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# Evaluating the use of lake sedimentary DNA in palaeolimnology: A comparison with long-term microscopy-based monitoring of the phytoplankton community

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## Abstract

Palaeolimnological records provide valuable information about how phytoplankton respond to long-term drivers of environmental change. Traditional palaeolimnological tools such as microfossils and pigments are restricted to taxa that leave sub-fossil remains, and a method that can be applied to the wider community is required. Sedimentary DNA (sedDNA), extracted from lake sediment cores, shows promise in palaeolimnology, but validation against data from long-term monitoring of lake water is necessary to enable its development as a reliable record of past phytoplankton communities. To address this need, 18S rRNA gene amplicon sequencing was carried out on lake sediments from a core collected from Esthwaite Water (English Lake District) spanning ~105 years. This sedDNA record was compared with concurrent long-term microscopy-based monitoring of phytoplankton in the surface water. Broadly comparable trends were observed between the datasets, with respect to the diversity and relative abundance and occurrence of chlorophytes, dinoflagellates, ochrophytes and bacillariophytes. Up to 20% of genera were successfully captured using both methods, and sedDNA revealed a previously undetected community of phytoplankton. These results suggest that sedDNA can be used as an effective record of past phytoplankton communities, at least over timescales of <100 years. However, a substantial proportion of genera identified by microscopy were not detected using sedDNA, highlighting the current limitations of the technique that require further development such as reference database coverage. The taphonomic processes which may affect its reliability, such as the extent and rate of deposition and DNA degradation, also require further research.

**KEYWORDS**
Lake, palaeolimnology, phytoplankton, sedDNA, time-series

## 1 | INTRODUCTION

Phytoplankton play a vital role in lake ecosystems as primary producers at the base of aquatic food webs. Changes in their community composition in response to environmental change can have extensive ecological and biogeochemical implications (Litchman et al., 2015). Many lakes worldwide are experiencing rapid rates of change in response to multiple interacting stressors, but our understanding of how phytoplankton communities respond is limited (Carpenter et al., 2011; Heino et al., 2009). Multi-decadal records of phytoplankton communities can enable us to understand how they have responded to past environmental change and provide insight for how they may respond in the future (Willis et al., 2010).

Detailed long-term monitoring of the phytoplankton community is restricted to a relatively small number of well-studied lakes (Burlakova et al., 2018; Hampton et al., 2008). Where long-term monitoring records are not available, a range of proxies can be used to produce historical records of the phytoplankton community, such as microfossils and pigments extracted from lake sediment cores (Davidson & Jeppesen, 2013). Microfossil analysis is a widely used technique but is limited to organisms with well-preserved and morphologically distinct remains, such as diatom frustules (Hembrow et al., 2014; Leira, 2005) and the resting cysts produced by some dinoflagellates (Drljepan et al., 2014). Photosynthetic pigments can provide a record of eukaryotic algal and cyanobacterial community composition, abundance and primary productivity (Griffiths et al., 2022; Kpodonu et al., 2016; Makri et al., 2019; Watanabe et al., 2012), but many pigments are not specific enough to enable taxonomic identification beyond the class level (Gong et al., 2020). These limitations of traditional palaeolimnological techniques highlight the need for complementary and improved methods which can be applied to a wider diversity of organisms, such as sedimentary DNA (sedDNA).

sedDNA is a promising palaeolimnological approach which can be used to reconstruct past communities using DNA preserved within lake sediment cores (Edwards, 2020). DNA from living organisms is deposited in the lake sediment, where it is preserved and progressively buried over time. This DNA can then be extracted from layers of a sediment core and sequenced to produce a temporal record of lake communities (Capo et al., 2021; Thorpe et al., 2022). sedDNA offers many potential benefits compared to traditional palaeolimnological techniques. For example, a relatively high taxonomic resolution can be achieved, and high-throughput amplicon sequencing can process many hundreds or even thousands of samples relatively quickly (Bohmann et al., 2022; Gong et al., 2020; Mejbel et al., 2021). A wider diversity of organisms can be studied using sedDNA, including those previously overlooked with microfossil analysis due to a lack of well-preserved and morphologically distinct remains (Domaizon et al., 2017). The applicability of sedDNA to the wider community, including eukaryotic algae (Capo et al., 2016), bacteria (Thorpe et al., 2022), zooplankton (Tsugeki et al., 2022) and macrophytes (Stoof-Leichsenring et al., 2022) allows a more complete reconstruction of ecosystem structure which may, in turn, facilitate inferences on past trophic interactions (Barouillet et al., 2022; Ellegaard et al., 2020).

sedDNA is becoming more widely used in palaeolimnology, but there are currently some uncertainties surrounding the deposition and taphonomy of DNA in lakes (Capo et al., 2021, 2022). The extent and rate of DNA degradation may vary among taxa and depend upon the state in which DNA is deposited. For example, intracellular DNA or DNA bound to mineral particles is typically better protected from degradation processes, such as oxidation, hydrolysis and bacterial degradation than free extracellular DNA (Giguet-Covex et al., 2019; Mauvisseau et al., 2022). The depositional and degradational processes DNA is subject to could affect the ability of sedDNA to provide a reliable record of past phytoplankton communities. Although sedDNA has previously been found to be broadly comparable with records from diatom frustules (Anslan et al., 2022) and photosynthetic pigments (Picard et al., 2022; Tse et al., 2018), these traditional palaeolimnological tools are also subject to pre- and post-depositional losses and subsequent biases. Validation of sedDNA against long-term monitoring of phytoplankton in the water column is therefore needed to further the development of sedDNA as a reliable and robust record of past microbial communities.

To address this need, we analyse and compare sedDNA and water column phytoplankton data from Esthwaite Water, a relatively small lake in the English Lake District which has experienced well-documented changes in human activity and has undergone substantial eutrophication in recent decades (Dong et al., 2011; Maberly et al., 2011). Lake physicochemical conditions and the phytoplankton community have been continually monitored since 1945, providing a detailed record against which palaeolimnological records can be compared and validated. Esthwaite Water has been the site of several studies investigating seasonal trends in phytoplankton communities in the water column (Feuchtmayr et al., 2012; Talling & Heaney, 2015), and palaeolimnological studies of the bacterial and cyanobacterial community as measured by sedDNA (Thorpe et al., 2022), and the microbial eukaryotic community as measured with photosynthetic pigments (Moorhouse et al., 2018) and diatom frustules (Bennion et al., 2000; Dong et al., 2011, 2012). Our study, which combines concurrent microscopy-based monitoring and sedDNA records, is therefore uniquely placed to determine whether sedDNA is a reliable tool for reconstruction of past trends in phytoplankton community composition.

