Shaokatan, Lake Carlos, Elk Lake, Portage Lake and Upper Red Lake; Table 1) which span the major ecoregions of the state of Minnesota, U.S.A. (western corn-belt plains, peatlands, central hardwood forest, boreal shield). Cores were collected during the open water and winter seasons of 2017–18 by means of a surface piston corer operated via a boat or from the frozen lake surface by rigid Mg-alloy drive rods (Wright Jr., 1991). An additional deeper core section was collected from Madison because previous work on this lake revealed very high sedimentation rates (>1 cm/year). All sediment cores were maintained in the vertical position and stored at 4°C until they were sectioned. Sectioning was performed in the laboratory at 1- or 2-cm intervals depending on lake trophic status, and thus, the plausible sedimentation rate. A subsample was removed from the center of each section using a clean, flame-sterilised spatula and each sample was stored at −20°C under a nitrogen (N_2) atmosphere until thawing for DNA extraction (Pal et al., 2015). The total number of subsamples from each sediment core was determined by sectioning resolution and sample availability, and is listed in Table 1.

TABLE 1 Core locations and the number of sediment sections that were included in this study.

Lake	Latitude (N)	Longitude (W)	Number of subsamples for DNA extraction
Elk Lake	47.1911	−95.2197	8
Lake Carlos	45.9413	−95.3669	22
Lake Shaokatan	44.4081	−96.3396	7
Madison Lake	44.1960	−93.8069	17
Pearl Lake	45.3998	−94.3088	4
Portage Lake	46.9620	−95.1083	12
St James Lake	45.3698	−92.8351	5
South Center Lake	44.9775	−94.6438	25
Upper Red Lake[a]	48.14XX	−94.60XX	15

[a]Exact location of Upper Red Lake core obscured by request of the Red Lake Nation Department of Natural Resources.

2.2 | Radiometric dating

The cores were analysed for ^{210}Pb activity to determine age and sediment accumulation rates for the past 150–200 years. Lead-210 was measured at 15–20 depth intervals in each core through its granddaughter product ^{210}Po, with ^{209}Po added as an internal yield tracer. The polonium isotopes were distilled from 0.5 to 1.0 g freeze-dried sediment at 550°C following pretreatment with concentrated hydrochloric acid (HCl) and plated directly onto silver planchets from a 0.5 M HCl solution (Eakins & Morrison, 1978). Activity was measured for 1–12 days on an Ortec alpha spectroscopy system (Ortec Inc.). Unsupported ^{210}Pb was calculated by subtracting supported activity from the total activity measured at each level; supported ^{210}Pb was estimated from the asymptotic activity at depth (the mean of the lowermost samples in a core). Dates and sedimentation rates were determined according to the constant rate of supply (CRS) model with errors calculated by first-order propagation of counting uncertainty (Appleby, 2001). St James Lake was dredged in 1971, making it impossible to date with the CRS model. For this core, we measured ^{137}Cs based on Ritchie and McHenry (1973) using an Ortec-EGG high-purity, germanium crystal well detector coupled to a Digital Gamma-Ray Spectrometer (Ortec Inc.) and used the abrupt disappearance of ^{137}Cs at 33 cm as a discrete dating marker for the dredging hiatus. Dates were then linearly interpolated from this depth to the surface of the core.

2.3 | DNA extraction

DNA was extracted from sediment samples using the DNeasy Powersoil Kit (Qiagen Inc.) after samples had thawed at 4°C for 12 hr and 0.2–0.5 g of wet sediment was weighed into Power Bead Tubes and recorded to be used for DNA extraction. DNA yield and purity were measured using a NanoDropTM 2000 Spectrophotometer (Thermo Fisher Inc.), and if the quality and quantity of extracted DNA did not meet standards (260:280 nm absorbance ratio between 1.8 and 2.0), wet weight was increased up to 0.9 g to increase yield. A modification to the protocol included removal of humic substances and divalent cations before DNA extraction. To remove these substances, a sterile 10 mM ethylenediaminetetraacetic acid (EDTA), 50 mM Tris–HCl and 50 mM sodium phosphate dibasic heptahydrate ($Na_2HPO_4{\cdot}7H_2O$) at pH 8.0 buffer was used (Zhou et al., 1996). The three-part buffer was added to the sediment, then samples were

vortexed at maximum speed using a Vortex-Genie 2 (Scientific Industries Inc.) for 30 s, centrifuged at 5,000 *g* for 3 min and the supernatant was discarded (Brazeau et al., 2013). The weight of sediments was recalculated following removal of humic substances and divalent cations, and the DNeasy Powersoil Kit protocol was followed yielding 80 μL of DNA extract that were stored at −20°C.

End-point PCR and gel electrophoresis were used to determine if the DNA had retained its proper base-pairing structure. Amplification was conducted using glutamine synthetase (*glnA*) and cyanobacterial 16S rRNA (CYA) targeting primers (Pal et al., 2015; Rinta-Kanto et al., 2005). The reaction mixture for amplification included 12.5 μl of EconoTaq PLUS green 2× master mix (Lucigen Inc.), 2.5 μl of forward primer, 2.5 μl of reverse primer, 6.5 μl of sterilised deionised nuclease-free water and 1 μl of DNA extract. Each amplification included a positive control with genomic DNA extracted from *Microcystis aeruginosa* (CPCC300; Canadian Phycological Culture Center) and a negative control with sterile water. Amplification protocols for *glnA* and CYA are outlined in Table S1. Once amplified, samples were separated by agarose gel electrophoresis (1.5% agarose, 107 V for 35–50 min), stained with GelRed nucleic acid dye (Biotium Inc.), accompanied by GeneRuler 1 kb DNA ladder (Thermo Fisher Inc.).

