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RESEARCH

Lake phytoplankton status and trends: a case study from Greek lakes, Eastern Mediterranean

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Abstract Until now, little information was available regarding phytoplankton communities of Greek lakes. In this study, we present the first comprehensive analysis of phytoplankton composition and biovolume, as well as their inter-annual variations, across 15 natural lakes in Greece from 2016 to 2021. The ecological status and trends of these lakes were assessed using HeLPhy, a phytoplankton index of the Water Framework Directive (WFD). Additionally, this study examined potential similarities among lakes based on the number of phytoplankton taxa identified, the composition of phytoplankton communities, and the ecological classification. A total of 462 phytoplankton taxa from 10 taxonomic groups were recorded in 287 phytoplankton samples collected between 2016 and 2021. The phytoplankton communities in all lakes were dominated mainly by Cyanobacteria, Bacillariophyta, and Chlorophyta. The highest number of phytoplankton taxa was recorded in Chlorophyta and Cyanobacteria taxonomic groups. Based on the HeLPhy index, the lakes were classified into four ecological status classes: high, good, moderate, and poor. Inter-annual variations in ecological classification were observed. Eight of the 15 lakes were classified as having good or better ecological status according to WFD criteria, with deep lakes generally exhibiting a better status than shallow lakes. Bacillariophyta were more prevalent in lakes with high and good ecological status, while Cyanobacteria were more abundant in poor status lakes and dominated in bad status lake-years. Lakes in moderate and poor status exhibited higher total phytoplankton and Cyanobacteria biovolume values. This research improves our understanding of the status and temporal variations of phytoplankton communities in Greek lakes.

Keywords Phytoplankton · Greek lakes · Ecological status · HeLPhy

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Introduction

Many lakes have suffered from degradation over the past decades, leading to a decline in their water quality, due to anthropogenic pressures (Reid et al., 2019). Lake ecosystems are subject to multiple pressures. The most dominant pressure seems to be eutrophication, as nutrients remain one of the primary factors influencing European lakes (Phillips et al., 2008; EEA, 2018; EEA, 2024; Weber et al., 2020). According to Nõges et al. (2016), nutrient stressors represent the predominant stressor group in around 78% of multi-stressor situations in the lakes studied. Phytoplankton taxa are sensitive bio-indicators of eutrophication in lakes (Järvinen et al., 2013), as individual taxa show different responses to nutrient concentrations. For instance, many chrysophyte species are indicators of oligotrophic waters, thriving in lakes with low nutrient levels (Brettum & Andersen, 2005). In contrast, colonial and filamentous Cyanobacteria, which are indicators of eutrophic waters, tend to be more abundant in nutrient-rich waters (Reynolds et al., 2002; Brettum & Andersen, 2005; Järvinen et al., 2013). Phytoplankton taxa attain their nutrients from the water column and have short generation times, making them direct and primary indicators of the effects of changing nutrient conditions (Lyche Solheim et al., 2013; Reynolds, 2006). As a result, phytoplankton taxa have long been studied as indicators of water quality over the years (e.g., Nygaard, 1949; Carlson, 1977; Rosen, 1981; Rott, 1984).

Phytoplankton was one of the first biological quality elements examined under the Water Framework Directive (WFD), (European Commission, 2000) due to its frequent use in evaluating eutrophication pressure in lakes (Birk et al., 2012; Carvalho et al., 2013). Lake eutrophication leads to changes in phytoplankton composition and generally results in high phytoplankton biomass, often dominated by Cyanobacteria (Ptacnik et al., 2009; Salonen et al., 2023). Phytoplankton composition, abundance, biomass, and bloom frequency and intensity are the key parameters used to assess the ecological status of lakes under the WFD. These parameters are therefore incorporated into national monitoring and assessment systems, in compliance with WFD (European Commission, 2000). In Greece, the Hellenic Phytoplankton Assessment System for Natural Lakes (HeLPhy) is applied (Tsiaoussi et al., 2017; European Commission, 2024). HeLPhy includes metrics indicative of taxonomic composition, biomass, and algal blooms, in line with the definitions outlined in Annex V to WFD. In particular, the system uses the modified Nygaard index (Ott & Laugauste, 1996) for taxonomic composition, the concentration of chlorophyll a (microgram per liter) and total phytoplankton biovolume (cubic millimeter per liter) for abundance and biomass, and the biovolume of Cyanobacteria (cubic millimeter per liter) for algal blooms. This system is applied in 15 lakes that belong to two types of high alkalinity lakes in Greece: (a) natural deep warm monomictic lakes (7 lakes) and (b) shallow polymictic lakes (8 lakes) (Tsiaoussi et al., 2017; Kagalou et al., 2021).

In Greece, phytoplankton monitoring in lakes was introduced in 2012 with the establishment of the National Monitoring Network (Mavromati et al., 2018), as required by article 8 of WFD (JMD No. 140384/2011, later replaced by the JMD No. 107168/1444/2021). Prior to the operation of the monitoring network, there was limited information available on phytoplankton composition of freshwater natural lakes. Available data were primarily focused on specific lakes (e.g., Megali Prespa, Kastoria, Koroneia) and/or covered only brief time periods, as part of short-term research projects (Katsiapi et al., 2012, 2013; Moustaka-Gouni et al., 2012). A long-term record of phytoplankton data for the major natural lakes of Greece was lacking, meaning that knowledge on the structure of phytoplankton communities across freshwater natural lakes and their inter-annual variations was constrained. This, in turn, prevented the comparative analysis of lakes over time, based on the similarities and differences in their phytoplankton composition. Moreover, the ecological status and trends of these lakes based on phytoplankton WFD-compliant monitoring and assessment methods were unknown. The above limitations restricted a comprehensive understanding of the variations in the classification of these lakes into distinct ecological status classes. Recent data collected during the second monitoring period under the WFD (2016–2021) help fill this gap in phytoplankton knowledge for lakes in Greece.

This study represents the first comprehensive investigation of phytoplankton communities in natural lakes within the under-represented Mediterranean region over a 6-year period. Further to describing phytoplankton community structure of Greek lakes, we hypothesize that inter-annual variations will occur in both the composition and biovolume of phytoplankton, with these variations differing across lakes and over time. Additionally, we expect that the WFD-compliant, phytoplankton-based method will provide a robust framework for assessing the ecological status of the lakes, supported by distinct differences in phytoplankton structure across ecological classes. Finally, we hypothesize that notable similarities in the phytoplankton communities of the lakes will mostly reflect comparable ecological status classes, and vice versa. Overall, we anticipate that our understanding of the status and temporal variations of phytoplankton communities across Greek lakes will be improved.

The objectives of this study are as follows: (a) to determine the composition and biovolume of phytoplankton communities and examine their inter-annual variations in 15 natural lakes from 2016 to 2021, (b) to assess the ecological status of these 15 natural lakes using a WFD-compliant method based on phytoplankton, and (c) to investigate potential similarities among the lakes based on their phytoplankton communities.

Materials and methods

Study area and sampling procedure

Fifteen natural lakes were sampled during the warm period from 2016 to 2021 (Fig. 1): 7 deep warm monomictic and 8 shallow polymictic ones. These lakes comprise 60% of all natural lakes included in the National Monitoring Network according to WFD. The main morphometric parameters (altitude, mean depth, size), typology, and ecological status, according to the one-out-all-out principle of the WFD, for all studied lakes are presented in Supplementary Table 1 (Zervas et al., 2021; Soria & Apostolova, 2022; WISE, 2024; Perivolioti et al., 2025). In particular, the highest altitude was recorded in Mikri Prespa (850 m) and the lowest in Lake Lysimacheia (15 m). The surface area of the studied lakes ranged from 94 km^2 in Lake Trichonida, the largest lake in Greece, to less than 1 km^2 in Lake Kournas on Crete island. The mean depth of the lakes ranged from 29 m in Lake Trichonida to 3 m (Lakes Kastoria and Lysimacheia).

