Interaction between temperature and nutrients: How does the phytoplankton community cope with climate change?

Flavia Dory [*], Veronica Nava, Morena Spreafico, Valentina Orlandi, Valentina Soler, Barbara Leoni [*]

University of Milano-Bicocca, Department of Earth and Environmental Sciences, Piazza della Scienza 1, Milan, Italy

HIGHLIGHTS

- Warming directly led to an increase in algal biovolume and a diversity loss.
- Warming indirectly affected phytoplankton community via nutrient variations.
- Nutrient variations caused taxonomic and functional reorganization of community.
- Climate change must be urgently integrated in deep lake's management strategies.

GRAPHICAL ABSTRACT
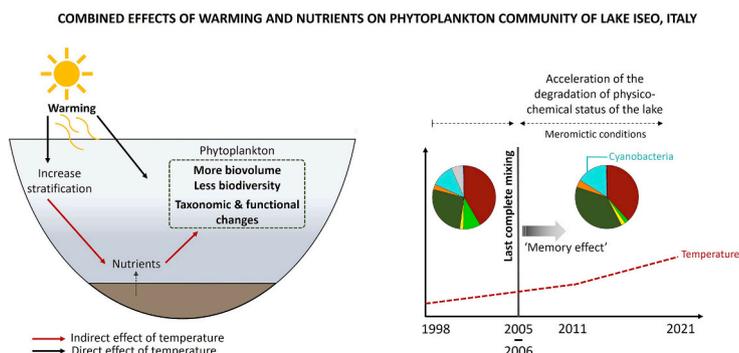
ABSTRACT

Climate change and increasing nutrient concentrations are two major threats to lake ecosystems. Furthermore, warming is exacerbating the symptoms of eutrophication in freshwaters. Facing both environmental challenges simultaneously is more urgent than ever to preserve and recover water quality and protect the remaining biodiversity. Here, we used long-term observational data to investigate the phytoplankton response to the interaction between temperature and nutrient variations in a deep mesotrophic subalpine lake (L. Iseo, Italy). Despite the existing management programs, we observed a deterioration of the physicochemical status of the lake between 1993 and 2021 in the water column. The average rate of temperature increase was 0.02 °C y^{-1} across the studied period and accelerated after the last complete mixing events (2005 and 2006), particularly during the last decade (0.08 °C y^{-1}). Water warming caused severe impacts on nutrient cycling, reflected by the overall increase in nutrient concentrations. The direct effect of warming was of great importance for phytoplankton, leading to an increase in total biovolume and a loss of diversity and promoting the dominance of Cyanobacteria. Nutrient variations, especially TN and the DSi:TP ratio, considerably structured the community composition. The modification of physicochemical parameters caused by the last complete mixing events led to a remarkable, long-term taxonomical and functional reorganization of the phytoplankton community. This study illustrates that phytoplankton in deep subalpine lakes will experience severe changes in the upcoming years, and that complete mixing events may constitute a threshold for community transformation. Our results stress the importance of using powerful interpretative models with multifaceted long-term datasets to disentangle the pathways by which

temperature and nutrients could regulate the phytoplankton community. Our conclusions will help to integrate climate change into mitigation strategies to preserve ecosystem structure and functions.

1. Introduction

Climate change and nutrient variations are the key drivers of changes in aquatic ecosystems (Deng et al., 2019; Jones and Brett, 2014). Many lakes are warming rapidly across the world, and long-term in situ monitoring data indicate an increase in surface water temperature worldwide (Kraemer et al., 2021; O'Reilly et al., 2015). Climate change, which acts globally and at regional/local scales, is promoting the symptoms of eutrophication in freshwaters (Meerhoff et al., 2022). Climate change, particularly warming, can affect nutrient dynamics in lakes through multiple direct and indirect processes. Warmer temperatures result in longer and more intense thermal stratification in deep lakes (Kraemer et al., 2015; Richardson et al., 2017). Higher stratification often results in prolonged periods of hypoxia in deep water (Jane et al., 2021), promoting the release of dissolved phosphorus and ammonium from the sediments into the water column, as well as the decrease in nitrate via denitrification (Farrell et al., 2020). More indirectly, warming may increase nutrient concentrations by enhancing mineralization (Malmaeus et al., 2006), or may decrease nutrient concentrations by promoting phytoplankton growth rates and nutrient uptake (Reynolds, 2006). Lakes, as sentinels of climate change, act as early warning systems (Adrian et al., 2009). Lakes are vulnerable and crucial, covering only a limited area worldwide but sheltering high levels of biodiversity and contributing significantly to ecosystem services (Heino et al., 2021). The indirect effects of climate change are overlooked and should be taken into account in policy strategies to improve the qualitative status of water bodies (Moss, 2011). Indeed, the interaction between temperature and nutrient increase will make the existing standards harder to achieve and increasingly invalid (Bennion et al., 2011). To mitigate the negative effects of global change, integrated studies disentangling the impacts of nutrients and climate change are urgently needed to maintain and recover water quality and to protect the remaining biodiversity and ecosystem services.