## 2 | MATERIALS AND METHODS

### 2.1 | Study site

Esthwaite Water (54°21.56′N, 2°59.15′W) is located within the Lake District National Park, Cumbria, UK, and has a catchment area of 17 $km^2$, surface area of 0.96 $km^2$, and mean and maximum depths of 6.9 m and 16 m respectively (Maberly et al., 2011; Mackay et al., 2012). Human activities in Esthwaite Water and its catchment, including construction of a wastewater treatment works in 1973 and

fish farming between 1983 and 2009, led to this lake becoming one of the most eutrophic lakes in the Lake District (Dong et al., 2011; Maberly et al., 2011).

## 2.2 | Long-term environmental monitoring record

Physiochemical conditions in Esthwaite Water have been continuously monitored on a weekly to fortnightly basis from 1945 by the Freshwater Biological Association (FBA), and then from 1989 by the UK Centre for Ecology & Hydrology (UKCEH). Measurements and depth integrated surface water samples (0–5 m) were collected from the deepest point of Esthwaite Water, including surface water temperature, pH and alkalinity, and the concentration of total phosphorus (TP), soluble reactive phosphorus (SRP), nitrate-nitrogen ($NO_3$–N), ammonium-nitrogen ($NH_4$–N) and chlorophyll *a* (Chl *a*). Winter SRP was calculated as the mean SRP from December to February. The full dataset is available at: https://doi.org/10.5285/87360d1a-85d9-4a4e-b9ac-e315977a52d3 (Maberly et al., 2017), and annual means for these variables have previously been described (Thorpe et al., 2022).

## 2.3 | Long-term phytoplankton microscopy record

Sub-samples of the surface water samples collected for physiochemical analysis between 1945 and 2010 were used to monitor the phytoplankton community at weekly to fortnightly intervals. These sub-samples were preserved with Lugol's iodine, concentrated by sedimentation, and then placed in a counting chamber under a microscope for identification and enumeration. Cells were counted within a sedimentation chamber until 1994, after which a Lund chamber was used for enumeration. Phytoplankton were identified to species level where possible, and quantified as the number of cells, colonies or filaments per mL of lake water.

## 2.4 | Sediment coring

A sediment core was collected from the deepest point of Esthwaite Water (54°21′54.2″N, 2°59′16.4″W) using a HTH 9 cm diameter gravity corer (Pylonex, Sweden) in August 2021. Coring equipment was thoroughly cleaned with ethanol and rinsed with deionised water three times before use. After collection, the 35 cm long sediment core remained intact within the sealed Perspex core tube and was kept upright on ice in a large cool box in the field and during transportation to UKCEH, Wallingford, where it was stored at 4°C in the dark prior to sectioning.

The sediment core was sectioned in 1 cm intervals using the extruding device (Pylonex, Sweden), beginning with recent sediment at the top and working downwards. Each 1 cm sediment section was pushed out the top of the core tube directly into a sterile petri dish of the same diameter to minimise contact with the air. A broad stainless-steel blade was used to cut between the core tube and the petri dish containing the extruded sediment section, which was then sealed with a lid and secured with parafilm. The blade was cleaned with bleach and ethanol and rinsed with deionised water between each section. Clean lab coats, gloves and masks were worn when handling the sediment core to minimise contamination risk. Each sediment core section was then sub-sampled in a UV-sterilised laminar flow cabinet. Using a sterilised spatula, a small amount of undisturbed sediment from the centre of each section which did not come into contact with the blade or core tube was transferred to a sterile Eppendorf tube for storage at −20°C prior to DNA extraction.

## 2.5 | Sediment core chronology

The chronology of the sediment core was estimated using the age-depth relationship of a separate reference core collected from the same location within Esthwaite Water (54°21′54.2″N, 2°59′16.4″W) in 2014 as described by Thorpe et al. (2022). Sample depths for the reference core were corrected to 2021 assuming a constant sedimentation rate (Appendix S1, Table S1), and the slope and intercept of the age-depth relationship were then used to estimate the age of each sediment core section (Appendix S1, Table S1). The full length of the 35 cm sediment core was estimated to cover 105 years from 1916 to the date of collection in 2021.

To evaluate whether the reference core chronology was accurately aligned with the 2021 core, 16S rRNA gene amplicon sequencing was carried out on sediments from the core and compared with the bacterial sedDNA record obtained from cores collected from the same location within Esthwaite Water in 2016 (Thorpe et al., 2022). Temporal trends in the relative abundance of the dominant bacterial groups were closely aligned between the two bacterial sedDNA records, supporting the use of the estimated chronology (refer to the Appendix S1 and Figure S1 for a detailed comparison).

## 2.6 | DNA extraction, PCR amplification and 18S rRNA gene sequencing

DNA was extracted from 0.25 g of each of the 35 sediment core samples using the Qiagen DNeasy PowerSoil Pro extraction kit (Qiagen, Germany) according to the manufacturer's protocol. DNA extractions were performed in small batches in a random order to minimise bias, and a negative control was included in every other batch. The concentration and purity of each DNA sample was checked on the NanoDrop 8000 spectrophotometer (Thermo Fisher Scientific, MA, USA). Extracted DNA samples were stored at −20°C.

The V4-V5 region of the 18S rRNA gene was amplified with universal forward and reverse eukaryotic primers, NSF563 (5′-CGCGGTAATTCCAGCTCCA-3′) and NSR951 (5′-TTGGYRAATGC TTTCGC-3′) (Mangot et al., 2012). Primers were adapted with the addition of Illumina sequencing primer and Nextera pre-adaptor sequences. Each 50 μL PCR mix contained 0.5 μL of 2000 units $mL^{-1}$ Q5

High-Fidelity DNA polymerase, 10 μL of 5× reaction buffer, 10 μL of 5× high GC enhancer (New England Biolabs, UK), 1 μL of a 10 mM dNTP mix (Bioline, UK), 26.3 μL of molecular grade water, 0.1 μL of each 100 μM forward and reverse primer and 2 μL of DNA. One PCR was performed per sample, and negative PCR controls were included. The PCR programme was set to an initial denaturing temperature of 94°C for 5 min, followed by 30 cycles of 94°C for 30 s, an annealing temperature of 60°C for 30 s, an extension temperature of 72°C for 30 s, and then a final extension temperature of 72°C for 10 min. Successful PCR amplification was confirmed with an agarose gel. PCR product was purified with the Millipore multiscreen PCR filter plate kit according to the manufacturer's protocol (Merck, Millipore, MA, USA), resulting in purified PCR product eluted in 35 μL of molecular grade water.

Second-step PCR was performed using a dual-indexing approach (Kozich et al., 2013), and 25 μL reactions contained 0.25 μL of Q5 DNA polymerase, 5 μL of reaction buffer, 5 μL of high GC enhancer, 0.5 μL of dNTPs, 7.25 μL of molecular grade water, 5 μL of the indexing primers (Kozich et al., 2013) and 2 μL of purified PCR product from the first PCR step. The second step PCR programme was set to an initial denaturing temperature of 95°C for 2 min, followed by 8 cycles of 95°C for 15 s, an annealing temperature of 50°C for 30 s, an extension temperature of 72°C for 30 s, and then a final extension temperature of 72°C for 10 min. Gel electrophoresis confirmed successful PCR amplification from the second PCR step and determined an average amplicon size of 525 bp including adaptors and index sequences.