Phytoplankton samplings were carried out at a single station in the pelagic zone. Three transboundary lakes (Megali Prespa, Mikri Prespa, and Doirani) had two monitoring stations each. Integrated samples were collected from the euphotic zone of the water column, defined as 2.5 × Secchi disk depth, using a Nansen-type sampler (de Hoyos et al., 2014). On average, three samples per year were collected from each station across all lakes. All lakes were sampled on average for 5 years from 2016 to 2021, and 287 samples were collected in total. The phytoplankton samples were preserved with Lugol's solution (CEN EN 16698, 2015a). In addition, qualitative samples were collected using a phytoplankton net (mesh size 20 μm) to further support the identification of the phytoplankton taxa of each lake. These qualitative samples were preserved in formaldehyde solution (3.9% v/v).

Sample analysis

A total of 287 phytoplankton samples were analyzed under inverted microscopes (Leica DM IL LED Fluo and Olympus CKX31). Quantitative analysis was conducted following the Utermöhl sedimentation method (Utermöhl, 1958; CEN EN 15204, 2006), where a minimum of 400 phytoplankton individuals were counted in each sample at ×10 or ×20 and ×40 or ×63 magnification, depending on the composition of each sample. Phytoplankton biovolume was estimated according to CEN EN 16695 (2015b). In particular, the dimensions of phytoplankton taxa required for biovolume estimation were measured using an inverted microscope and an eyepiece micrometer or microscope camera (Leica MC170 HD Camera). The required dimensions of the relevant geometrical shape were measured for each taxon of interest. At least 20 individuals per taxon were measured to ensure that the standard error of cell or counting unit volume was generally < 10%.

The identification of phytoplankton taxa was carried out at the lowest possible taxon, in order to avoid misclassifications, based on taxonomic keys and papers (Komárek & Fott, 1983; Komárek & Anagnostidis, 1999, 2005; John et al., 2011).

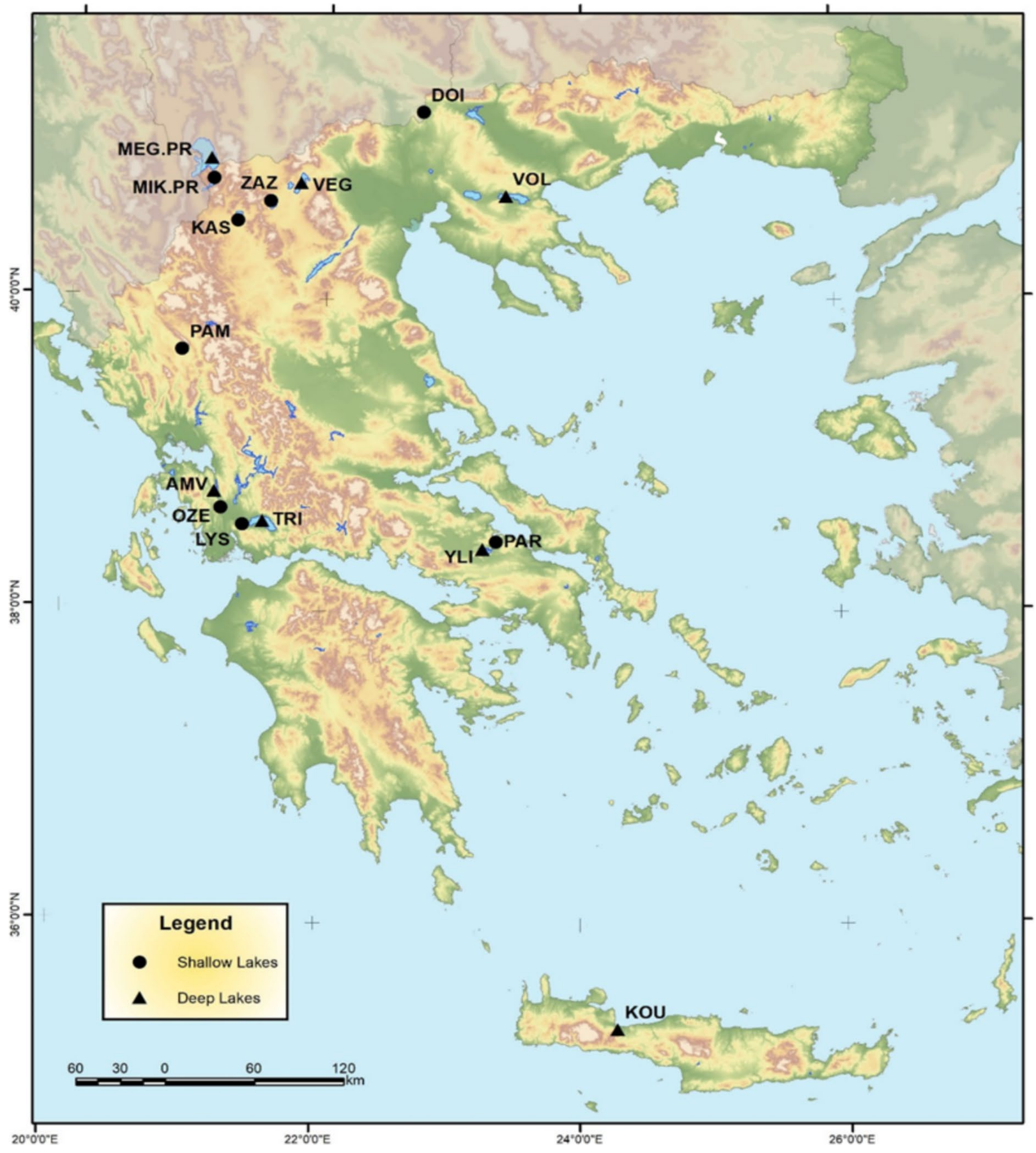

Fig. 1 Map of Greece showing the locations of 15 lakes included in the study (Amv: Amvrakia, Doi: Doirani, Kas: Kastoria, Kou: Kournas, Lys: Lysimacheia, Meg.Pr: Megali Prespa, Mik.Pr: Mikri Prespa, Oze: Ozeros, Pam: Pamvotida, Par: Paralimni, Tri: Trichonida, Veg: Vegoritida, Vol: Volvi, Yli: Yliki, Zaz: Zazari)

The concentrations of chlorophyll a were determined spectrophotometrically according to standard methods (APHA, 2017).

Data analysis

Differences in the number of taxa identified between lakes were tested by one-way analysis of variance (ANOVA). Prior to analysis, data were tested for normality, homogeneity of variance, and independence to ensure they met the assumptions of ANOVA. Tukey's honestly significant difference (HSD) was applied for post hoc multiple comparisons to identify specific lakes that differed significantly from others.

The Hellenic Phytoplankton Assessment System for Natural Lakes (HeLPhy) was applied to all 15 studied natural lakes, both deep and shallow (Tsiaoussi et al., 2017; European Commission, 2024). This system is sensitive to the pressure of eutrophication and consists of four parameters, which are aggregated in a multimetric index, with equal weighting assigned to each parameter. These parameters are as follows: chlorophyll a (microgram per liter), total biovolume (cubic millimeter per liter), modified Nygaard index, and biovolume of Cyanobacteria (cubic millimeter per liter). In particular, with regard to the composition index, the modified Nygaard index is used to determine the taxonomic composition, using biomass of major groups. Ott and Laugaste (1996) have added two additional elements to the original formula: Cryptophyta and Chrysophyceae. The modified calculation (by Ott & Laugaste, 1996) was further amended to exclude Centrales (Tsiaoussi et al., 2017), since they are substantially represented both in high and good quality lakes, suggesting that they could not always validate eutrophic conditions in Greek lakes (Katsiapi et al., 2016; Moustaka-Gouni & Nikolaidis, 1992). The final formula used is given below:

$$PCQ = \frac{Cyanophyta + Chlorococcales + Euglenophyceae + Cryptophyta + 1}{Desmidiales + Chrysophyta + 1} \tag{1}$$

The value of each metric is divided by its reference value, and after normalization, the final lake score is calculated by averaging the normalized EQRs of the metrics according to Eq. 2.

$$HeLPHy = \frac{\left(\frac{nEQR_{Chl} + nEQR_{BV}}{2} + \frac{nEQR_{modNygaard} + nEQR_{CyanoBV}}{2}\right)}{2} \tag{2}$$

$HeLPhy_i$ Final value of HeLPhy assessment method, which is a normalized EQR for the assessment of lake *i*;
$nEQR_{Chli}$ Normalized EQR value of chlorophyll a for lake *i*;
$nEQR_{BVi}$ Normalized EQR value of total biovolume for lake *i*;
$nEQR_{modNygaardi}$ Normalized EQR value of modified Nygaard for lake *i*;
$nEQR_{CyanoBV}$ Normalized EQR value of Cyanobacteria biovolume for lake *i*.