Previous studies have investigated the relative importance of temperature and nutrients on phytoplankton assemblage variations. In many cases, studies concluded that nutrients have a higher effect on phytoplankton biovolume and composition than temperature (Brookes and Carey, 2011; Moss et al., 2003; Wagner and Adrian, 2009), whereas others argued that the effect of water temperature increase is highly dependent on the trophic state of the lake (Rigosi et al., 2014). Warming trends directly affect phytoplankton assemblages by prolonging the duration of the growing season and strengthening the stratification of the water column (Kundzewicz et al., 2007). It has also been shown that increasing temperature accelerates phytoplankton growth rates and overall metabolic activity, depending on the species optimum (Reynolds, 2006). Previous studies have observed changes in community composition with warming, with a dominance of phytoplankton taxa that have higher optimum temperatures for growth, with strong repercussions on diversity and community structure (Salmaso and Tolotti, 2021). For example, Rasconi et al. (2017) experimentally observed a decrease in phytoplankton diversity with warming, caused by the increasing dominance of Cyanobacteria and Chlorophyta, which are better adapted to warmer temperatures.

Nitrogen (N) and phosphorus (P) concentrations are key drivers of phytoplankton community variations, as they constitute limiting nutrients for phytoplankton growth in freshwater (Reynolds, 2006). An increase in their concentration traditionally results in a dramatic increase in the density of algal cells suspended in water (Smith and Schindler, 2009). An increase in the dominance of Cyanobacteria with increasing nutrient concentrations is a common result of numerous studies assessing the effects of eutrophication (Salmaso and Tolotti, 2021). However,

the respective roles of N and P in phytoplankton community changes are not clearly understood. Phosphorus has traditionally been identified as the main cause of the deterioration of water quality and one of the main factors controlling phytoplankton development, while the role of N in phytoplankton growth has been much less investigated (Schindler, 2012). Nevertheless, the importance of N limitation for lake productivity has recently been more intensively discussed, and recent works suggest that higher N concentrations may also have significantly adverse effects on lake water quality and algal development (Moss et al., 2013; Olsen et al., 2015; Paerl et al., 2016; Søndergaard et al., 2017). Additionally, variation in silica concentrations is rarely considered in determining the role of nutrients on phytoplankton, but this nutrient is of major importance for silica-user taxa, mainly diatoms and chrysophytes (Tolotti et al., 2007; Yankova et al., 2017). Further levels of complexity are added when, in addition to N and P, other nutrients and environmental conditions are taken into consideration, highlighting how the relationship between algal growth and resources is of indisputable importance but is still poorly comprehended (Reynolds, 1997; Salmaso and Tolotti, 2021). Interpretative models that include the direct and indirect effects of climate variability on phytoplankton ecology are still scarce and are needed to disentangle climate impacts from responses triggered by other ecological drivers (Salmaso and Tolotti, 2021).

Deep Southern subalpine Lakes (DSLs: Maggiore, Lugano, Como, Iseo, and Garda) are located south of the Alps and constitute a fundamental water source for several uses, such as agriculture, industry, potabilization, fishing, recreation, and tourism (Rogora et al., 2018). These lakes are particularly sensitive to climate change, especially temperature increases (Fenocchi et al., 2018; Rogora et al., 2018; Valerio et al., 2015). In addition, DSLs are positioned in one of the most densely inhabited and industrialized areas of Europe and thus are highly influenced by local anthropic pollution loading from their watersheds. In the last decade, management programs have permitted a reduction in phosphorus concentrations in some of these lakes (Lepori and Roberts, 2017). However, a reduced tendency for the total mixing of the whole water column in DSLs leads to hypoxic conditions in the bottom layers, resulting in a release of phosphorus from sediments (Leoni et al., 2019). Directly linked to the change in the thermal behavior of lakes toward more meromictic conditions, silica segregation in the deepest layers may also occur, as was already observed in Lake Iseo (Leoni et al., 2019; Scibona et al., 2022). Prolonged phases of stratification due to global warming should seriously impact the planktonic communities if extreme climate events favoring water circulation occur. Indeed, even if nutrient segregation at the bottom could have apparent beneficial reoligotrophication effects on surface waters, future complete mixing with subsequent nutrient replenishment of the epilimnion may have detrimental effects on the entire lake ecosystem, as has already occurred in Lake Lugano (Holzner et al., 2009). DSLs thus represent optimal systems to study the interaction among different drivers (e.g. warming and nutrients) on phytoplankton communities. However, there is a lack of knowledge concerning the potential consequence of long-term warming coupled with the observed trends in nutrients for the ecological functions of these lakes.