2.4 | Gene copy quantification

The quantity of target genes in the extracted sediment DNA was measured on all subsamples (Table 1) using the QX200 ddPCR system (Bio-Rad Laboratories Inc.). The genes chosen for analysis included: *glnA* (Hurt et al., 2001), CYA (Rinta-Kanto et al., 2005), *mcyE Microcystin* ADDA subunit (*mcyE*) (Fortin et al., 2010) and *Microcystis*-specific 16S rRNA (MICR) (Rinta-Kanto et al., 2005). The primer sequences for each target gene are outlined in Table S1. To measure the quantity of each gene target for each sediment sample, we used the ddPCR reader and present/absent digital format to clearly separate between positive and negative droplet clusters, where thresholds to separate the target positive and negative droplets were set manually by visual inspections. Each PCR reaction included 11.5 μl of QX200 Evagreen Super Mix (Bio-Rad Laboratories Inc.), 0.23 μl of both forward and reverse primers, 6.04 μl of sterile deionised water and 5 μl of DNA extract. All primers were a stock solution concentration of 10 μM with the following dilution factors for the PCR reaction for each target gene: *glnA* – 1,000×, CYA – 100×, *mcyE* - 4× and MICR – 2×. Before processing the samples through ddPCR, pooled DNA (5 μl from each extraction) was used to optimise the reaction conditions through temperature and dilution gradients. This was performed for each sediment core. Twenty microlitres of reaction mixture were loaded into a DG-8 cartridge with 60 μl of Droplet Digital droplet generation oil and run through the QX200 Droplet Generation machine. Samples then were transferred to a ddPCR 96-well plate and sealed with foil using a PX1 Plate Sealer (Bio-Rad Laboratories Inc.). The sealed plates then were run through a C1000 Touch Thermocycler (Bio-Rad Laboratories Inc.) with different conditions optimised to each primer pair. Once amplified, ddPCR plates were transferred to a QX200 Droplet Reader and analysed using QuantaSoft software (Bio-Rad Laboratories Inc.). In particular, using a precision needle and capillary suction to take in each droplet from the reaction, each droplet was recorded individually based on the fluorescence generated when excited by the double-stranded DNA-bound fluorescing dye from the Supermix. This signal is interpreted as a binary positive for fluorescence, as more dye can bind and fluoresce when more of the target sequence is replicated. By contrast, a negative or null amplification would result in no change in bound fluorescence, indicating no DNA replication and, by extension, the absence of the target sequence. An output file then was generated indicating positive and non-positive amplification peaks and provided with the droplet results numerically, using Poisson distribution statistics to generate a copy number per μl value. Because each droplet is 1 μl in volume and approximately 20,000 droplets are analysed in each reaction, this gives a theoretical detection of 1 copy/μl and translates into approximately 10^3 gene copy numbers per g of wet sediment, for an average limit of detection in gene copy number. Final gene copy numbers were normalised using starting wet weight recorded during DNA extraction.

2.5 | DNA sequencing and analysis

Samples from a subset of three lakes were extracted for DNA and sent for sequencing using Illumina MiSeq PE 2×300 (Illumina Inc.) for the cyanobacterial 16S rRNA genic region (Jungblut et al., 2005; Nübel et al., 1997). These lakes represented deep mesotrophic (Lake Carlos) and eutrophic (South Center Lake) systems, as well as a shallow eutrophic system (Upper Red Lake). Aliquots of 10 μl were submitted if their integrity had been confirmed using agarose gel electrophoresis and the DNA quantity in the sample exceeded 50 ng. Samples were aliquoted into a 96-well plate (Eppendorf Inc.) and shipped at −20°C to Génome Québec (Montréal, Canada) for analysis. Primers used to target cyanobacterial 16S rRNA for HTS are outlined in Table S1.

For sequence data analysis, primers and adapters were removed using Cutadapt (default values) (Martin, 2011). The DADA2 pipeline (Callahan et al., 2016) then was used as a workflow for recovering single-nucleotide resolved amplicon sequence variants (ASVs). Forward and reverse read pairs were trimmed and filtered (parameters: maxN = 0, maxEE = (4, 6), trunclen = (260, 240)), dereplicated, chimera-checked and merged. Taxonomic assignment was performed against the Greengenes (version 13.8) database (DeSantis et al., 2006) via the assignTaxonomy function in DADA2 (Wright, 2016). Because Greengenes is no longer updated, the most significant cyanobacteria also were further phylogenetically assigned using the Cydrasil database (version 3) (Roush et al., 2021) – a manually-curated cyanobacterial database composed of ~1,300 16S rRNA gene sequences. Placements with like weight ratios >0.75 were used to correct Greengenes taxonomic assignments. Significant ASVs were selected based on a redundancy analysis (RDA) of the ASV community matrix constrained to time as ^{210}Pb dates where

taxa were selected if they explained a significant ($p < 0.05$) amount of the variance in the ordination. The RDA was performed using the *vegan* package in R (Oksanen et al., 2013).

2.6 | Fossil pigment analysis

Sedimentary pigments were extracted, filtered and dried under N_2 gas following the procedures of Leavitt and Hodgson (2001). Carotenoids, chlorophylls and pigment-derivative concentrations were quantified using a 1100 HPLC system (Agilent Inc.) following the reversed-phase procedure of Leavitt and Hodgson (2001). Spectral characteristics, chromatographic mobility, and calibration with authentic standards were used to identify pigments from all sources (Leavitt & Carpenter, 1989). Pigment analysis focused on carotenoids characteristic of cyanobacteria (lutein-zeaxanthin), all cyanobacteria (echinenone), filamentous or colonial cyanobacteria (myxoxanthophyll), Nostocales (canthaxanthin) and potentially N_2-fixing cyanobacteria (aphanizophyll), as well as the major parent and derivative compounds of chlorophyll-*a* (Chl-*a*). Pigment concentrations were expressed as nmoles pigment g^{-1} organic matter, an index that is linearly related to algal biomass in the water column in whole-lake experimental calibration (Leavitt & Findlay, 1994).

2.7 | Data analysis

All statistical analyses were performed using the statistical software R (R Core Team, 2013). All plots were created using the R package *ggplot2* unless otherwise noted (Wickham, 2016). The correlation of ddPCR gene copy numbers was assessed using linear regression and Pearson's correlation coefficient. Specifically, we tested the correlation of total cyanobacteria (CYA) and the genus *Microcystis* (MICR) with the toxin-producing gene *mcyE*. Gene copy numbers were $\log_{10}$-transformed to minimise heteroscedasticity of variance. Temporal trends in the sequencing data were analysed using stratigraphic plotting of the significant ASVs (based on an RDA constrained to time), combined with constrained hierarchical clustering analysis (CONISS) of all ASVs. The latter looks at the most similar periods of time in a stratigraphic sequence while preserving the chronological order of the sequence (Grimm, 1987). The broken stick method was used to determine the number of significantly different time periods and distinguish the amount of dissimilarity between groups against that which would be expected by random chance (Bennett, 1996). Stratigraphic plotting and CONISS were performed using the R package *rioja* (Juggins, 2017).

Finally, to measure community-level changes and identify collections of taxa that tended to co-occur in specific proportions, we conducted a Latent Dirichlet Allocation (LDA; Blei et al., 2003) of the ASV matrix. Following the methods presented by Christensen et al. (2018), we used the *topicmodels* package in R to reduce the high-dimensional ASV matrix to a few "topics", or community types, and track how these different community types varied over time. Before running the LDA, we removed the permanently rare taxa by only keeping ASVs with >2% relative abundance in at least one time point. To determine the number of community-types to retain, we compared models with up to five community-types and kept the number with the lowest Akaike information criterion (AIC). This showed that four (Lake Carlos) to five (South Center and Upper Red lakes) community-types fitted the data well. Once again, following Christensen et al. (2018), we then applied change-point models using multinomial generalised linear models fit with the *nnet* package in R (Venables & Ripley, 2002) to identify breakpoints in community types over time. Briefly, the approach tests for rapid changes in the mean value of each community type; a rapid change in the mean value and a narrow distribution of the estimated change-point location is interpreted as more certainty about the timing of the change point. We fitted up to four change points for each lake time series and determined the optimal number of change points by comparing the log-quasi-likelihood of the four competing models.