PCR product from the second PCR step was normalised using the Invitrogen SequalPrep normalisation kit according to the manufacturer's protocol (Thermo Fisher Scientific, MA, USA), resulting in 1–2 ng $\mu L^{-1}$ of DNA per sample. Samples were pooled, gel-extracted using the Qiagen MinElute gel extraction kit according to the manufacturer's protocol, and the purified DNA concentration was quantified using the Invitrogen Qubit dsDNA high sensitivity assay kit with the Qubit 3.0 fluorometer. The amplicon library was denatured with NaOH, neutralised with HCl, combined with 10% denatured PhiX and then diluted with HT1 buffer (Illumina, CA, USA). The library was heat denatured at 96°C for 2 min and immediately transferred to an ice bath prior to sequencing on the Illumina MiSeq Platform with a 500-cycle v2 MiSeq reagent kit.

## 2.7 | Sequence data processing

The DADA2 pipeline was implemented to process the sequences (Callahan et al., 2016). Samples were demultiplexed and primers and adaptors were removed. The read quality profiles were inspected and the reverse reads were truncated at position 248 where the quality score fell below Q30. The reads were filtered with a maximum expected error of 5. Forward and reverse reads were merged with a minimum overlap of 12 identical bases. An amplicon sequence variant (ASV) abundance table was generated and taxonomy was assigned to each exact ASV using the naive Bayesian classifier with a minimum bootstrap confidence of 50 (Wang et al., 2007) against the $PR^2$ database v4.14.0 (Guillou et al., 2012). The sequences were rarefied to a uniform sequencing depth of 14,936 reads. Two sediment core samples (4 and 33 cm) and the extraction and PCR negative controls did not meet the rarefaction depth and were therefore excluded from further analysis.

ASV abundance was converted to relative abundance, and ASVs were filtered according to taxonomy to remove those unidentified at the phylum level. Heterotrophic groups that were outside of the scope of the microscopy-based monitoring record were excluded from analysis. Chlorophytes, dinoflagellates, ochrophytes and bacillariophytes were well-represented in both the microscopy and 18S rRNA sedDNA records and were therefore included for in-depth analysis.

## 2.8 | Data analysis

Many reference databases use their own taxonomic nomenclature which can lead to conflicting taxonomy assignments when comparing multiple datasets (Canino et al., 2021). To allow for comparisons between the microscopy and sedDNA records, taxonomy was homogenised using Phytool v2 (Canino et al., 2021) which is based on the taxonomic classifications used in AlgaeBase (Guiry & Guiry, 2022). This ensured that taxa in both records were classified according to the same taxonomic nomenclature and names were updated to the current taxonomically accepted name.

To account for potential inaccuracies in species identification, taxa in both records were grouped at the genus level. As the counting method sometimes varied by size or form (e.g. single cell, colony or filament), the microscopy-based counts were converted to a binary presence-absence value for each genus on each sampling occasion. The total number of sampling occasions on which each genus was observed was calculated for each year as a measure of occurrence, and then normalised to the number of sampling occasions per year to account for variable sampling effort.

Non-metric multidimensional scaling (NMDS) was performed based on a beta diversity Bray–Curtis dissimilarity matrix of genus relative abundance as measured using sedDNA and genus occurrence as measured by microscopy from 1945 to 2010. Correlations between each dissimilarity matrix and lake physicochemical conditions were assessed with a permutation test and fitted to the ordination space using the vegan R package v2.6-2 (Oksanen et al., 2019). The vegan package was also used to calculate Shannon's alpha diversity at the genus level in both records. Generalised additive models (GAMs) with Gamma error distributions and a log link were fitted to the temporal trend in alpha diversity using the mgcv R package v1.8-40 (Wood, 2020). As there was not a sediment sample corresponding to each year of the microscopy-based monitoring record, annual values of alpha diversity from 1945 to 2010 as measured by sedDNA were estimated using the GAM fitted to the temporal trend. These GAM-estimated annual values were then correlated with GAM-estimated annual values of alpha diversity as measured

by microscopy using a model II regression with the lmodel2 R package v1.7–2 (Legendre, 2018).

GAMs were fitted to the temporal trends in phylum relative abundance as measured by sedDNA using Beta error distributions with a logit link, which is suitable for proportion data. For the trends in phylum occurrence as measured by microscopy, GAMs were fitted using Gamma error distributions with a log link, which is suitable for positively skewed, non-negative data (Anderson et al., 2010; Simpson, 2018). Restricted maximum likelihood (REML) was used as the smoothness selection method for all GAMs (Simpson, 2018). Annual values of relative abundance from 1945 to 2010 were estimated using the GAM fitted to the temporal trend and correlated with the GAM-estimated annual values of occurrence using a model II regression.

For each phylum, Venn diagrams were used to illustrate which genera were uniquely detected using sedDNA, which were uniquely detected by microscopy, and which were detected in both records. Venn diagrams were produced with the eulerr R package v7.0.0 (Larsson, 2022), and all data analysis was performed in R v4.2.1 (R Core Team, 2022).

## 3 | RESULTS

### 3.1 | Beta diversity

The NMDS of the Bray–Curtis dissimilarity matrix based on beta diversity as measured by sedDNA and microscopy both displayed a similar trajectory of community change from older to more recent samples (Figure 1a,b). Pre-1981 sediment core samples were positioned on the left side of the ordination space, and post-1982 samples were on the right. More recent sediment core samples from 1997 to 2010 were closely clustered in the bottom right quadrant (Figure 1a). Water samples collected for microscopic analysis prior to 1978 were positioned on the left half of the ordination space, and those collected after 1979 were positioned on the right with more recent samples from 1994 to 2010 in the bottom quadrant (Figure 1b). The lake physiochemical conditions that correlated significantly with the sedDNA dissimilarity matrix included alkalinity, SRP and pH ($p < .05$) with $R^2$ values of 0.53, 0.50 and 0.44 respectively. The microscopy dissimilarity matrix also correlated significantly with alkalinity ($p < .001$) and SRP ($p < .05$), in addition to NH4–N ($p < .01$), NO3–N, and TP ($p < .05$), with $R^2$ values of 0.61, 0.23, 0.31, 0.19 and 0.25 respectively. Mean annual trends in lake physiochemical conditions are presented in Appendix S1, Figure S2, and statistics for the correlations between these conditions and the dissimilarity matrices are provided in Appendix S1, Table S3.