For each lake, the final EQR was calculated by averaging the values from all samples and monitoring stations for each year, thereby minimizing within-year temporal and spatial variations. Consequently, the 287 phytoplankton samples were aggregated into 80 lake-years, with the mean HeLPhy values for 15 lakes calculated over the period from 2016 to 2021. The final HeLPhy score was then assigned to an ecological status class (high, good, moderate, poor, and bad) with threshold values defined at 0.8, 0.6, 0.4, and 0.2, respectively.

Regulatory thresholds, consistent with the definitions of WFD, were intercalibrated among Member States. As a result of the intercalibration exercise, the Commission Decision (EU) 2024/721 establishes the values of the Member State monitoring system classifications to be applied across Europe. This phytoplankton assessment system, HeLPhy, is referred to in the decision, as the national monitoring and assessment method. Its sensitivity to eutrophication was demonstrated through its significant correlation with total phosphorus concentrations (Tsiaoussi et al., 2017).

Non-metric multidimensional scaling (NMDS) was applied to compare lakes assigned to different ecological status classes based on their phytoplankton community composition. The similarity between lake groups was tested using analysis of similarities (ANOSIM). For both analyses, the Bray–Curtis distance measure was applied to square root–transformed data. Intermediate-level data transformations, such as square root transformation, are often used to reduce the disproportionate influence of the most abundant taxa, which otherwise tend to dominate the dissimilarity matrix, relative to less abundant ones (Anderson et al., 2008). This approach ensures that less abundant taxa are adequately considered in the calculation of Bray–Curtis measure, allowing for a more balanced evaluation of their contribution to the phytoplankton community composition.

The similarity percentage (SIMPER) analysis was performed to quantify the contribution of individual taxa to each ecological class and to present the average dissimilarity between different ecological status classes based on phytoplankton taxa (Clarke & Gorley, 2015). The analysis was conducted at the lowest level of ecological classification (lake-years). A cutoff criterion of 90% was applied in the analyses, which were computed using PRIMER v7 software (Clarke & Gorley, 2015). Box plots, NMDS, and statistical analyses (ANOSIM, ANOVA, and Tukey's HSD tests) were carried out using R version 3.5.3 (R Core Team, 2021).

Results and discussion

Results

A total of 462 phytoplankton taxa from 10 taxonomic groups were recorded from 287 samples (Chlorophyta (211), Cyanobacteria (97), Bacillariophyta (39), Euglenophyta (24), Charophyta (28), Xanthophyta (21), Chrysophyta (13), Dinophyta (17), Cryptophyta (9), and Haptophyta (3)). The highest number of phytoplankton taxa was recorded in Chlorophyta and Cyanobacteria taxonomic groups. Cryptophyta and Haptophyta were represented only by a small number of taxa. Although only a few taxa from Cryptophyta were recorded, these were present in 260 out of 287 samples. In contrast, Euglenophyta were only present in 82 samples. Taxa from Xanthophyta and Chrysophyta were relatively rare, being present in 95 and 139 samples, respectively. The most common taxa

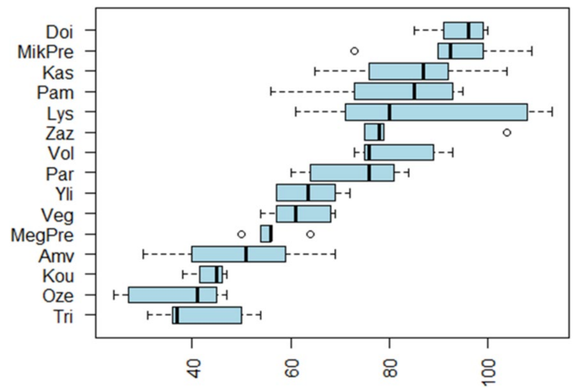

Fig. 2 Box and whisker plots of number of taxa identified in the 15 studied lakes for the period 2016–2021. In the box plots, the central represents the median; the lower and upper limits correspond to the 25 th and 75.th percentiles and the upper and lower whiskers extending to 1.5 and − 1.5 times the interquartile range, respectively (Amv: Amvrakia, Doi: Doirani, Kas: Kastoria, Kou: Kournas, Lys: Lysimacheia, Meg.Pr: Megali Prespa, Mik.Pr: Mikri Prespa, Oze: Ozeros, Pam: Pamvotida, Par: Paralimni, Tri: Trichonida, Veg: Vegoritida, Vol: Volvi, Yli: Yliki, Zaz: Zazari)

recorded in each taxonomic group are presented in Supplementary Table 3.

The number of phytoplankton taxa identified in the 15 studied lakes for the period 2016–2021 is presented in Fig. 2. Lakes Doirani and Mikri Prespa had the highest median number of taxa identified (96 and 93, respectively), and Lake Trichonida had the lowest (37) (Fig. 2). Lake Lysimacheia displayed the greatest variability of taxa identified during this period, whereas Lake Megali Prespa had most of its taxa concentrated around the median, with two outliers. ANOVA analysis revealed statistically significant differences in mean values of taxa identified among the studied lakes [$F(14, 65) = 14.62$, $p < 0.001$]. Tukey's HSD test for multiple comparisons compared the values of each lake with all the others and identified two groups of lakes with no statistically significant differences: one group with a small number of taxa (Lakes Trichonida, Ozeros, Kournas, Amvrakia, and Megali Prespa) and one group with a large number of taxa (Lakes Doirani, Mikri Prespa, Kastoria, Pamvotida, Lysimacheia, Zazari, Volvi, and Paralimni). Two lakes, Lakes Yliki and Vegoritida, did not clearly belong to either of these groups, based on the analysis.