3 | RESULTS

3.1 | *Microcystis* as potential main microcystin producer

Sedimentary DNA was successfully extracted from all sections of cores, including those which were estimated to be up to 250 years old. In general, the concentration of DNA increased up-core with a sharp spike at the top-most sections (Figure 1). Analysis of temporal patterns of the specific genic regions amplified via ddPCR showed that *glnA*, a broadly conserved bacterial housekeeping gene, was detected in all lakes and at all ages (Figure 2). This indicates that bacterial DNA was successfully extracted even in the oldest of sediments and also mirrors the pattern of increased concentration over time seen in the total *sed*DNA results (Figure 1). CYA was correlated to *glnA* ($R^2 = 0.51$, $p < 0.001$), indicating that total bacterial productivity increased at the same time as cyanobacteria abundance. Both CYA ($R^2 = 0.31$, $p < 0.001$) and the MICR ($R^2 = 0.49$, $p < 0.001$) were significantly correlated to the concentrations of *mcyE* gene, required for microcystin production. Overall, these results showed a stable background rate of microbial production in these lakes from the late 18th or early 19th Century until the 1950s. This was followed by a period of increasing total bacterial, cyanobacterial and particularly *Microcystis* production in most lakes, which in turn led to a higher toxin-producing potential. This trend was not monotonic in all lakes, and some showed a decrease at c. 1970 (e.g., Madison Lake, South Center Lake, Lake Carlos).

3.2 | Cyanobacterial community *sed*DNA metabarcoding reveals key compositional transitions

High-throughput sequencing was conducted on three of the nine lakes examined in this study (Lake Carlos, South Center Lake, Upper

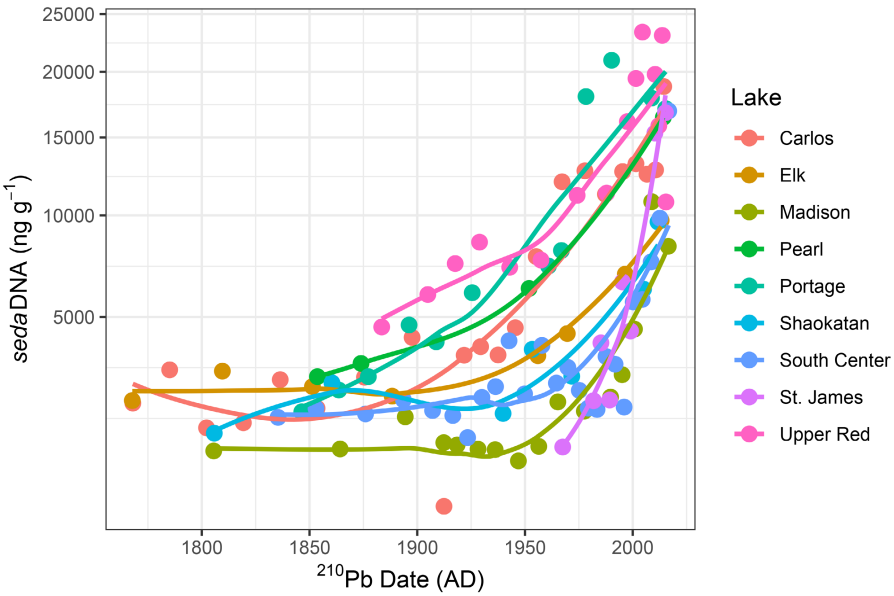

FIGURE 1 Concentration of total sedimentary DNA (*sed*DNA) versus ^{210}Pb date in sediment cores from nine Minnesota lakes in ng/g wet sediment.

Red Lake). ASVs were assigned to the lowest possible taxonomic unit; however, many species and strains of cyanobacteria are not yet represented in the available microbial taxa libraries. In an attempt to improve the taxonomic resolution for cyanobacteria obtained with the Greengenes database, cyanobacterial ASVs were phylogenetically assigned to reference sequences from the Cydrasil database (Roush et al., 2021). Following this, any unassigned ASVs previously classified with 100% bootstrap confidence assignments within the cyanobacteria phylum using Greengenes were generically designated as "cyanobacteria". All ASV read counts were converted to absolute abundances by multiplying each ASV relative abundance by the total number of CYA gene copies as determined by ddPCR above (Jian et al., 2020; Tettamanti Boshier et al., 2020).

As shown in the ddPCR results (Figure 2), total cyanobacteria increased through time in all three lakes. Sequencing data revealed that this primarily was a result of the increase of the orders Nostocales and Chroococcales (Figure 3). Within Nostocales, *Dolichospermum* showed the greatest increase across all three lakes, with the largest changes in abundance taking place after 1990 (Figure 3). South Center Lake is the only lake which had DNA from the invasive *Sphaerospermopsis aphanizomenonoides* (ASV 42; Table S2), a species found to co-occur with the sub-tropical exotic cyanobacterium *Raphidiopsis raciborskii* (ASV 45; Table S2) (Jovanović et al., 2016). This species was abundant in this system since the 1990s and detectable since 1950 (Figures 3 and 4b). In all three lakes, the majority of the identified Chroococcales were of the genus *Microcystis*; however, an even larger percentage of this order was represented by ASVs which could not be resolved at the genus level (Figure 3). Although placed under Chroococcales by Greengenes, these ASVs may come from several genera of morphologically similar unicellular coccoid picocyanobacteria based on Cydrasil (e.g., *Radiocystis* [ASV 32, 36 and 61]; Table S2).

Analysis of the sequenced cyanobacterial community using CONISS identified two to three historical zones in the study lakes (Figure 4). The historical record of Lake Carlos was split into significant stratigraphic zones before and after ~1990 (Figure 4a), with sustained levels of the microcystin-producing genera *Dolichospermum/Anabaena* (ASVs 16, 18 and 54) and *Microcystis* (ASV 83), in the uppermost samples. These increases were correlated ($R^2 > 0.55$) with both the *mcyE* and MICR genes. South Center Lake exhibited three significant stratigraphic zones, distinguishing material deposited after c. 1988, where ASVs from the order Nostocales, particularly *Dolichospermum*, became more common. A secondary distinction of DNA deposited after c. 2004 was marked by elevated concentrations of most ASVs and the *mcyE* gene (Figure 4b). The historical record of Upper Red Lake exhibited two significant zones, split at c. 1990, and a third zone dating back to c. 1930 (Figure 4c). Sediments deposited after c. 1990 reflect elevated markers for several genera of Nostocales, as well as the genus *Microcystis*. Furthermore, there were two spikes in *mcyE* correlated with increases in total *Microcystis* spp. (ASVs 38 and 58). Unlike the other two lakes, Upper Red Lake was the only lake to have markers for the benthic cyanobacterium *Limnolyngbya* (ASVs 6 and 53).