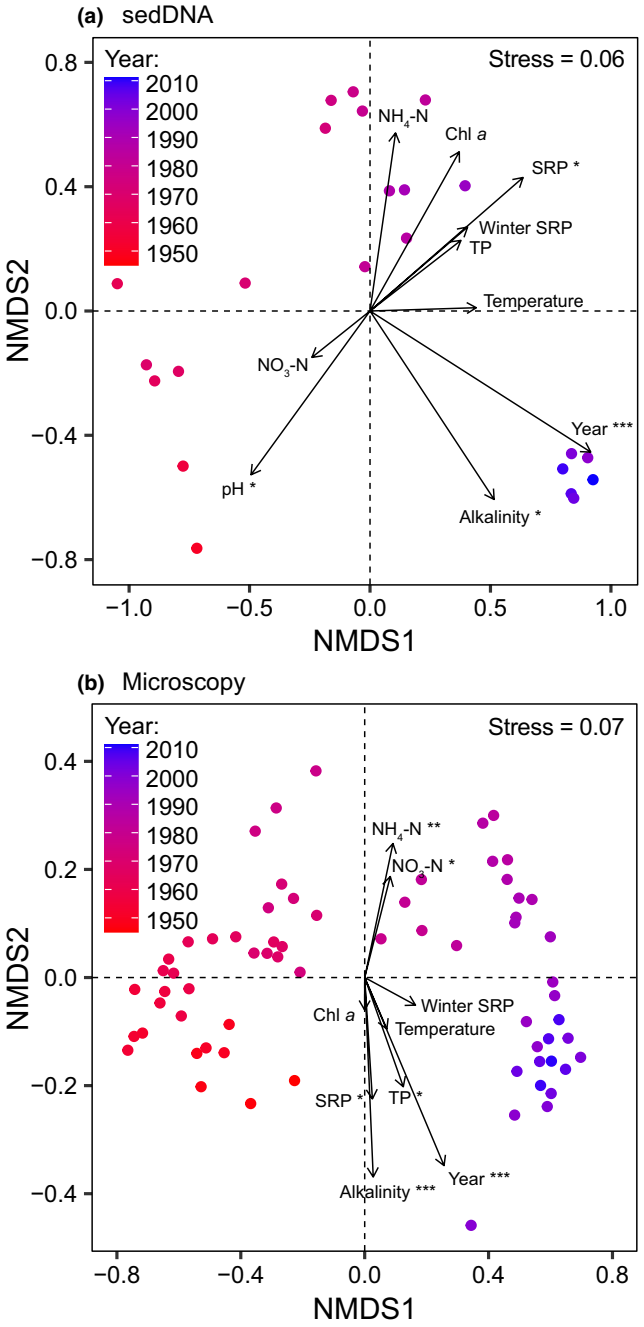


FIGURE 1 NMDS of a Bray–Curtis dissimilarity matrix based on beta diversity as measured by sedDNA (a) and microscopy (b) from 1945 to 2010. The red to blue gradient indicates older to more recent samples. Vectors for sample year and the lake physiochemical conditions that correlated with each dissimilarity matrix are fitted. Vector length is proportional to the strength of the correlation. ***$p < .001$, **$p < .01$, *$p < .05$. NMDS stress values are shown.

### 3.2 | Alpha diversity

Shannon's diversity index at the genus level was used as a measure of alpha diversity throughout the sedDNA and microscopy records. Both records displayed a general increasing trend in alpha diversity from the 1970s which began to plateau from the 1990s (Figure 2). Diversity in the most recent sediment core section was 2.75, which was similar to that in the oldest section with a diversity of 2.50. Alpha diversity as measured by sedDNA ranged between 1.47 and 2.76 and was consistently lower than that measured by

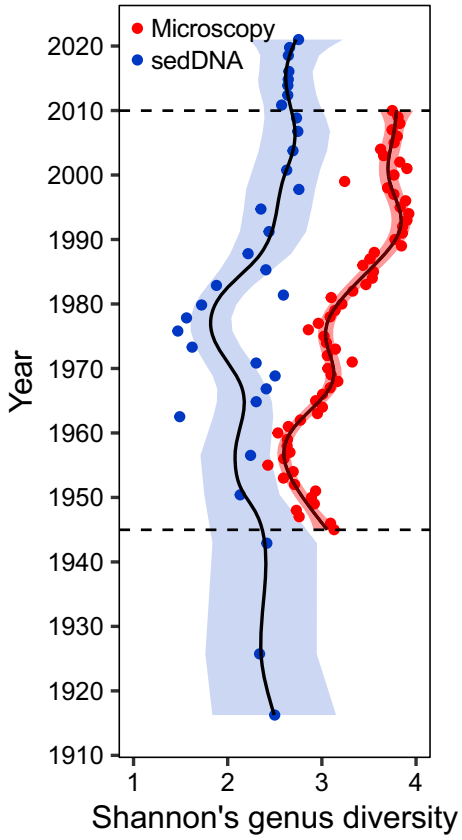


**FIGURE 2** GAMs fitted to the trend in Shannon's genus diversity as measured by sedDNA (blue) and microscopy (red). Shaded areas show the 95% confidence intervals.

microscopy, which ranged between 2.43 and 3.92. There was a significant positive correlation between the sedDNA and microscopy GAM-estimated annual values of alpha diversity between 1945 and 2010 with an $r$ value of .75 ($F_{1,64} = 81.45$, SE = 0.19, $p < .001$).

### 3.3 | Temporal trends in community composition

Chlorophytes, dinoflagellates, ochrophytes and bacillariophytes were well-represented in both the sedDNA and microscopy records (Figure 3a–d). Generally, data derived from sedDNA and microscopy showed broadly similar long-term trends for these phyla, but with some differences in the exact timing of the onset of change. Of these dominant phyla, chlorophytes and dinoflagellates made up the largest proportion of the sedDNA community. Chlorophytes were initially present in the sedDNA record with a relative abundance between 0.03 and 0.10 from 1916 to 1994. Their relative abundance then increased abruptly to between 0.20 and 0.27 in more recent samples from 1997 to 2021. In the microscopy-based monitoring record, chlorophytes had a low occurrence initially, but increased sharply from the 1980s to become the group with the highest occurrence (Figure 3a).

In the sedDNA record, dinoflagellates had a relative abundance <0.01 until 1970. Their relative abundance then increased to two distinct peaks in 1980 when they reached a relative abundance of 0.17, and in 2000 when they reached a relative abundance of 0.25. Dinoflagellates in the microscopy record had three main peaks in 1967, 1986 and 2002 when they reached an occurrence of 2.35, 2.67 and 2.92 respectively (Figure 3b).

The relative abundance of ochrophytes in the sedDNA record was below 0.006 and relatively stable until the 1980s when there was a slight increasing trend to the 2000s. The occurrence of ochrophytes in the microscopy record remained below 1.00 until 1983, but then increased throughout the 1980s and 1990s to their highest occurrence of 4.08 in 2001 (Figure 3c).