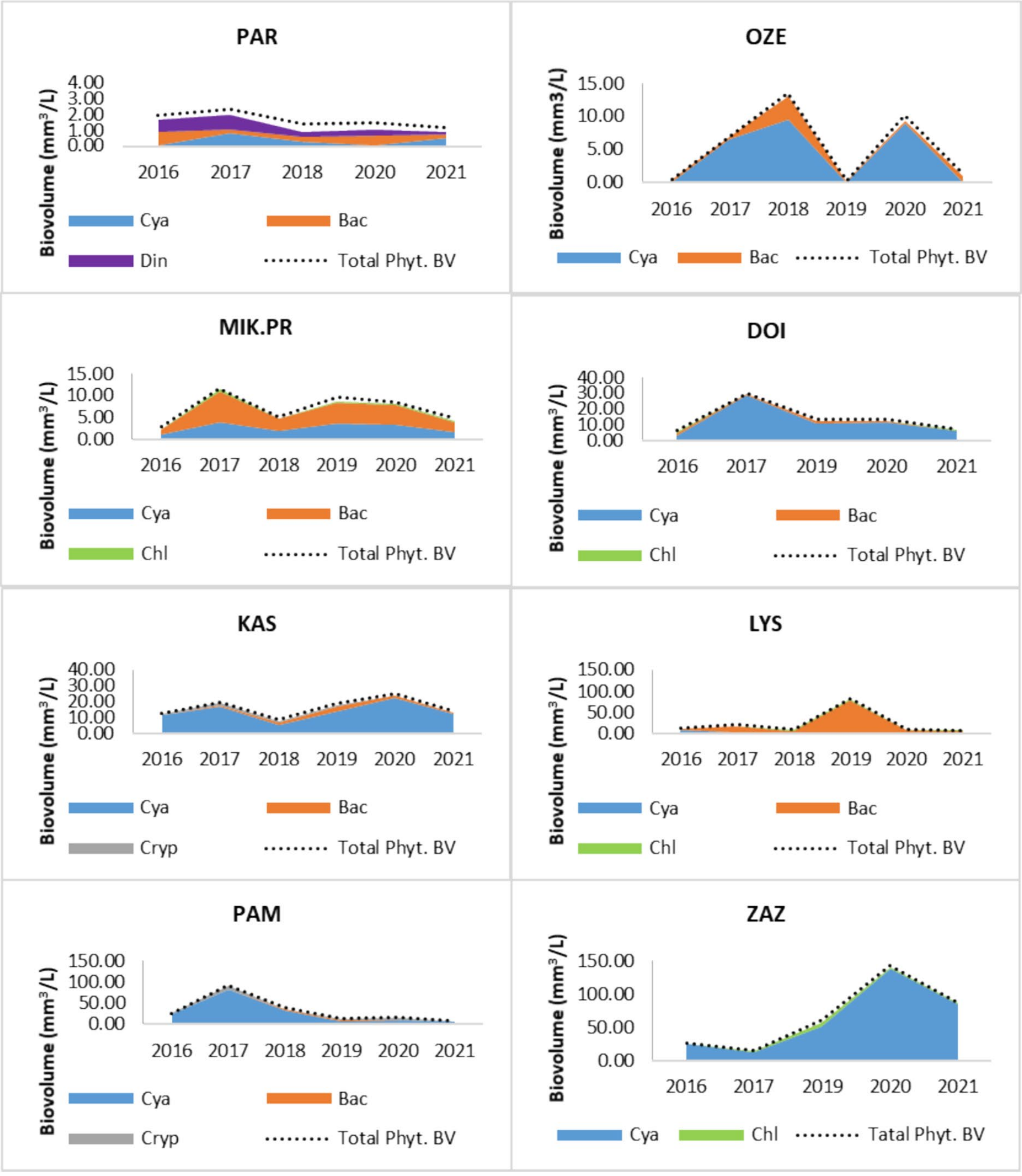

Fig. 3 Biovolume values of the main phytoplankton taxonomic groups for the period 2016–2021 in shallow lakes (Cya: Cyanobacteria, Bac: Bacillariophyta, Chl: Chlorophyta, Din: Dinophyta, Cry: Cryptophyta; Doi: Doirani, Kas: Kastoria, Lys: Lysimacheia, Mik.Pr: Mikri Prespa, Oze: Ozeros, Pam: Pamvotida, Par: Paralimni, Zaz: Zazari)

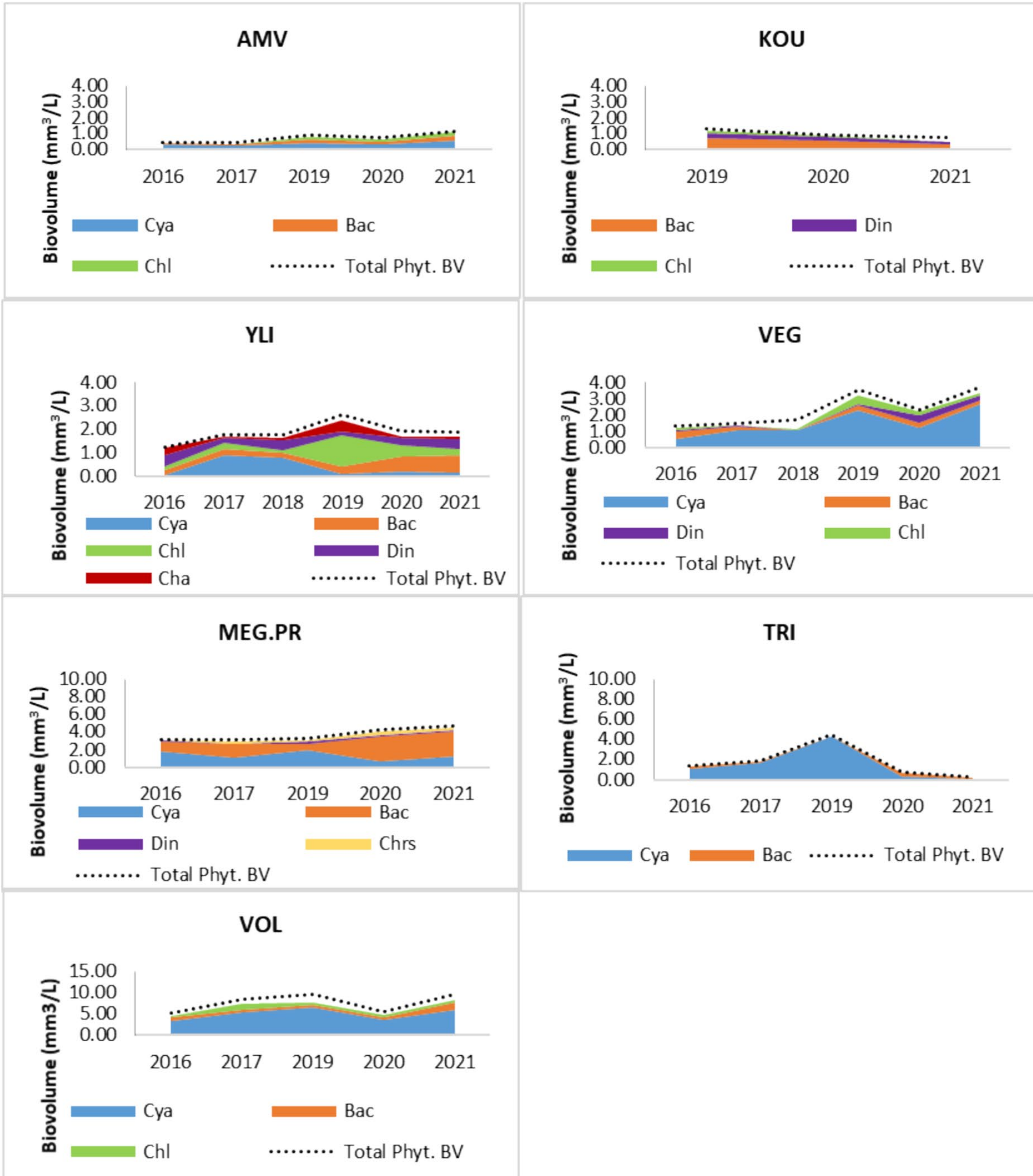

Fig. 4 Biovolume values of the main phytoplankton taxonomic groups for the period 2016–2021 in deep lakes (Cya: Cyanobacteria, Bac: Bacillariophyta, Chl: Chlorophyta, Din: Dinophyta, Cry: Cryptophyta, Chrs: Chrysophyta, Cha: Charophyta; Amv: Amvrakia, Kou: Kournas, Meg.Pr: Megali Prespa, Tri: Trichonida, Veg: Vegoritida, Vol: Volvi, Yli: Yliki)

In Figs. 3 and 4, the total phytoplankton biovolume values as well as the biovolume values of the main taxonomic groups are presented for each sampling year and for each lake (separated into deep and shallow lakes, respectively). Overall, during the growing season, the phytoplankton communities in all lakes were dominated mainly by the taxonomic groups of Cyanobacteria, Bacillariophyta, and Chlorophyta. Cyanobacteria dominated the phytoplankton biovolume in shallow lakes such as Kastoria, Doirani, Pamvotida, and Zazari, as well as in deep lakes, with lower biovolume values, such as Vegoritida, Trichonida, and Volvi over the years. Bacillariophyta were most abundant in terms of biovolume in Lakes Megali Prespa, Mikri Prespa, and Lysimacheia. Chlorophyta also contributed to the phytoplankton community composition, with the highest biovolume values recorded in shallow lakes such as Zazari, Lysimacheia, and Pamvotida. Dinophyta had a more substantial contribution to the phytoplankton biovolume of Lakes Paralimni and Yliki. The remaining taxonomic groups (Charophyta, Chrysophyta, and Cryptophyta) contributed less than 10% to the total biovolume. For instance, the highest contribution of Chrysophyta to the total phytoplankton community, though still at low percentages, was observed in Lakes Paralimni (1.2%), Yliki (1.5%), Megali Prespa (5.3%), and Kournas (6.3%).