The LDA analysis and change-point models suggested that the timing of major community-level change (Figure 5, S1 and S2) occurred earlier than the major taxa-level change detected by CONISS (Figure 4). When comparing the community-type change points among the three lakes, there was a greater certainty in the deeper lakes (narrow distribution, centred on c. 1940 AD) relative to the shallower Upper Red Lake (wider distribution, centred on c. 1960 AD) (Figure S2). Across all three lakes, however, the change points in community-types followed the changes in dominant taxa shown in the stratigraphic plots (Figure 4). For instance, in Lake Carlos, the

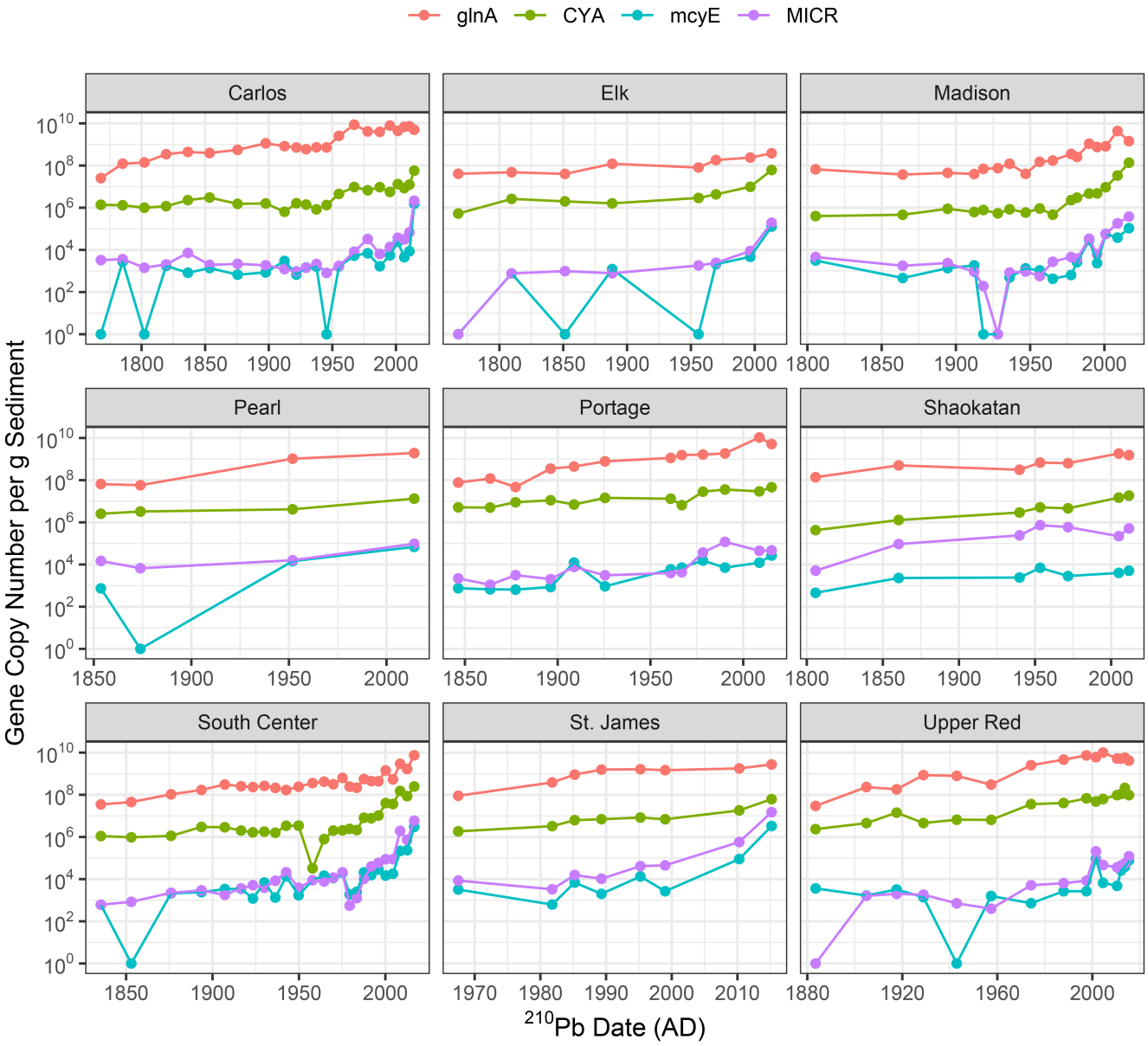

FIGURE 2 Gene copy number (GCN/g wet sediment) versus ^{210}Pb date for all lakes in this study, where the limit of detection is approximately 10^3 GCN/g wet sediment. The four primers measured were glutamine synthetase (*glnA*; orange), cyanobacterial 16S rRNA (CYA; green), *Microcystis*-specific 16S rRNA (MICR; purple) and the microcystin toxin-producing subunit *mcyE* (blue).

1940 change point was marked by a switch from community-type 1 (mainly Chroococcales: ASVs 22, 25, 31, 33, 37 and 46) towards the dominance of community-type 2 (mainly Nostocales: ASVs 1, 5, 7, 9 and 16) (Figures 5a, S1a). Historically, South Center Lake was dominated by community-types 1 and 4, where ASVs 3 and 10 (Chroococcales) were most common and replaced by community-type 2 (ASV 2) c. 1950 AD, and most recently by community-types 3 and 5 (dominated by *Dolichospermum*) (Figures 5b, S1b). In Upper Red Lake, the change point tracked the more gradual change from a community-type 4 dominance (largely Nostocales: ASVs 1, 4, 6, 23, 49 and 41), to a time where community types 4 and 5 (largely Chroococcales and Synechococcales: ASVs 6, 14 and 26) were found in equal proportions (Figures 5c, S1c).

3.3 | Corroborated trends in fossil pigments

Stratigraphic profiles of fossil pigments specific to cyanobacteria were compared to both the ddPCR and HTS results to quantify the relationship between *sed*DNA and traditional biomarkers of phototroph abundance (Figure 6). In general, both the total cyanobacteria pigment (as echinenone) and the CYA gene copies increased