Bacillariophytes had the lowest relative abundance of the four phyla analysed in the sedDNA record which was consistently below 0.003. There was a general increasing trend in the relative abundance of bacillariophytes from the 1970s, although there was some scatter around this trend. In the microscopy record, bacillariophytes displayed a slight decreasing trend to the 1980s, and then increased to a period of higher occurrence from the 1990s. Bacillariophytes had the highest occurrence of any phylum in the microscopy record until 1980, after which the only phylum with a higher occurrence was chlorophytes (Figure 3d).

There was a significant positive correlation between the sedDNA and microscopy GAM trends for all four phyla ($p < .001$). The correlation between the two records was strongest for ochrophytes, with an $r$ value of .93, followed by .76 for chlorophytes and bacillariophytes, and .75 for dinoflagellates (Figure 3a–d). All GAM trends for each dataset were significant ($p < .01$), and statistics associated with the GAMs are provided in Appendix S1, Tables S4 and S5.

Charophytes, cryptophytes and haptophytes were also recorded by microscopy. However, charophytes and haptophytes were only detected with a relative abundance >0.001 in three sediment core samples, and cryptophytes were absent from the sedDNA record. The GAM-fitted trends in occurrence as measured by microscopy for charophytes, cryptophytes and haptophytes are presented in Appendix S1, Figure S3.

### 3.4 | Shared and unique genera

Across the four main phyla studied, a total of 215 genera were identified with both sedDNA and microscopy. Of these genera, 113 (52.6%) were uniquely detected by microscopy, 66 (30.7%) were uniquely detected by sedDNA and 36 (16.7%) were detected in both the sedDNA and microscopy records (Figure 4a–d). More chlorophyte, ochrophyte and bacillariophyte genera were uniquely detected by microscopy compared to sedDNA, whereas more dinoflagellate genera were uniquely detected by sedDNA.

Outside of these four phyla, microscopy also detected 15 charophyte genera, five cryptophyte genera and one haptophyte genus. sedDNA only detected three charophyte genera, two of which were also detected by microscopy.

A total of 669 phytoplankton ASVs were detected in the sedDNA record, and 410 (61%) of these ASVs were grouped into 105 genera.

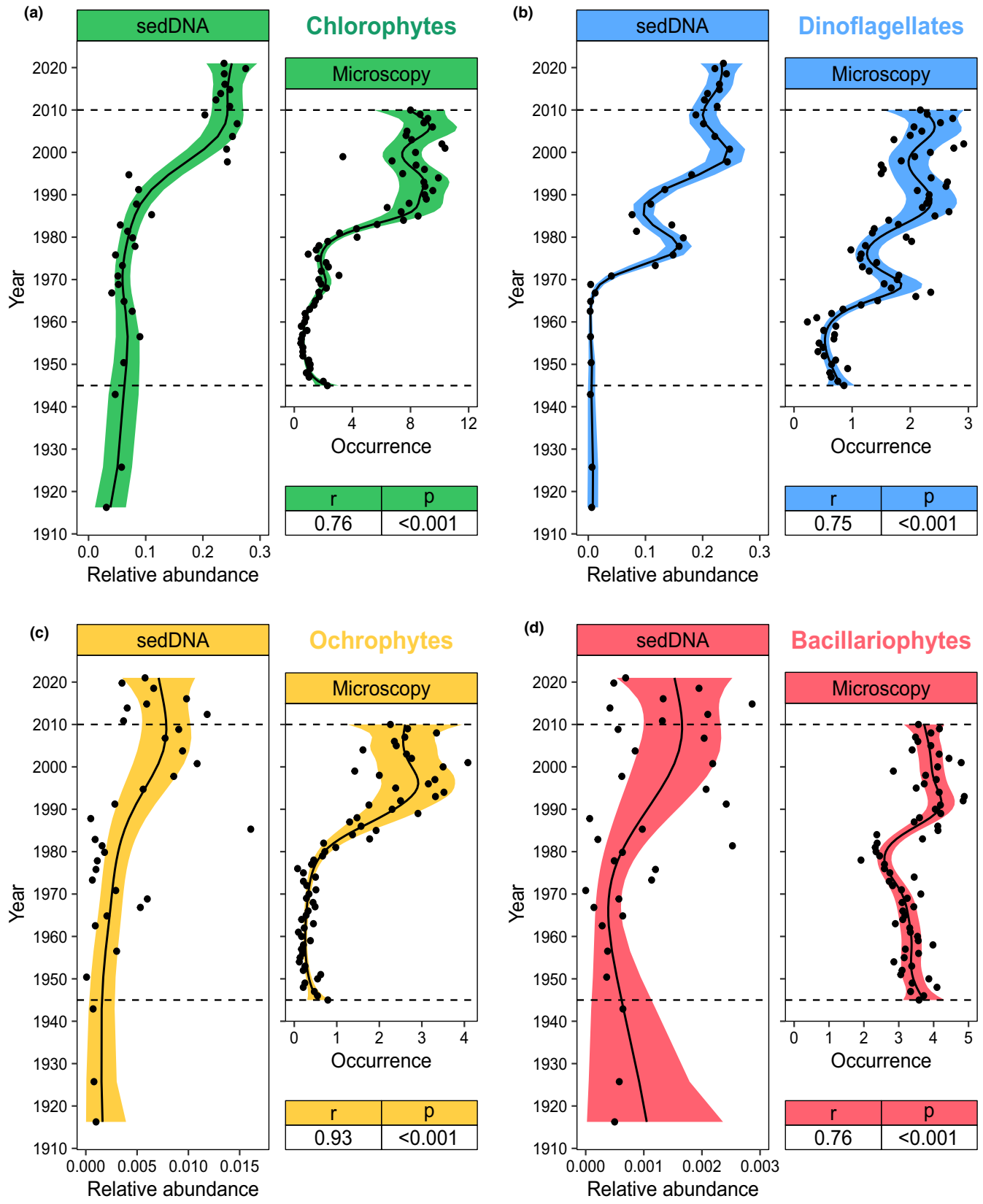


FIGURE 3 GAMs fitted to the trend in relative abundance as measured by sedDNA and occurrence relative to sampling frequency as measured by microscopy for chlorophytes (a), dinoflagellates (b), ochrophytes (c) and bacillariophytes (d). For each phylum, *r* values and significance levels are shown for the correlation between sedDNA and microscopy GAM-estimated annual values from 1945 to 2010. Shaded areas show the 95% confidence intervals.

However, 259 phytoplankton ASVs (39%) had no definitive taxonomic assignment at the genus level. Within the microscopy-based monitoring record, there were 928 phytoplankton records, and 407 (44%) of these records were grouped into 170 genera. The remaining 521 microscopy records (56%) could not be identified to the genus level (Appendix S1, Table S6).