Lake Iseo is one of the Italian DSLs and seems particularly sensitive to temperature increases. For example, the climate change scenario predicts an overall average increase in the lake water temperature of 0.012 $^{\circ}$C yr^{-1} and a reinforced Schmidt's thermal stability of the water column in the winter for the period 2012–2050 (Valerio et al., 2015). Indeed, since the last complete turnovers in 2005 and 2006, the lake has no longer mixed, resulting in the longest recorded phase of anoxia in deep water experienced by the lake (Rogora et al., 2018). The increasing phosphorus concentrations over the past 40 years brought this naturally

oligotrophic phosphorus-limited lake into meso-eutrophic conditions (Leoni et al., 2014a, 2014b; Salmaso et al., 2014). Similar to others DSLs, Lake Iseo has long been studied and monitored, resulting in a high quantity of physical, chemical and biological data availability and a relatively extensive knowledge of ecosystem functioning. These sets of knowledge and the long-term datasets (1998–2021) constitute an enormous benefit to performing interpretative models including the direct and indirect effects of climate variability.

This study aims to disentangle the pathways by which climate change and nutrient variations regulate the phytoplankton community in a deep mesotrophic lake, L. Iseo. We used powerful pathway analysis to understand the respective significance of these ecological drivers in shaping both the diversity and biovolume of phytoplankton. We expected direct and indirect effects of water warming on phytoplankton biovolume and, at the genus level, on richness, evenness and diversity, with long-term taxonomical and functional reorganization of the phytoplankton community. Specifically, we hypothesized that (a) warming would increase the phytoplankton biovolume and favor the dominance of taxa with higher temperature optima. Furthermore, we hypothesized that (b) warmer temperatures would induce more intense thermal stratification that should in turn affect nutrient dynamics, resulting in changes in phytoplankton community structure.

2. Materials and methods

2.1. Study site

Lake Iseo, the fourth largest subalpine Italian lake, has a surface area of 61.8 km^2 and a maximum depth of 258 m (Leoni et al., 2019). The lake is located in northern Italy, at the end of the pre-alpine valley Val Camonica. Due to its water temperature that never drops below 4 °C, Lake Iseo should be classified as warm monomictic. However, the classification of the lake as holo-oligomictic should be more correct, as complete circulation only occurs irregularly during extremely windy and cold winters (Garibaldi et al., 2003). Standardized phytoplankton studies in the euphotic layer (0–20 m depth) of Lake Iseo have existed since the 1990s. The main algal groups dominating the phytoplankton community of the lake are Bacillariophyceae, Conjugatophyceae, and Cyanobacteria, with seasonal variations in community composition following the changes in meteoclimatic and nutrient conditions (Garibaldi et al., 2003; Leoni et al., 2014a, 2014b). Chemical and physical data in Lake Iseo have also been documented since the 1990s, leading to a high quantity of data available: A long-term series of samples has been collected monthly at different depths (0, 10, 20, 30, 50, 75, 100, 150, 200 and 250 m) (Leoni et al., 2019).

2.2. Sampling and measurement of abiotic parameters and phytoplankton

Initially, our analysis included 19 physico-chemical variables of the long-term series: temperature, transparency, conductivity, alkalinity, total phosphorus (TP), soluble reactive phosphorus (SRP), total nitrogen (TN), nitrate (NO_3), ammonia (NH_4), dissolved silica (DSi), calcium (Ca), magnesium (Mg), sodium (Na), potassium (K), sulfate (SO), and chloride (Cl), and ratios of TN:TP, DSi:TN and DSi:TP. To identify the most significant drivers influencing phytoplankton dynamics and to avoid collinearity among environmental variables, we performed the statistical analyses described in Section 2.3.1 (Fig. A1). The selection procedure allowed us to identify six physicochemical drivers, namely, temperature, transparency, DSi, SRP, TN, and DSi:TP, for which the detailed methodology is described below. For the other parameters, the analytical procedure is described in Table A1.