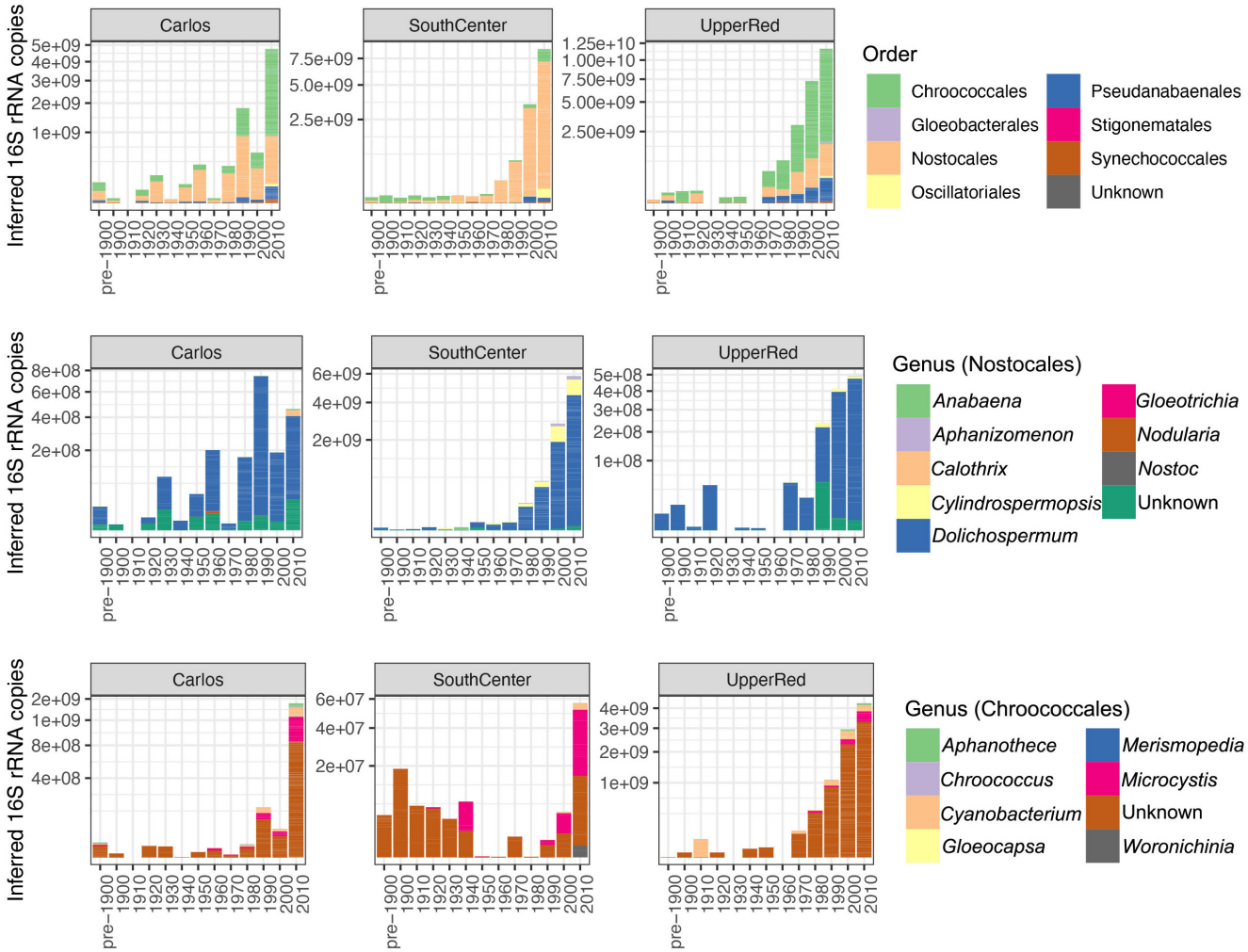

FIGURE 3 Inferred cyanobacterial 16S rRNA abundance (GCN/g wet sediment) versus time from high-throughput sequencing of sediment cores from three lakes. Top panels show the distribution by order, and middle and bottom panels show the genera present within the Nostocales and Chroococcales, respectively. Abundance data are binned within each core by decade. White lines represent subdivisions of the next lowest taxonomic level within each coloured bar (e.g., genus within order). The *y*-axes are square-root transformed for better data visualisation. If order or genus was unknown, the next highest taxonomic assignment was indicated.

steadily after c. 1930–1950 AD in Lake Carlos and South Center Lake (Figures 2 and 6a,b). Likewise, a bimodal peak in aphanizophyll, a pigment produced by diazotrophic cyanobacteria (Leavitt & Hodgson, 2001), began to increase much later (c. 1950), which coincided with an increase in many ASVs associated with Nostocales, such as the N_2-fixing genus *Dolichospermum* (Figures 3, 4a and 6a). In South Center Lake, the concentration of the colonial cyanobacteria pigment myxoxanthophyll also was correlated to the abundance of ASV 2, that was likely to have been a species of *Dolichospermum* ($R^2 = 0.54$, $p = 0.002$). By contrast, the pigment profile from Upper Red Lake was less closely related to changes in *sed*DNA, as pigments suggested a period of declining cyanobacterial abundance between c. 1950 and 1980, whereas there was no evidence for this decline in the *sed*DNA profiles. The rebound and peak of echinenone at the surface of Upper Red Lake is accompanied by the appearance and rise of the pigment aphanizophyll. Although there are increases in several ASVs of the genus *Dolichospermum* in this core, most began well before (c. 1970 AD) the peak in aphanizophyll (c. 2000). Finally, the Chl-*a* to phaeophytin-*a* ratio is shown for all cores as a relative measure of degradation in labile organic biomarkers. This measure shows a sharp peak in the topmost two to three samples of all cores (Figure 6).

4 | DISCUSSION

4.1 | Trajectory of cyanoHAB abundance and toxicity

By combining ddPCR (to target gene copy numbers) with HTS, we reconstructed the history of cyanoHABs toxicity on a scale from decades to centuries. Our results indicate that cyanoHABs