## 4 | DISCUSSION

Here, we have compared a sedDNA record with long-term microscopy-based monitoring to determine whether sedDNA can be used to reliably reconstruct past phytoplankton communities. Temporal trends in diversity and relative abundance and occurrence at the phylum level were broadly comparable between the sedDNA and microscopy records. However, each method detected a distinct composition of genera, with only a small proportion of genera detected by both methods.

### 4.1 | Data considerations

Differences between the sedDNA and microscopy records may arise from the way the data are collected, and this must be considered when comparing the two temporal records. For example, changes and improvements to the methods used throughout long-term monitoring schemes are to be expected. In the present study, the type of counting chamber used to produce the microscopy record changed from a sedimentation chamber to a Lund chamber in 1994. The way counts were recorded also varied throughout the monitoring scheme as cells were sometimes counted according to size, form or whether they were found in colonies. To alleviate some of the possible biases that may arise from changes to the counting method, the counts were converted to binary presence-absence values as a measure of temporal occurrence. A consequence of converting counts to occurrence is that this measure may not be directly comparable with the relative abundance values used in the sedDNA record, although a positive relationship between species occurrence and abundance has been widely observed (Gaston & He, 2011). Some issues could remain such as the ability of the observer to identify phytoplankton to genus level by microscopy. This may have varied with the counting method used and the expertise and time investment of the observers, and the counts may have been biased towards more easily identifiable taxa or taxa of particular scientific interest.

There are also methodological factors associated with the sedDNA record which must be considered when making comparisons with the microscopy-based record. For example, the chronology of the sediment core was estimated based on the chronology of a separate sediment core collected in 2014 from the same location within Esthwaite Water. Application of this chronology required the assumption that the sedimentation rate remained constant since 2014, but variation in the sedimentation rate could lead to inaccuracies in the estimated chronology and therefore cause a discrepancy between the sedDNA and microscopy records. Only phytoplankton residing in the surface water were examined in the monitoring scheme, but sedDNA had the potential to record taxa originating from deeper within the water column and at the sediment surface. While contribution from active benthic phytoplankton may be relatively low at the depth the sediment core was collected due to low light availability, benthic taxa originating from littoral areas may have been transported to the sediment in the deeper basin during sediment resuspension and focussing (Mackay et al., 2012). The choice of 18S rRNA amplicon primers influences the composition of the community detected, and the accuracy of taxonomic assignment is limited by completeness of the reference database (Francioli et al., 2021). The 18S rRNA gene copy number can vary between taxa and lead to over-estimations in relative abundance for some groups (Gong & Marchetti, 2019). Despite these data considerations, there were still remarkable similarities between the sedDNA and microscopy records, but possible explanations for the discrepancies between the records are explored further in the following sections.

### 4.2 | Temporal trends in diversity

The NMDS of the dissimilarity matrices based on beta diversity as measured by sedDNA and microscopy both displayed comparable trajectories of change from older to more recent samples. The temporal trends in alpha diversity were also similar between the two records, with both showing an increase in diversity from the 1970s which coincided with the intensification of nutrient enrichment. A trend that is driven by the accumulation of DNA degradation with age could be expected to be a monotonic decline in diversity with sediment depth (Dommain et al., 2020). However, alpha diversity measured at the core surface was similar to that measured at the bottom of the core, and the temporal trends observed in the sedDNA record were accompanied by similar trends in the microscopy record. This provides evidence that the trends in diversity throughout the sediment core may represent a community response to environmental conditions and not a trend that is primarily driven by an accumulation of DNA degradation with age. Previous studies have also shown that temporal trends in phytoplankton diversity as measured by sedDNA are consistent with environmental change and not necessarily DNA degradation (Capo et al., 2017; Huo et al., 2022; Zhang et al., 2021). However, alpha genus diversity as measured by sedDNA was lower compared to that measured by microscopy. A lower diversity could be evidence of at least some DNA degradation, the extent of which may vary with conditions within the sediment (Torti et al., 2015), but sedDNA diversity could also be underestimated due to an incomplete reference sequence database.

### 4.3 | Temporal trends in community composition

In both records, chlorophytes, dinoflagellates, ochrophytes and bacillariophytes displayed general increasing trends beginning