Most lakes exhibited inter-annual variations in the biovolume of specific taxonomic groups and in total phytoplankton biovolume. In particular, among deep lakes, Lake Trichonida showed a peak in Cyanobacteria biovolume in 2019 and Lake Vegoritida experienced small increases in Cyanobacteria biovolume in years 2019 and 2021, although biovolume values remained relatively low. On the other hand, shallow lakes displayed variations not only in Cyanobacteria biovolume but also in Bacillariophyta biovolume on multiple occasions. The highest Cyanobacteria biovolume rise was recorded in Lake Zazari during 2020 (138.43 mm^3/L). Lake Doirani also experienced a high peak in Cyanobacteria biovolume in 2017 (29.02 mm^3/L). Regarding Bacillariophyta, the highest biovolume value (79.08 mm^3/L) was recorded in Lake Lysimacheia in 2019, while in Lake Mikri Prespa, there was a modest increase in 2017 (7.18 mm^3/L). Lake Zazari exhibited the most pronounced inter-annual variations based on its phytoplankton biovolume, followed by Lakes Pamvotida and Lysimacheia. These variations were mostly attributed to changes in the biovolume of Cyanobacteria in Lakes Zazari and Pamvotida and of Bacillariophyta in Lake Lysimacheia. Moreover, inter-annual variations in terms of total phytoplankton biovolume and Cyanobacteria biovolume values were recorded in Lake Ozeros in 2018, 2019, 2020, and 2021. In contrast, the lowest variations were recorded in Lakes Kournas and Amvrakia. The highest variations in phytoplankton taxonomic composition throughout 2016–2021 were observed in Lake Yliki, where different taxonomic groups dominated each year. On the other hand, the taxonomic composition of Lake Kournas was the most stable throughout the years.

The mean biovolume values of the three main phytoplankton taxonomic groups (Cyanobacteria, Bacillariophyta, Chlorophyta) of all 15 studied lakes during the period 2016–2021 are presented in Fig. 5. Lake Amvrakia exhibited the lowest annual average total phytoplankton biovolume (0.76 mm^3/L), whereas Lake Zazari displayed the highest annual average phytoplankton biovolume (66.8 mm^3/L). In general, lakes with the highest phytoplankton biovolume values tended to have their phytoplankton community dominated mostly by Cyanobacteria, as observed in Lakes Zazari and Pamvotida where the highest mean values of Cyanobacteria were recorded. The Cyanobacteria taxa with the highest biovolume values mostly observed in these two lakes were *Microcystis* spp. and *Dolichospermum* spp. The highest mean biovolume of Bacillariophyta was recorded in Lake Lysimacheia (17.90 mm^3/L), where the phytoplankton community was mainly dominated by Stephanodiscaceae, *Aulacoseira granulata* (Ehrenberg) Simonsen 1979, *Cyclotella* spp., *Acanthoceras zachariasii* (Brun) Simonsen 1979, and *Fragilaria* spp.

The values of HeLPhy EQRs (mean $\pm$SD) for shallow and deep lakes during the period 2016–2021 are shown in Figs. 6 and 7, respectively. For shallow lakes, based on phytoplankton, two lakes were assigned to good, four to moderate, and two lakes to poor status (Fig. 6). Overall, Lake Ozeros exhibited the highest inter-annual variations (SD $=0.24$), as EQRs ranged from 0.45 to 0.96 crossing the good/moderate boundary. In contrast, the EQR values of Lakes Doirani and Kastoria showed the lowest inter-annual variations (SD $=0.03$). The two lakes assigned to poor status were classified as bad for one year each (see Tables 1 and 2).

Deep lakes (Fig. 7) seemed to have scored higher; two lakes were classified in high status, four in good, and one in moderate status. Lakes Vegoritida and Megali Prespa slightly crossed the good/moderate boundary, each with an average EQR value of

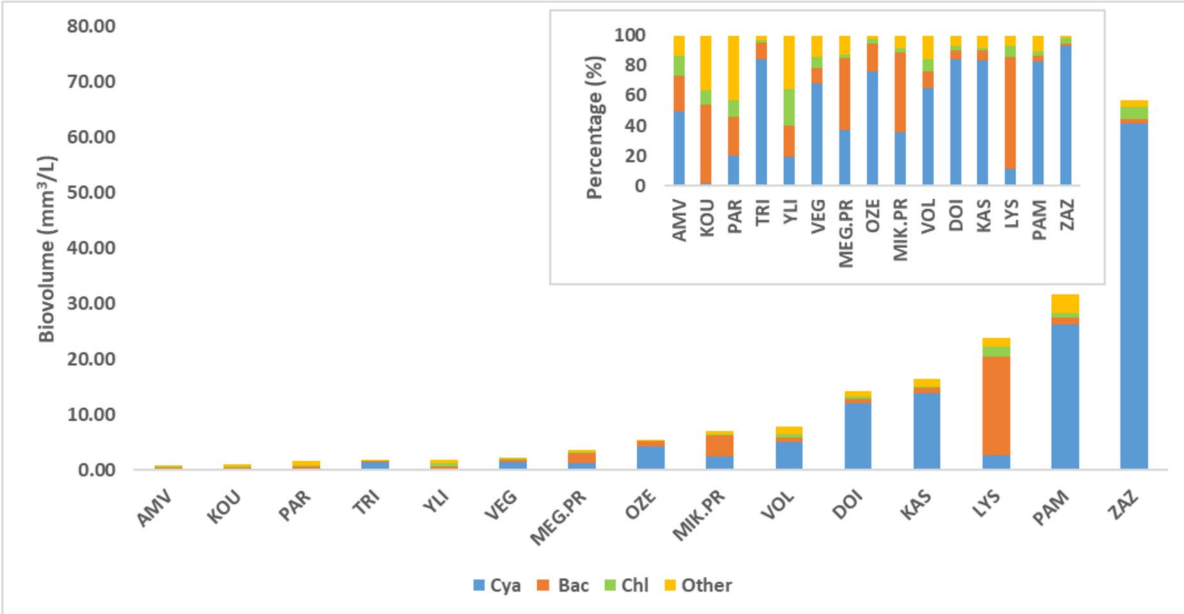

Fig. 5 Mean biovolume values of the three main phytoplankton taxonomic groups in the 15 studied lakes for period 2016–2021 and the percentage share of phytoplankton groups in each lake (Cya: Cyanobacteria, Bac: Bacillariophyta, Chl: Chlorophyta; Amv: Amvrakia, Doi: Doirani, Kas: Kastoria, Kou: Kournas, Lys: Lysimacheia, Meg.Pr: Megali Prespa, Mik.Pr: Mikri Prespa, Oze: Ozeros, Pam: Pamvotida, Par: Paralimni, Tri: Trichonida, Veg: Vegoritida, Vol: Volvi, Yli: Yliki, Zaz: Zazari)