Water temperature was measured using an underwater multiparametric probe (WTW Multi 3410) and water for nutrient analysis was collected at each sampling depth using a 1.7-L Niskin bottle. Transparency values were assessed by Secchi measurements. At the laboratory, DSi, TP, and SRP concentrations were analyzed using the

molybdenum reaction and spectrophotometry, and TN by spectrophotometry at 272/220 nm.

To evaluate the effect of the length and strength of stratification on phytoplankton dynamics, we calculated the annual maximum mixing depths and Schmidt's thermal stability (Idso, 1973). For each month, the maximum mixing depth was determined by analyzing discrete physical and chemical data across the water column (Salmaso et al., 2014). The resistance to mixing due to the potential energy inherent in the stratification of the water column was assessed by Schmidt's stability calculation (package *rLakeAnalyzer*) based on temperature values and bathymetry data from 1998 to 2021.

As a proxy of runoff, precipitation can play a role in nutrient concentrations and thus indirectly in phytoplankton dynamics. Precipitation data measured at Costa Volpino (North of Lake Iseo) were collected online (https://www.arpalombardia.it), and we calculated the cumulated monthly precipitation (mm/month) for the whole studied period.

Phytoplankton biovolume and community composition were analyzed from integrated water samples using a Zullig bottle for integrated samples (0–20 m, sampled volume: 40 cm^3·m^{-1}) collected monthly from 1998 to 2021 in the upper layers (between 0 and 20 m). Data were almost continuous, except for 2013 and 2020 which are missing from the dataset. Subsamples of water (~200 mL) were fixed with acetic Lugol's solution immediately after collection and sent to the laboratory for phytoplankton counting and identification. Phytoplankton cells were counted using an IM35 inverted microscope following the Utermöhl (1958) method. Phytoplankton biovolume was estimated by shape assimilation to known geometric forms and direct measurement of the main cell dimensions. Phytoplankton taxa were identified at the genus levels using appropriate taxonomic guides (mainly from the Süßwasserflora von Mitteleuropa and specific manuals of Das Phytoplankton des Süßwassers, see Leoni et al., 2007). Each phytoplankton organism was individually described using (i) the commonly used phytoplankton groups, (ii) the taxonomic affiliation at the genus level, and (iii) the morpho-functional groups (MFG) (Salmaso and Padisák, 2007) (Table A2). The Shannon-Wiener index, Pielou evenness, and richness (number of genera) were calculated at the genus level, as some phytoplankton species were only seldom observed and morphological criteria for species designation were not always clear.

2.3. Statistical analyses

2.3.1. Selection of environmental drivers

Multicollinearity among the 19 physicochemical variables was identified using variance inflation factors (VIF), and the redundant environmental variables with VIF > 10 were removed before analysis (Zuur et al., 2010). Then, we used generalized boosted regression models with the *gbm* package in R (Ridgeway et al., 2013) to identify the most important environmental variables explaining phytoplankton biovolume, and only variables with a relative influence >5 % were kept in the analysis (Fig. A1). For these most influential drivers, we calculated the volume-weighted value between 0 m and 20 m (hereafter termed the 'upper water column').

We used Pearson correlations (r) to check the role of precipitation in lake nutrient concentrations. Any weak or negative correlations would result in precipitation being excluded as an explanatory variable of changes in lake nutrient concentrations during the study period.

Pearson correlations were also performed among water temperature, Schmidt's stability and mixing depth to reduce the redundancy due to a high correlation between features to select variables for statistical analysis. When the correlation is high, the redundancy is considered high, and such features will be removed.

2.3.2. Long-term trends in environmental drivers

To assess the temporal (years) and spatial (depth) dynamics of environmental predictors, as well as their interaction, linear regressions were performed on the following selected volume-weighted variables:

temperature, DSi, SRP, TN, and DSi:TP (Fig. A1). Environmental variables were log-transformed if this resulted in a more uniform spread of data points. For temperature, which controls many processes, including stratification, we also used the nonparametric Mann–Kendall test (Helsel and Hirsch, 1992) to test the statistical significance of the trend in the annual series of average temperature for the whole water column (0–250 m) from 1993 to 2021. Thus, we used the *Kendall* package in R (McLeod, 2011) to calculate the statistical test τ (tau) and the *p* value. The τ statistic ranges from -1 to $+1$, with positive values indicating an increasing trend and negative values indicating a decreasing trend. The magnitude of the trend (change per unit time) was estimated by the nonparametric method of Sen's slope (Sen, 1968) using the *trend* package (Pohlert et al., 2016).