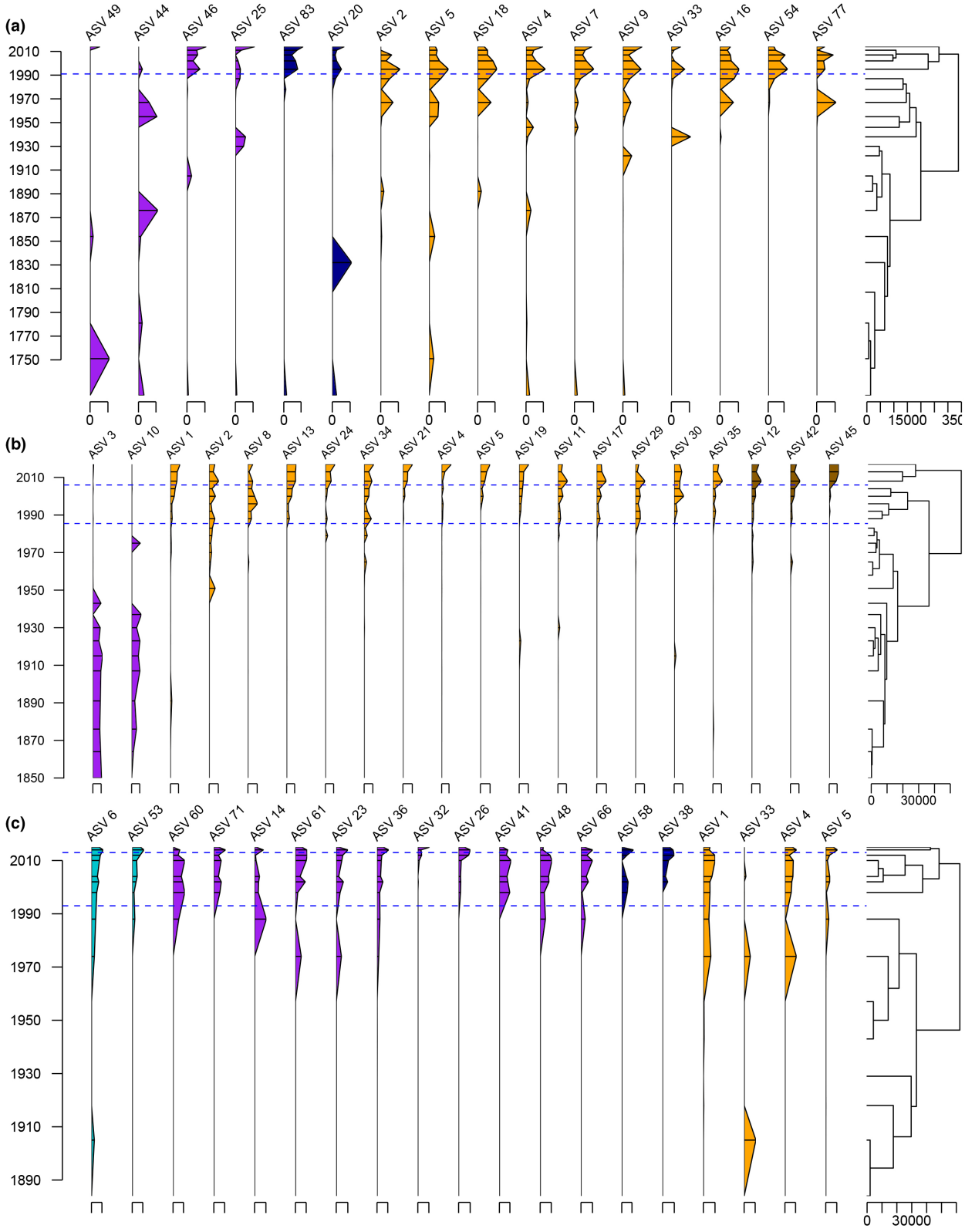

FIGURE 4 Stratigraphic diagrams of high-throughput sequencing data from sediment cores of (a) Lake Carlos, (b) South Center Lake and (c) Upper Red Lake. Coloured polygons represent species most correlated to time based on a redundancy analysis constrained by sediment age. Purple polygons represent Chroococcales, dark blue *Microcystis*, orange *Dolichospermum*, brown *Raphidiopsis/Sphaerospermopsis* and turquoise *Limnolyngbya*. Dendrogram represents constrained cluster analysis (CONISS) results with significant zones separated by blue dashed lines. Inferred 16S rRNA abundances (*x*-axis) were square-root transformed.

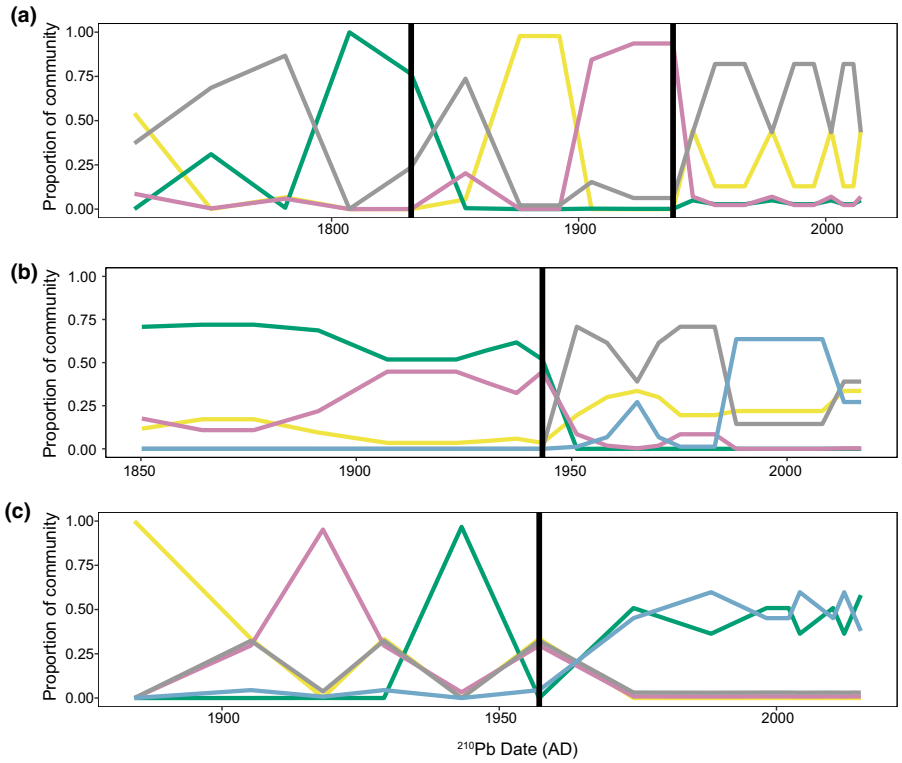

FIGURE 5 Latent Dirichlet Allocation (LDA) results showing the estimated prevalence of each community-type before and after major change points (black bar) in (a) Lake Carlos, (b) South Center Lake and (c) Upper Red Lake.

increased during the 20th century through quantitative analysis of genes specific to cyanobacteria abundance (CYA) and the toxin-producing genus *Microcystis* (MICR) across the majority of the nine lakes examined in this study. This increase occurred during a backdrop of agricultural intensification and urbanisation in this region that significantly increased the supply of nutrients to lakes (Anderson et al., 2013). These data corroborate the previously assumed trajectory of lakes in the Midwestern US towards greater primary productivity and cyanobacterial dominance (Heathcote et al., 2014), but offer more direct evidence through *sed*DNA-based cyanobacteria proxies.

The increase in MICR and CYA was accompanied by elevated concentrations of *mcyE* associated with microcystin production. However, this reconstructed toxin-producing potential (*mcyE*) was more strongly correlated to MICR than CYA, indicating a coupling of potential microcystin toxicity and the abundance of the genus *Microcystis*, despite the fact that other genera common to these lakes may produce this toxin (e.g., *Dolichospermum*) (Otten & Paerl, 2015), and that not all *Microcystis* species or strains of *Microcystis* produce the *mcyE* gene (Pérez-Carrascal et al., 2019). Related to this coupling, there are examples of the occurrence of MICR and the absence of *mcyE* during certain time periods in Carlos, Elk, Pearl, South Center and Upper Red lakes, which could indicate periods of low or no potential toxicity. There also is one core section where MICR and *mcyE* DNA were at the limit of detection, but CYA was still detected. The nested nature of these genetic proxies that range from all prokaryotic organisms (*glnA*), to all cyanobacteria (CYA), to the genus *Microcystis* (MICR), to a toxin-producing gene (*mcyE*), provides insight into both the long-term trajectory of cyanobacterial abundance and toxicity in these lakes as well as episodic events (spikes or disappearances).