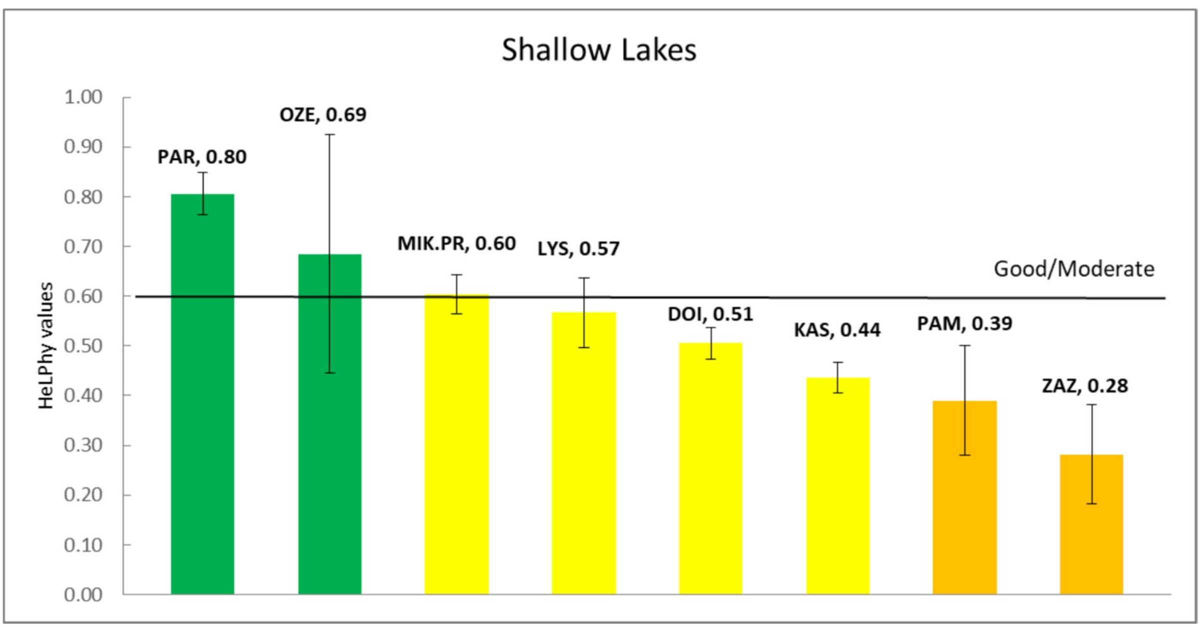

Fig. 6 HeLPhy values (mean ± SD) for 2016–2021 for shallow lakes (Doi: Doirani, Kas: Kastoria, Lys: Lysimacheia, Mik.Pr: Mikri Prespa, Oze: Ozeros, Pam: Pamvotida, Par: Paralimni, Zaz: Zazari)

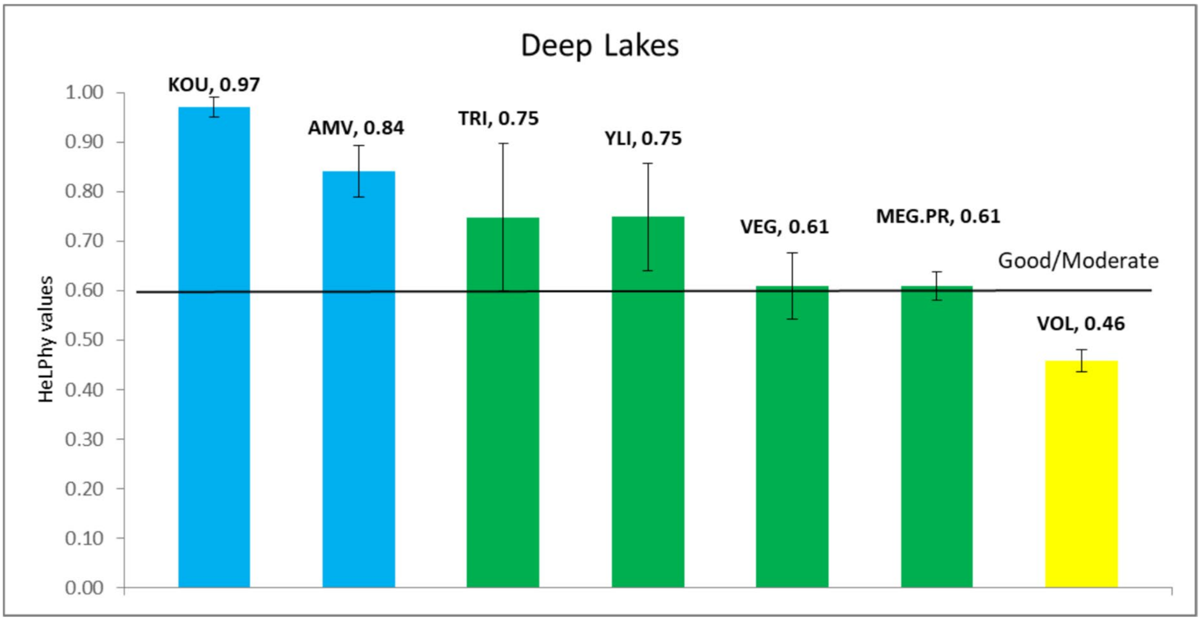

Fig. 7 HeLPhy values (mean ± SD) for 2016–2021 for deep lakes (Amv: Amvrakia, Kou: Kournas, Meg.Pr: Megali Prespa, Tri: Trichonida, Veg: Vegoritida, Vol: Volvi, Yli: Yliki)

Table 1 SIMPER results for average dissimilarity between different ecological status classes (PRIMER 7 software)

Groups of different ecological status	Average dissimilarity (%)
High and good	83.35
High and moderate	92.05
High and poor	97.69
High and bad	99.48
Good and moderate	88.32
Good and poor	95.66
Good and bad	98.61
Moderate and poor	90.72
Moderate and bad	95.97
Poor and bad	68.99

0.61. Among deep lakes, Lake Trichonida showed the highest inter-annual variations (SD = 0.15), with EQR values ranging from 0.58 to 0.95. All lakes but one scored above good. On the other hand, Lake Volvi was consistently classified as moderate, with the lowest standard deviation (SD = 0.02).

Overall, across the 15 lakes, 8 lakes met the WFD good status target according to HeLPhy (2 in high and 6 in good status), 5 were classified as moderate, and 2 as poor, as aggregated for the period 2016–2021. At the lake-year level, all five ecological status classes were represented (Tables 1 and 2).

The lakes were plotted in the NMDS based on their ecological status (Fig. 8). The stress value was 0.06 indicating a strong representation of the lakes in the reduced dimensions, and the ANOSIM analysis ($R = 0.61$) further confirmed the differences between the groups. Ellipses could only be calculated for lakes with good and moderate ecological status, as there were only two lakes with high and poor ecological status, respectively. The lakes spread out in the ordination plot, mostly reflecting the ecological gradient of HeLPhy. Only Lake Lysimacheia appeared to be differentiated from its group, as it was positioned at the bottom of the plot and separated from the other lakes classified with moderate ecological status.

The results of the SIMPER analysis were based on phytoplankton taxa according to the ecological status at the lake-year level ($n = 80$) (Tables 1 and 2).

The average dissimilarities between pairs of different ecological status classes ranged from 68.99 to 99.48%, with the dissimilarity between good and moderate status being 88.32%. The SIMPER analysis showed that the contributions of phytoplankton taxa at high status were consistent with undisturbed conditions, characterized by low contributions of the most common taxa, with

Table 2 Summary table of the SIMPER results for taxa contribution to similarity within each ecological status class (PRIMER 7 software). A cutoff at a cumulative similarity (%) of 90% was applied

Ecological status: high ($n = 14$)			Ecological status: good ($n = 22$)			Ecological status: moderate ($n = 36$)			Ecological status: poor ($n = 6$)			Ecological status: bad ($n = 2$)		
Taxa	Contrib (%)	Cum (%)	Taxa	Contrib (%)	Cum (%)	Taxa	Contrib (%)	Cum (%)	Taxa	Contrib (%)	Cum (%)	Taxa	Contrib (%)	Cum (%)
Stephanodiscaceae	65.02	65.02	Stephanodiscaceae	32.13	32.13	Stephanodiscaceae	13.45	13.45	*Microcystis* cf. *wesenbergii*	63.05	63.05	*Microcystis* spp.	50.10	50.10
Peridiniales	5.10	70.12	*Cyclotella* spp.	9.54	41.67	*Dolichospermum* spp.	12.76	26.21	*Microcystis* spp.	14.15	77.20	*Microcystis* cf. *wesenbergii*	47.62	97.72
Ceratium hirundinella (O. F. Müller) Dujardin 1841	4.24	74.35	Peridiniales	6.02	47.69	*Aphanizomenon* spp.	7.46	33.66	*Cryptomonas* spp.	4.45	81.65			
Oocystis sensu lato	3.48	77.83	*Ceratium hirundinella*	5.53	53.22	*Cryptomonas* spp.	6.27	39.94	*Dolichospermum* spp.	3.70	85.35			
Chrysochromulina spp.	2.70	80.53	*Oocystis* sensu lato	5.10	58.32	*Microcystis* spp.	5.70	45.63	Stephanodiscaceae	2.06	87.41			
Aphanocapsa spp.	2.60	83.14	*Cryptomonas* spp.	4.68	63	*Raphidiopsis raciborskii* (Wołoszyńska) Aguilera et al. 2018	4.88	50.51	*Microcystis* cf. *aeruginosa*	2.02	89.44			
Oscillatoriales	2.40	85.53	*Microcystis* spp.	4.02	67.02	Peridiniales	3.79	54.30	*Chroococcus* spp.	1.02	90.46			
Plagioselmis nannoplanctica (Skuja) G. Novarino, I. A. N. Lucas, and Morrall 1994	2.34	87.87	*Cyanodictyon* spp.	3.38	70.4	*Cuspidothrix issatschenkoi* (Usachev) P. Rajaniemi, Komárek, R. Willame, P. Hrouzek, K. Kastovská, L. Hoffmann, and K. Sivonen 2005	3.56	57.87						
Green coccoid	1.87	89.74	*Aphanocapsa* spp.	2.97	73.37	*Cyanodictyon* spp.	3.49	61.36						