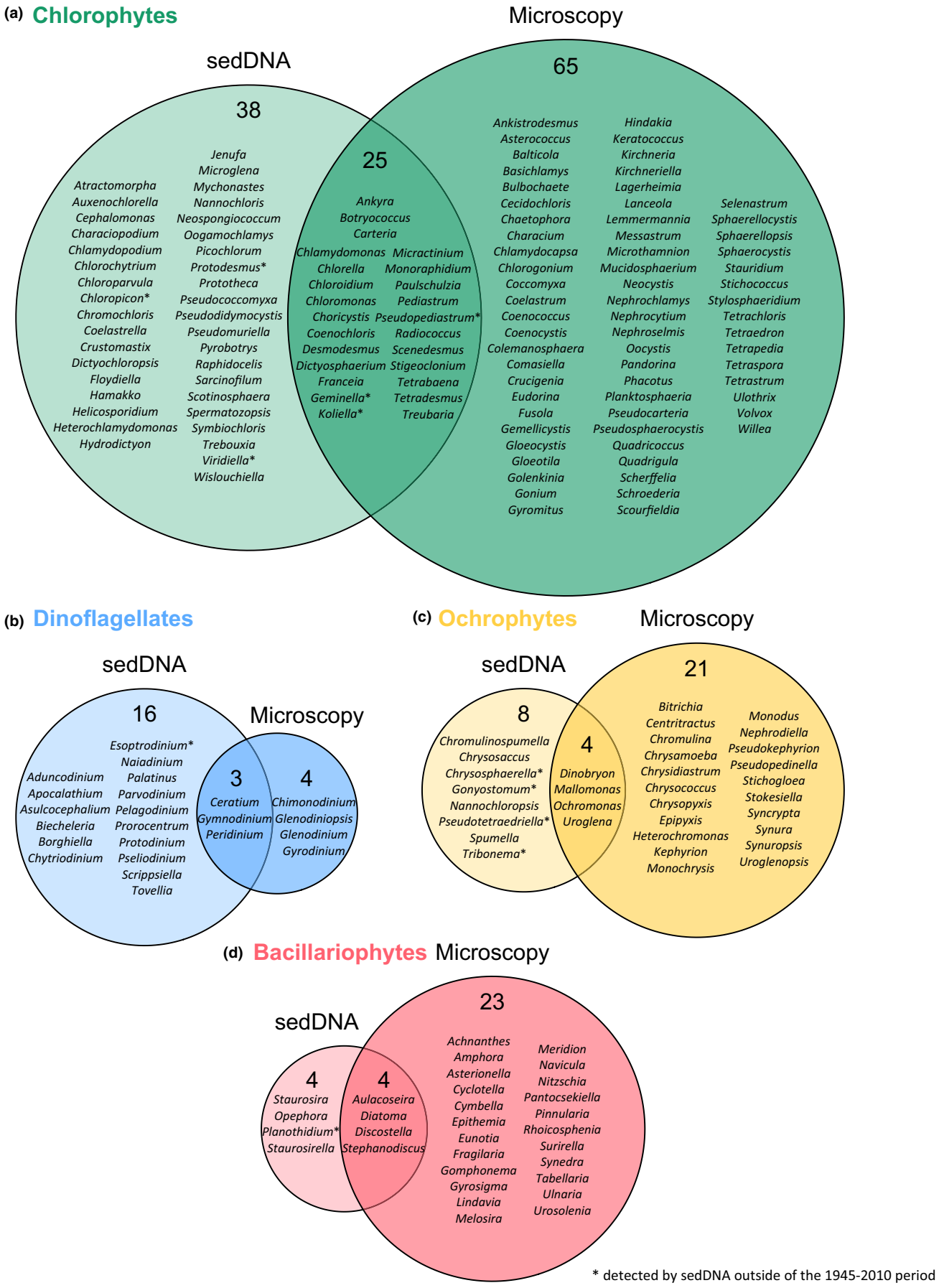


FIGURE 4 Shared and unique chlorophyte (a), dinoflagellate (b), ochrophyte (c) and bacillariophyte (d) genera detected by sedDNA and microscopy. * indicates genera detected by sedDNA outside of the 1945–2010 period covered by the microscopy record.

around 1970–1990. These could be responses to nutrient enrichment, which accelerated in Esthwaite Water from the 1970s and remained high until the early 2000s (Appendix S1, Figure S2). A sediment core has previously been collected from Esthwaite Water for sedimentary pigment analysis. In this record, many algal pigments also displayed increasing trends over time from the 1800s to 2011 with their highest concentrations detected after the 1970s, including chlorophyll *b* and lutein, which are indicative of chlorophytes, and diatoxanthin, which is indicative of bacillariophytes. However, there was a large peak in the concentration of diatoxanthin around the 2000s, and this was not reflected in the sedDNA or microscopy records for bacillariophytes (Moorhouse et al., 2018).

Co-occurring patterns in the microscopy record could support sedDNA as a reliable record of past community change. The relative abundance and occurrence of chlorophytes in the sedDNA and microscopy records, respectively, both increased sharply in more recent samples. However, the increase in chlorophyte relative abundance in the sedDNA record occurred over a decade later than the increase in occurrence in the microscopy record. Distinct peaks in the relative abundance and occurrence of dinoflagellates were observed in the sedDNA and microscopy records, but the timing of these peaks was also not aligned. sedDNA and microscopy may have recorded the same trends, but they may have been off-set due to uncertainties in the chronology of the sediment core. Multiproxy analysis of the same sediment core may provide a useful means of assessing the performance of sedDNA relative to other proxies such as diatom microfossils or pigments, independent of radiometric dating and its associated uncertainties.

Taphonomic processes could also have affected the ability of sedDNA to provide a reliable temporal record. For example, it was possible that there was a delay in the time taken for cells in the surface water to deposit in the sediment, particularly for smaller and more buoyant cells. Recently deposited cells and DNA may have become resuspended before complete burial and compaction within the sediment, and DNA may have migrated between sediment layers due to processes such as bioturbation or leaching which could have disrupted the vertical organisation of DNA (Giguet-Covex et al., 2019). However, it has been suggested that substantial DNA leaching between layers is unlikely to occur in the permanently saturated sediments of lakes (Anderson-Carpenter et al., 2011).

Degradation of DNA over time could limit the reliability of sedDNA reconstructions. Prior to 1970, the relative abundance of chlorophytes, dinoflagellates, ochrophytes and bacillariophytes was low and stable in the sedDNA record. Their occurrence in the microscopy record was also relatively low prior to 1970, but there were indications of a slightly higher occurrence in the earlier monitoring records between 1945 and 1950 which were not reflected in the sedDNA record. This could be evidence of some DNA degradation and a reduced ability of sedDNA to detect phytoplankton community change in older sediments. However, separating the effect of DNA degradation from an increase in the relative abundance of phytoplankton with intensification of nutrient enrichment is complex as both factors could be expected to show a change in the same direction (i.e. an increase from older to more recent sediments). Furthermore, heterotrophic eukaryotes that may have been active within the sediment such as fungi were also sequenced with the 18S rRNA amplicon primers, and the number of reads assigned to the phytoplankton phyla relative to total reads generally declined with sediment depth (Appendix S1, Table S7). The heterotrophic community within the sediment therefore likely contributed to the lower relative abundance of these phytoplankton groups detected in deeper sediments.

Cryptophytes were absent in the sedDNA record but were well-represented in the microscopy-based record, and alloxanthin, the diagnostic pigment of cryptophytes, was detected in the sediment core pigment record from Esthwaite Water (Moorhouse et al., 2018). Cryptophytes could therefore be expected to be detected using sedDNA, but similar to the present study, Capo et al. (2015) also reported that cryptophytes were poorly represented by sedDNA and suggested that the absence of a cell wall made their DNA vulnerable to degradation, and their high nutritional content made them vulnerable to grazing by zooplankton so that cells did not reach the sediment surface (Capo et al., 2015, 2021). Haptophytes were also poorly represented by sedDNA, and an underrepresentation of haptophytes in Lake Bourget, France, as measured by sedDNA has previously been reported (Capo et al., 2015). However, haptophyte temporal dynamics in an Antarctic lake throughout the Holocene have successfully been reconstructed using sedDNA (Coolen et al., 2004), but the low temperatures in the Antarctic lake may have promoted DNA preservation. Haptophytes were not consistently counted throughout the monitoring scheme, so determining whether this group was underrepresented because they experienced greater rates of DNA degradation, or because they had a low abundance in Esthwaite Water is challenging. The reliability of sedDNA reconstructions depends on the extent of DNA degradation, which may occur at varying rates for different taxa in different environments (Capo et al., 2021). Previous efforts have been made to explore DNA degradation patterns in dinoflagellates and bacillariophytes in an Antarctic lake core record (Boere et al., 2011), and for cyanobacterial taxa within microcosms (Mejbel et al., 2022). However, the extent of DNA degradation that different taxa may be subject to in temperate lake sediments requires further research, particularly for groups that were not well-represented by sedDNA, such as cryptophytes and haptophytes.