4.2 | Cyanobacteria community change

The increases in cyanobacterial abundance described above was primarily attributed to two orders, Nostocales and Chroococcales, which contain the most abundant genera of toxin-producing cyanobacteria in the region. In all three lakes, HTS results showed that Nostocales was primarily composed of the genus *Dolichospermum*, which includes a number of microcystin-producing species common to the region (*D. flosaquae*, *D. crassum*, *D. lemmermannii*) (Heiskary et al., 2016). The taxonomic resolution of Chroococcales was more uncertain, with the majority of ASVs being assigned to an unknown genus, although *Microcystis* was the next most common assignment with several species being common to the region (*M. aeruginosa*, *M. wesenbergii*) (Heiskary et al., 2016). The uncertainty of taxonomic assignment within Chroococcales could be related to the lack of available sequences for many of the picoplanktonic species of that order in existing cyanobacteria taxa libraries; however, it also could point to better preservation of DNA in the Nostocales, where species may produce degradation resistant resting cells (akinetes).

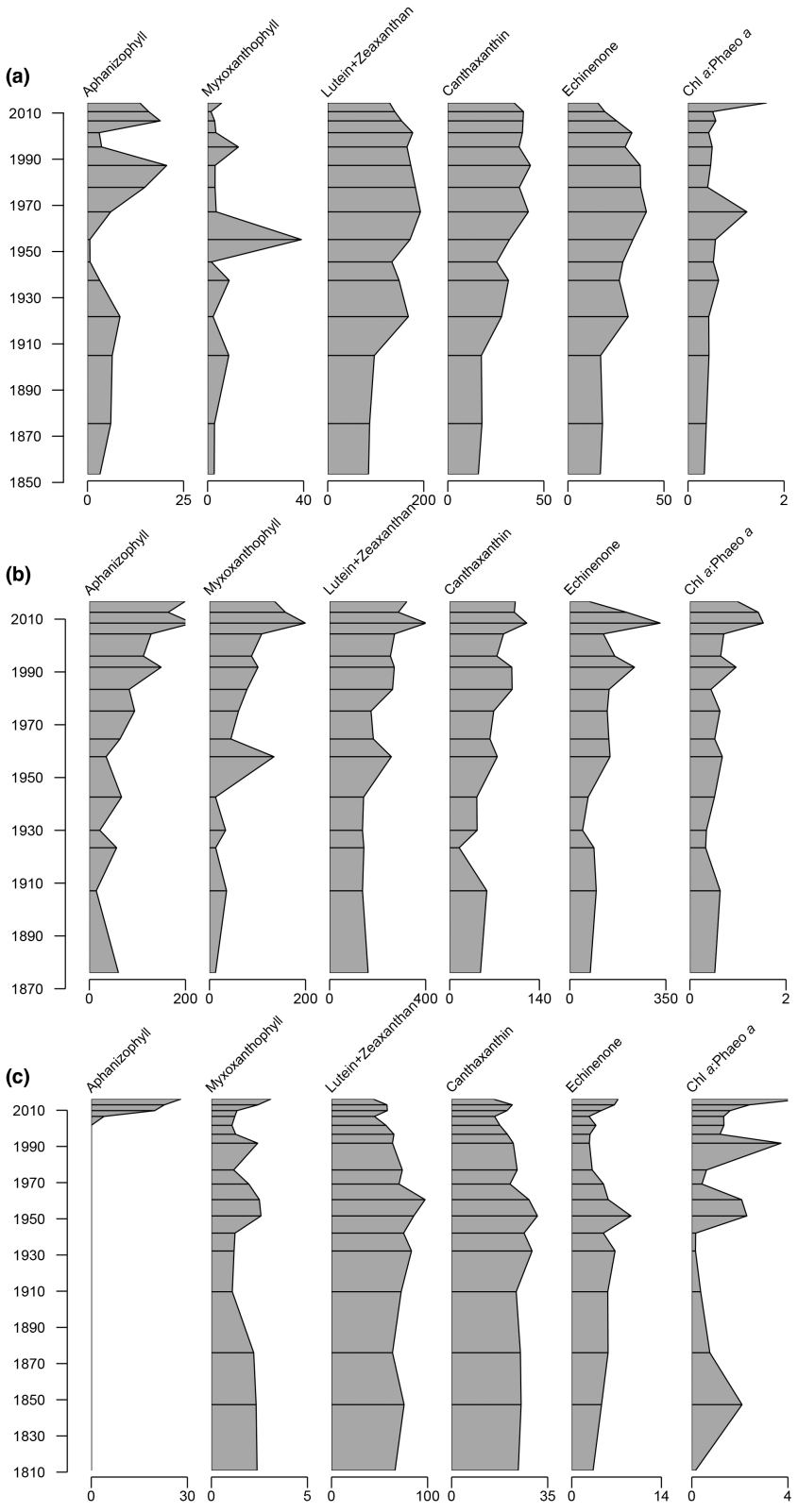

FIGURE 6 Stratigraphic diagram of fossil pigments specific to cyanobacteria in sediment cores from (a) Lake Carlos, (b) South Center Lake, and (c) Upper Red Lake. The ratio of chlorophyll-*a* to phaeophytin-*a* also is shown as an indicator of the relative influence of degradation within each section. Pigment concentrations are in ng/g dry organic matter.

CONISS results suggest a common stratigraphic zone of community change across all three lakes that occurred in the late 1980's to early 1990's. This timing coincides with the period of most rapid warming in the Upper Midwest (USA) (Edlund et al., 2022), and is over a century after initial land clearance and Euro-American settlement of the region (Anderson et al., 2013). In Carlos and Upper Red lakes this zone was characterised by increases in unknown genera of Chroococcales, *Microcystis* and *Dolichospermum*, although in many cases the onset of this increase predated the stratigraphic zone by several decades. Additionally, Upper Red Lake showed an

increase in the benthic genus *Limnolyngbya* at c. 1970, highlighting the importance of considering both planktonic and benthic toxin-producers in shallow lakes.

South Center Lake likewise had a stratigraphic demarcation in the late 1980's that coincided with the rise of a number of ASVs from the genus *Dolichospermum*; however, this also was preceeded in this system with a decline in the most abundant ASVs from the order Chroococcales. In contrast to Upper Red and Carlos lakes, this transition represents a clear switch from non-diazotrophic to diazotrophic cyanobacteria as the dominant DNA preserved in the sediments. Although long-term monitoring data are not available for South Center Lake, spot-sampling has occurred and demonstrates that the lake does appear to be N-limited for at least parts of the year (Summer N:P by mass = 10; Sauer et al., 2022).

There is a second transition in South Center Lake arising from the increasing abundance of ASVs associated with the subtropical cyanobacteria *Raphidiopsis raciborskii* and *Sphaerospermopsis aphanizomenonoides* at c. 1960 (ASVs 12, 42 and 45). These species have gained attention since the early 2000's as they are increasingly found in lakes outside their previous temperature range globally (Antunes et al., 2015), and have been reported in other states in the Upper Midwest US (Hong et al., 2006). *Raphidiopsis raciborskii* was first identified in South Center Lake via microscopy by state agency personnel in 2013 (Heiskary et al., 2016); however, earlier published reports from other lakes in Minnesota exist dating back to 1966 (Hill, 1969). The results from this study support a much earlier introduction of these sub-tropical species to South Center and perhaps other north temperate lakes in the region, indicating that it was abundant going back to the 1990's with the first detectable DNA in sediment sections dated to c. 1950. In terms of the identification of exotic species via *sed*DNA, it must be noted that 16S rRNA is insufficient to obtain a precise taxonomic assignment of cyanobacteria at the species level due to the relatively short sequences (~300 bp) used here (Salmaso et al., 2022). However, the lack of other closely-related native species (e.g., no other species of the genus *Raphidiopsis* are known to be native to Minnesota) and the confirmation of its presence in contemporary water samples from this lake via light microscopy (Heiskary et al., 2016) independently corroborate this taxonomic identification.