Table 2 (continued)

Ecological status: high (*n* = 14)			Ecological status: good (*n* = 22)			Ecological status: moderate (*n* = 36)			Ecological status: poor (*n* = 6)			Ecological status: bad (*n* = 2)		
Taxa	Contrib (%)	Cum (%)	Taxa	Contrib (%)	Cum (%)	Taxa	Contrib (%)	Cum (%)	Taxa	Contrib (%)	Cum (%)	Taxa	Contrib (%)	Cum (%)
Cyclotella spp.	1.20	90.94	*Plagioselmis nannoplanctica*	2.61	75.99	*Planktolyngbya* spp.	3.39	64.75						
			Dolichospermum spp.	2.61	78.6	*Microcystis* cf. *wesenbergii*	3.13	67.88						
			Oscillatoriales	2.09	80.69	Oscillatoriales	2.66	70.54						
			Chrysochromulina spp.	1.83	82.52	*Aphanocapsa* spp.	2.53	73.07						
			Dinobryon spp.	1.40	83.92	*Ceratium hirundinella*	2.27	75.34						
			Aphanizomenon spp.	1.33	85.25	*Oocystis* sensu lato	2.24	77.58						
			Chlorellaceae	1.13	86.38	*Plagioselmis nannoplanctica*	1.99	79.56						
			Fragilaria spp.	1.07	87.45	*Aulacoseira granulata*	1.96	81.52						
			Green coccoid	1.00	88.45	*Lyngbya* spp.	1.85	83.37						
			Planktothrix spp.	0.97	89.42	*Raphidiopsis* spp.	1.81	85.18						
			Staurastrum spp.	0.96	90.38	*Chrysochromulina* spp.	1.77	86.96						
						Acanthoceras zachariasii	1.01	87.97						
						Chlamydomonas spp.	0.99	88.96						
						Green coccoid	0.88	89.84						
						Fragilaria spp.	0.77	90.60						

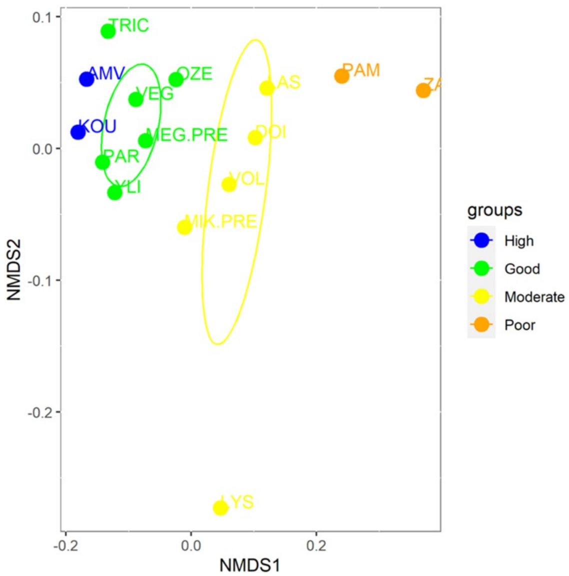

Fig. 8 NMDS plot showing the relative position of lakes based on the Bray–Curtis similarity index using phytoplankton biovolume of taxonomic groups (stress value 0.06). Ellipses are standard deviations from the mean; each ellipse and color represent different ecological status (Amv: Amvrakia, Doi: Doirani, Kas: Kastoria, Kou: Kournas, Lys: Lysimacheia, Meg. Pr: Megali Prespa, Mik.Pr: Mikri Prespa, Oze: Ozeros, Pam: Pamvotida, Par: Paralimni, Tri: Trichonida, Veg: Vegoritida, Vol: Volvi, Yli: Yliki, Zaz: Zazari)

Bacillariophyta being persistent throughout the growing season. According to SIMPER, phytoplankton communities at good status were dominated by Bacillariophyta, followed by Cyanobacteria and Dinophyta. Cyanobacteria taxa increased their contribution compared to high status, though still at low levels. Bacillariophyta taxa were also present at moderate status, but their contribution was less than half compared to good status. In the poor ecological status class, Cyanobacteria contributed almost exclusively to the average similarity of the lakes (83.94%), with *Microcystis* taxa being the ones that contributed most. At bad status, *Microcystis* cf. *wesenbergii* (47.62%) and other *Microcystis* taxa (50.10%) were the only contributing taxa. According to SIMPER analysis, a greater diversity of taxa contributed to the intermediate ecological classes, notably moderate and good status.

Discussion

This research is the first comprehensive investigation of phytoplankton community composition and biovolume for 15 Greek natural lakes over the period 2016–2021. The analysis covered several aspects for the studied lakes, including phytoplankton composition and diversity patterns, inter-annual variations in the biovolume of the main taxonomic groups, classification of lake ecological status, assessment of their similarity according to phytoplankton taxa, and contribution of phytoplankton taxa to the classification of ecological classes. Similar efforts have been undertaken in other countries with respect to their natural lakes (e.g., Cellamare et al., 2012; Hutorowicz & Pasztaleniec, 2014; Phillips et al., 2010), although it could be argued that the limnological research in the Mediterranean remains less extensive compared to other European regions (Alvarez Cobelas et al., 2005; Beklioglu et al., 2007).

Chlorophyta participated in the phytoplankton community composition of the lakes with the highest number of taxa (211). The highest biovolume values were found in shallow lakes in moderate and poor status, such as Zazari, Lysimacheia, and Pamvotida. Green algae and Cyanobacteria have similar environmental requirements for their growth, and they are natural competitors (Jensen et al., 1994; Yang et al., 2018). Chlorophyta dominance in hypertrophic shallow lakes may be attributed to continuous input of nutrients and carbon from the sediment and external sources (Jensen et al., 1994). This makes the fast-growing Chlorophyta a superior competitor compared with the relatively slow-growing Cyanobacteria, even when inorganic nutrient concentrations are low and the pH is high.

We identified 97 taxa belonging to Cyanobacteria. Cyanobacteria thrive in a wide range of nutrient concentrations, including oligotrophic waters (Sorichetti et al., 2014; Reinl et al., 2021), although high abundances are most typically associated with high nutrient concentrations (Salonen et al., 2023). Furthermore, Cyanobacteria are more favored in shallow lakes because the production of algal biomass in deeper lakes is limited by the poor light availability (Nixdorf & Deneke, 1997). As a result, a higher proportion of Cyanobacteria can be expected in shallow lakes (Scheffer, 1998; Scheffer & van Nes, 2007). Moreover, Cyanobacteria taxa are known for responding to increasing nutrient concentrations, while forming dense blooms during high temperatures in eutrophic waters (Carvalho et al., 2011; Watson et al., 2016). This pattern was observed in our results, as most shallow lakes, in lower ecological status classes, exhibited a high abundance of Cyanobacteria taxa, with the highest recorded in Lake Zazari, a shallow lake in poor status. Cyanobacteria taxa were represented mainly by the genera *Aphanizomenon* and *Microcystis*, typical of nutrient-rich lakes (Poniewozik & Lenard, 2022).

Bacillariophyta were also of great importance in Greek lakes, with numerous taxa being present in most lakes. Reynolds (2006) suggests that freshwater diatoms prefer cold and nutrient-poor water bodies. Notably, Stephanodiscaceae were mainly recorded in high and good status lakes, and their contribution declined in lower ecological status classes, as shown by SIMPER analysis.