### 4.4 | Shared and unique genera

More genera were detected by microscopy compared to sedDNA within each phylum except dinoflagellates. For chlorophytes, ochrophytes and bacillariophytes, microscopy may have been more sensitive when distinguishing between genera. Only a small proportion of genera occurred in both records. This was highest for chlorophytes, with 19.5% of chlorophyte genera detected by both methods, but only 16.7% for dinoflagellates, 12.1% for ochrophytes and 12.9% for bacillariophytes. The majority of genera were uniquely detected by

each method, and each method may be capable of recording a different component of the phytoplankton community. Genera unique to the sedDNA record could include taxa that occupied deeper layers of the water column or littoral areas and were therefore beyond the scope of the surface water monitoring scheme, or those that were difficult to identify based on morphology. Depositional or degradational processes could explain why a large proportion of the phytoplankton community were missed by sedDNA. Previous studies have shown that certain groups of eukaryotic algae (Gauthier et al., 2021) and cyanobacteria (Nwosu et al., 2021) were differentially represented in surface lake sediments compared to the water column, and this could be because some taxa did not readily deposit. The deposition potential of phytoplankton could be affected by grazing pressure and whether the cells form colonies or aggregate with organic matter which make them heavier and more likely to deposit and could also protect the DNA from degradation (Gauthier et al., 2021; Mauvisseau et al., 2022; Nwosu et al., 2021).

A larger number of dinoflagellate genera were detected in the sedDNA record compared to the microscopy record, and sedDNA may therefore be a particularly valuable method for studying past dinoflagellate communities. Many dinoflagellates form a robust cyst during the resting stage of their lifecycle which may protect their DNA from grazing by zooplankton and other extracellular degradation processes (Bravo & Figueroa, 2014). Dinoflagellates have previously been shown to be well-represented by sedDNA, but it is possible that their relative abundance is overestimated due to their large genomes and high 18S rRNA gene copy number (Gong et al., 2020).

Bacillariophyte DNA could also be expected to be preserved in sediments due to the presence of the protective silica frustule (Aguirre et al., 2018). However, this group was present at the lowest relative abundance of the four main phyla in the sedDNA record, despite being one of the groups with the highest occurrence in the microscopy record, and a larger number of bacillariophyte genera were detected by microscopy. A sediment core previously collected from Esthwaite Water for microfossil analysis found *Asterionella*, *Aulacoseira* and *Fragilaria* to be the most dominant genera between 1945 and 2005 (Dong et al., 2012), and microfossil analysis of another Esthwaite Water sediment core revealed that *Aulacoseria* and *Asterionella* were among the dominant genera from the 1900s to 2000 (Bennion et al., 2000). These genera were also detected by microscopy in the monitoring record from 1945 to 2010, but *Asterionella* and *Fragilaria* were absent from the sedDNA record. Targeted primers may be more capable of distinguishing a larger number of bacillariophyte genera compared to the broad range 18S rRNA amplicon primers selected in the present study, such as primers targeting the rbcL gene (Anslan et al., 2022; Dulias et al., 2017; Kang et al., 2021). Although a substantial number of bacillariophytes were missed by sedDNA, a small number of genera were detected which may have been overlooked in the microscopy-based monitoring and microfossil records. This included *Staurosira*, *Opephora*, *Planothidium* and *Staurosirella*. *Planothidium* are typically benthic taxa (Lange-Bertalot et al., 2017), and may therefore have been outside of the scope of the surface water monitoring scheme, although *Planothidium* was only detected by sedDNA after 2010. The bacillariophyte community sequenced in lake surface sediments has previously been compared with microscopy-based methods, and also revealed that while microscopy could detect more genera, each method detected a distinct proportion of the community (Dulias et al., 2017; Kang et al., 2021).

A substantial proportion of ASVs detected by sedDNA (39%) and records in the microscopy-based monitoring scheme (56%) were unidentified at the genus level. In the sedDNA record, the majority of ASVs unassigned at the genus level were chlorophytes and dinoflagellates. Taxonomy assignment in the sedDNA record may be limited by reference database coverage (Anslan et al., 2022) as 37 of the 113 genera uniquely identified using microscopy did not have a representative sequence in the $PR^2$ database (v4.14.0). In the microscopy record, a significant number of bacillariophytes were unidentified at the genus level and taxonomic identification with microscopy may be limited by microscope resolution and the expertise and time investment of the observers, which may vary throughout the monitoring scheme. Separation of the influence of these variables in long-term monitoring schemes from an environmental response is complex (Straile et al., 2013). While palaeolimnological tools such as sedDNA typically do not suffer from method changes, they may be subject to other limitations such as reference database completeness and taphonomic biases. Each method has its own limitations and biases, and multi-proxy analysis is likely the most reliable approach for reconstructing past phytoplankton communities.

### 4.5 | Conclusions and recommendations for the use of sedDNA in palaeolimnology

Validation of sedDNA against concurrent lake monitoring is crucial to further its development and evaluate its performance as a palaeolimnological tool. Our comparison with long-term microscopy-based monitoring of phytoplankton in the lake surface water revealed broadly similar trends in the diversity and relative abundance and occurrence of chlorophytes, dinoflagellates, ochrophytes and bacillariophytes, and up to 20% of genera detected by microscopy were also detected by sedDNA. These results support the use of sedDNA as an effective tool for the reconstruction of past phytoplankton communities, at least within the time period investigated in this study. However, DNA degradation may occur in older sediments which could limit the reliability of reconstructions over longer time periods, and a substantial proportion of the phytoplankton community detected by microscopy were missed by sedDNA. Based on these results, we recommend that sedDNA reconstructions over time periods exceeding 100 years or of groups such as cryptophytes that were poorly resolved with sedDNA are treated with caution, and future research should focus on identifying the key determinants of variable DNA degradation and deposition among taxa. Furthermore, due to incomplete reference databases, it is important that future studies consider the fact that phytoplankton sedDNA reconstructions may

only represent a subset of the total community in lakes. Continued improvements to reference database coverage, in addition to the combined use of multiple targeted primers may enable the wider phytoplankton community to be captured with sedDNA.

## AUTHOR CONTRIBUTIONS

ACT and EBM performed the fieldwork, and ACT and TG performed the laboratory work. SJT and SCM contributed to data curation, and ACT was responsible for data analysis and wrote the original draft. DSR and JAB supervised ACT. All authors contributed to editing of the draft and approved the final version of the manuscript.

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## CONFLICT OF INTEREST STATEMENT

Authors declare no conflicts of interest.

## OPEN RESEARCH BADGES

This article has earned an Open Data badge for making publicly available the digitally-shareable data necessary to reproduce the reported results. The data is available at https://datadryad.org/stash/dataset/doi:10.5061/dryad.q83bk3jpw.

## DATA AVAILABILITY STATEMENT

Raw sequence reads have been deposited in the European Nucleotide Archive (ENA) at EMBL-EBI under accession number PRJEB66188 (https://www.ebi.ac.uk/ena/browser/view/PRJEB66188). The microscopy-based monitoring record of phytoplankton genus occurrence from 1945 to 2010 is available at: https://doi.org/10.5061/dryad.q83bk3jpw. The physicochemical record from Esthwaite Water is available at: https://doi.org/10.5285/87360d1a-85d9-4a4e-b9ac-e315977a52d3 (Maberly et al., 2017).

## BENEFIT-SHARING STATEMENT

Not applicable.

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## SUPPORTING INFORMATION

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