In addition to measuring taxa-level change via CONISS, this study also examined community-level changes from each lake using LDA and change-point models. Interestingly, community-level changes were detected earlier (c. 1940–1960) than taxa-level changes (c. 1980–1990), and are more in line with previously noted periods of change in primary producer palaeoindicators attributed to land-use change in the region (Anderson et al., 2013; Heathcote et al., 2014; Heathcote & Downing, 2012). This contrast highlights the complementarity of using both approaches, in that while CONISS tests for finer taxonomic changes (by considering all taxa) and distinct temporal clusters (i.e., divisive clustering where a time frame is equated to one cluster), LDA follows coarser changes in groups of associated taxa and allows the relative proportion of these groups to co-vary (i.e., fuzzy clustering where a time frame could be composed of multiple clusters a.k.a. community-types). Furthermore, whereas CONISS and the stratigraphy plots did not allow us to view the dynamics of all taxa, the LDA identified the taxa, rare or common, that tended to rise and fall together. Overall, the LDA helped highlight when the community as a whole was first destabilised, whereas CONISS focused on the timing of pronounced change in the abundance of taxa within these communities.

4.3 | Comparing pigments and *sed*DNA metabarcoding for identifying cyanoHABs

Fossil pigment results from the deeper lakes (Lake Carlos, South Center Lake) supported the trend seen in both total cyanobacteria gene copy number (CYA) and the cyanobacteria community based on *sed*DNA metabarcoding. Fossil pigment concentrations from the shallower Upper Red Lake were more difficult to reconcile with our *sed*DNA results. The majority of the fossil pigments associated with cyanobacteria (lutein-zeaxanthin, canthaxanthin, echinenone) decreased after 1950, contrasting with increased CYA and MICR gene copy numbers and the abundance of Nostocales and Chroococcales over this same time period. There also was an appearance and spike in aphanizophyll that appeared after 2000, near the top of the core, that is not explained by a coincident increase in any identified diazotrophic cyanobacteria taxa. Presently, we cannot explain this discrepancy, and future studies providing a more robust comparison of trends in shallow, polymictic lakes versus deep, dimictic lakes are required to evaluate whether the observed stratigraphic differences between *sed*DNA and other organic biomarkers in the former lake type are a consequence of preservation biases (i.e., warmer, more oxygenated bottom waters and frequent mixing at the sediment–water interface in polymictic lakes). Future studies should include direct comparisons of long-term monitoring, *sed*DNA, and other fossil metrics to better constrain the relative importance of production and preservation as controls of stratigraphic patterns of fossil variation (e.g., Leavitt & Findlay, 1994).

4.4 | Potential for biomarker degradation

Our results showed sharp increases across multiple *sed*DNA-based biomarkers related to microbial production, including *sed*DNA, amplified microbial gene primers and cyanobacterial ASVs generated from 16S rRNA HTS. Examination of the temporal trends in the *sed*DNA concentrations, from which all subsequent analyses are based, showed an increase after c. 1990 that may be influenced by first-order loss degradation processes routinely seen in labile organic molecules (i.e., exponential decay over time) (Gälman et al., 2008; Leavitt & Carpenter, 1990). Further research is needed on the controls of *sed*DNA preservation and its similarity or difference relative to other organic geochemical proxies. Nonetheless,

overall patterns also are consistent with a period of prolonged land disturbance and eutrophication during the 20th Century, with elevated *sed*DNA concentrations beginning as early as 1950 in most cores, similar to trends seen in other organic markers (e.g., organic carbon accumulation, biogenic silica flux) and the effects of land-use change and fertiliser application in this region (Anderson et al., 2013; Heathcote et al., 2014; Heathcote & Downing, 2012). Additionally, organic components of sediments >10 years of age generally were stable with very low decomposition rates (<1% by mass per year) (Gälman et al., 2008), and work by Capo et al. (2017) demonstrated the surprising stability of *sed*DNA in sediments of a dimictic lake where water temperatures above the sediment remain cool, and oxygen and light are limited.

Fossil pigment records provide additional information in terms of the relative importance of first-order decomposition processes, and here we have included a direct measure of pigment preservation as the ratio of labile Chl-*a* to its immediate product, chemically-stable pheophytin-*a* (Leavitt & Carpenter, 1990). In the three cores where pigment profiles are available, we see only limited evidence of changes in organic matter preservation downcore, with a similar pattern of sharp increase in this ratio in the upper one to three sections. These patterns suggest not only that there is not a prolonged period of post-depositional diagenesis, but also that increasing trends in the youngest sediments should be interpreted with caution (Leavitt & Hodgson, 2001).

5 | CONCLUSIONS

The combination of quantitative gene frequency data (ddPCR) with HTS allowed the reconstruction of the history of cyanoHABs toxicity on a scale from decades to centuries. We demonstrated that cyanoHABs increased during the 20th Century across the majority of the nine lakes in this study through quantitative analysis of both CYA and MICR genes. This increase was accompanied by elevated concentrations of the toxin-producing subunit *mcyE*, a biomarker which was correlated more strongly to the genus-specific primer for *Microcystis* (MICR) than to the marker for total cyanobacterial abundance (CYA). With the addition of HTS data for three of the nine lakes, the strains of *Microcystis* and other important cyanobacterial genera known to produce microcystin (e.g., *Dolichospermum*) were identified, although these data could be further improved by increasing the diversity of genome sequences available in public cyanobacteria taxa libraries. Invasive subtropical cyanobacteria species also were documented to have been abundant in South Center Lake since the 1990s, and its introduction date to this lake, and region, pushed back several decades (c. 1960). When these data were compared to analyses of sedimentary pigment concentrations, consistent patterns were seen in the deeper lakes (maximum depth >20 m; Lake Carlos and South Center Lake), but issues with preservation may have made comparison difficult in the shallower and naturally eutrophic Upper Red Lake. These results highlight how combining multiple *sed*DNA methods using a multi-proxy palaeolimnological approach can advance our understanding of the timing and toxicity of cyanoHABs in freshwater systems.

AUTHOR CONTRIBUTIONS

Conceptualization: AJH, ZET, FRP. Developing methods: AJH, ZET, NT, MM, PRL, FRP. Data analysis: AJH, ZET, NT. Conducting the research, data interpretation, writing: AJH, ZET, NT, MM, PRL, FRP.

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DATA AVAILABILITY STATEMENT

Raw sequence data have been deposited into the NCBI GenBank under BioProject no. PRJNA720698. All other data will be made available upon reasonable request to the corresponding author.

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