In contrast, Chrysophyta and Xanthophyta did not seem to be quantitatively important in the phytoplankton community of Greek lakes. Their maximum mean biovolume value was only 0.20 mm^3/L (6% of the mean biovolume) for Chrysophyta and 0.09 mm^3/L (1% of the mean biovolume) for Xanthophyta, as it has also been reported in some Danish lakes (Olrik, 1998). The largest contribution of this group to the total phytoplankton community occurred in the high and good status Lakes Paralimni, Yliki, Megali Prespa, and Kournas. Despite the high richness of Chrysophyta floras recorded in localities that are neutral to slightly acidic (Siver & Hamer, 1989), low in specific conductance, alkalinity, and nutrient content (Cronberg & Kristiansen, 1980; Durrschmidt, 1980, 1982; Roijackers & Kessels, 1986; Sandgren, 1988; Siver & Hamer, 1989; Siver, 1991), our dataset lakes display high alkalinity (Mavromati et al., 2018). Xanthophyta typically have low contribution in the phytoplankton community; they are usually present with small numbers of taxa and low biomass (Kostryukova et al., 2019; Nweze, 2006; Zalocar de Domitrovic, 2003). For instance, Padisák et al. (2003) found that Xanthophyceae predominated only once in their study of the phytoplankton assemblages in 80 shallow Hungarian lakes.

Dinophyta, Cryptophyta, Charophyta, and Chlorophyta exhibit no specific preferences for ecological classes in our dataset. This finding aligns with research conducted on European lakes, which suggests that the observed wide variation within these phyla can be attributed to differences in nutrient preferences among genera and species within each group (Phillips et al., 2013).

Temporal variations in phytoplankton taxonomic composition and biomass occur on seasonal and interannual scales (Sommer et al., 2012; Talling, 1993). In Greek lakes, inter-annual variations in both phytoplankton composition and biovolume have been frequently observed, particularly in Lakes Zazari, Pamvotida, Lysimacheia, and Ozeros, with all but one (Lake Ozeros) assigned to lower ecological classes. The concentration of phosphorus and light conditions, which are indicators of eutrophic conditions that prevail in lower ecological classes, are considered key drivers of phytoplankton variation on these temporal scales (Anneville et al., 2004). Moreover, these fluctuations seem to affect the EQR values of the HeLPhy index, most notably in Lake Ozeros where the EQRs exceeded the good/moderate boundary in successive years. This finding supports the recommendation that three samples from each year, over a minimum of three years, are necessary to ensure an acceptable level of uncertainty in the assessment of ecological status according to phytoplankton in accordance with WFD (de Hoyos et al., 2014; CEN EN 16698, 2015a; Tsiaoussi et al., 2017).

Phytoplankton is generally considered an extremely sensitive, early warning bio-indicator of lake water pollution (Carvalho et al., 2013). As phytoplankton presents short generation time periods and derives its nutrients from the water column, it is the most direct and rapid indicator of eutrophication on lake ecosystems (Lyche Solheim et al., 2013). Therefore, it serves as a key biological indicator for in situ monitoring and assessment under the WFD (Birk et al., 2012). The target of WFD is for water bodies to achieve at least good status (European Commission, 2000). In our dataset, 8 out of the 15 lakes met the WFD good status target according to HeLPhy, a WFD-compliant phytoplankton index (European Commission, 2024). The remaining lakes were classified in moderate (five lakes) and poor status (two lakes).

The NMDS ordination demonstrated a strong model, allowing the confident separation between ecological classes. The distinction of Lake Lysimacheia from all lakes and in particular from the group of lakes exhibiting moderate ecological status can be attributed to the high inter-annual variability in the number of taxa recorded and to the substantial contribution of Bacillariophyceae to the phytoplankton biovolume in the lake in 2019. The SIMPER analysis, conducted at lake-year level, further confirmed the discrimination of ecological classes, based on compositional shifts in phytoplankton communities, and showed high average dissimilarity between them. As expected, high and bad ecological classes showed the highest dissimilarity. Notably, the average dissimilarity at the good/moderate threshold is 88.32%, much higher than the dissimilarity based on littoral zoobenthos of Greek lakes (Mavromati et al., 2021). The good/moderate boundary is the most important threshold on the ecological condition gradient for the WFD (Phillips et al., 2024; Poikane et al., 2014), making the high discrimination between these classes essential. Although a high number of phytoplankton taxa were identified in lakes of poor ecological status, their contribution to the similarity within this status class was minimal, comprising only seven taxa.

The number of phytoplankton taxa identified varied among the lakes. Lakes classified at high and good status exhibited a lower number of taxa compared to those at moderate and poor status. This pattern appears to be associated with the influence of disturbances, such as nutrient availability and eutrophication pressure (Quinlan et al., 2021; Borics et al., 2021). Notably, the results of the SIMPER analysis in our dataset revealed that the highest number of taxa contributing to the within-group similarity was observed in the moderate ecological status class. As expected, phytoplankton biovolume followed a similar pattern, with lower nutrient concentrations in high and good status classes supporting lower phytoplankton diversity and biomass (Quinlan et al., 2021) and fewer, if any, algal blooms (Ho & Michalak, 2020). In contrast, lakes with moderate and especially poor status exhibited elevated phytoplankton biovolume, primarily driven by Cyanobacteria, as also shown by SIMPER analysis. Cyanobacteria are key indicators of water quality deterioration (Brooks et al., 2016).

Stephanodiscaceae were the most important taxon in high and good status lakes according to SIMPER analysis. SIMPER analysis also reveals a clear tendency for Bacillariophyta to decline in importance as the ecological status deteriorates.

As the ecological status declined, we observed a distinctive pattern of increasing Cyanobacteria contribution, and especially *Microcystis* taxa, consistent with previous research showing that Cyanobacteria genera *Microcystis*, *Aphanizomenon*, and *Anabaena* tend to become more abundant in response to nutrient enrichment (Reynolds, 1984). Filamentous Cyanobacteria have the ability to adapt to different environmental conditions, are sensitive to light penetration, and compete against other photosynthetic organisms (Poniewozik & Lenard, 2022; Reynolds, 1987). *Microcystis* cf. *wesenbergii* and other *Microcystis* taxa dominated at bad status (2 lake-years).

Although Chlorophyta participated in the phytoplankton community composition of the lakes with the highest number of taxa, their contribution to similarity within each status class was not remarkable.

Greek lakes, as most Mediterranean lakes, face multiple pressures including nutrient loading from point and non-point sources, water abstraction, and morphological changes (Latinopoulos et al., 2016). Phytoplankton, as a well-established biological quality element that captures changes in the trophic gradient resulting from nutrient loading, effectively assesses the ecological status and trends of the main natural Greek lakes, with a robust and meaningful ecological interpretation by HeLPhy. An accurate assessment of the water quality of lakes using phytoplankton is crucial for establishing management objectives and measures to protect and, when required, restore aquatic ecosystems (Birk et al., 2013).

Conclusions

In this research, we analyzed a recent time series of phytoplankton data from 15 natural Greek lakes, collected as part of the national water monitoring network (period 2016–2021) in compliance with the Water Framework Directive. A total of 462 phytoplankton taxa from 10 taxonomic groups were recorded in 287 phytoplankton samples. The highest number of phytoplankton taxa was recorded in Chlorophyta and Cyanobacteria taxonomic groups. Overall, during the growing season, the phytoplankton communities in all lakes were dominated mainly by the taxonomic groups of Cyanobacteria, Bacillariophyta, and Chlorophyta. Inter-annual variations occurred in both the composition and biovolume of phytoplankton, with these variations differing across lakes and over time. Based on the HeLPhy index, the lakes were classified into four ecological status classes: high, good, moderate, and poor. Inter-annual variations in ecological classification were observed. The HeLPhy index effectively discerned lake ecological classes, with a robust and meaningful ecological interpretation. Comprehensive knowledge of phytoplankton composition and biomass and their temporal variability in Greece's natural lakes improves our understanding of these ecosystems, also contributing to the designing of effective management measures in the context of the Water Framework Directive. This analysis provided valuable insights into the status and trends of phytoplankton communities in lake ecosystems of Greece.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Ethics approval All authors have read, understood, and have complied as applicable with the statement on "Ethical responsibilities of Authors" as found in the Instructions for Authors.

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