2.3.3. Phytoplankton community dynamics and relationship with environmental variables

To analyze the temporal dynamics of phytoplankton, we evaluated how total biovolume and taxonomical diversity (Shannon index) varied annually and seasonally (Fig. A1). To take into account the probable autocorrelation among seasons, we used generalized least squares (GLS) models with the *correlation* argument to incorporate temporal autocorrelation into the model. Similarity percentage (SIMPER) analyses (Clarke and Warwick, 1994) were performed to estimate the percentage contribution of phytoplankton taxonomic groups and functional groups and their temporal variations.

A structural equation model (SEM) was performed to assess direct and indirect temperature and nutrient effects on phytoplankton biovolume, evenness, and richness. For the environmental descriptors, we used the volume-weighted values calculated between 0 and 20 m of the selected physicochemical drivers (temperature, TN, DSi:TP; DSi, SRP), transparency, and mixing depth. We developed a preliminary model based on the postulate that temperature directly influences stratification dynamics (represented by mixing depth) and phytoplankton biovolume, evenness, and richness (Fig. A2). Second, we assumed that stratification directly influences the distribution of nutrients in the epilimnion, which in turn affects phytoplankton. We also tested a possible relationship between changes in underwater light conditions (represented by transparency) and phytoplankton, including the direct effect of mixing depth on transparency. The total phytoplankton biovolume was log-transformed because of the nonnormal distribution of the data.

Then, we used a stepwise backward selection to eliminate the hypothesized links that were not significant. Standardized path coefficients (β) indicate the strength of the causal effect of one variable on another (Kline, 2015). The path coefficients vary between -1 and $+1$, and a β value of ± 0.1 represents a weak effect. Furthermore, transparency was eliminated from the final model because it did not act as an explanatory variable of phytoplankton. Indeed, transparency resulted as a dependent variable, reducing the robustness of the statistical coefficients of the model (Fig. A2). SEM analysis was performed using R (version 4.1.3) with the *Lavaan* package (Rosseel et al., 2017). Model fit was assessed using the comparative fit index (cfi), the standardized root Mean square residual (srmr), and the root mean square error of approximation (rmsea) indices. Cfi values > 0.90 and srmr and rmsea values < 0.08 generally indicate a good model fit.

Variation in phytoplankton community composition in relation to the environmental variables was analyzed by nonmetric multidimensional scaling (NMDS) (Fig. A1). The NMDS analysis was based on Bray & Curtis dissimilarity matrices (Legendre and Legendre, 1998) calculated from the biovolumes of functional groups (MFG) and taxa at the class level. We used the metaMDS procedure in the R package *vegan*, which uses adequate dissimilarity measures (function vegdist), runs NMDS repeatedly with random starting configurations, compares results (function procrustes), and stops after finding a similar minimal stress solution twice (Oksanen et al., 2013). The final configuration was rotated so that the variance of points was maximized on the first dimension (Oksanen et al., 2013). Environmental variables were fitted

to the configuration by vector fitting (envfit). Permutational multivariate analysis of variance was performed to partition the Bray-Curtis distance matrix by the environmental variables using the adonis function in the R package *vegan*.

3. Results

3.1. Temporal dynamics of phytoplankton

The SIMPER analysis showed that the most representative classes were Bacillariophyceae (37 % of the total biovolume, mainly represented by *Fragilaria* spp.), Conjugatophyceae (30 % of the total biovolume, mainly represented by *Mougeotia* sp.), and Cyanobacteria (14 % of the total biovolume, mainly represented by *Planktothrix* spp.) (Figs. 1 and A3). Bacillariophyceae was the most dominant group in winter (November to March), spring (April, May), and autumn (September, October), while Conjugatophyceae dominated during the summer months (June to August). Dinoflagellates were also well represented in the community until 2010, and then almost disappeared from the phytoplankton assemblage (Fig. 1). The peak of dinoflagellates mainly occurred in early summer and/or early autumn, approximately the same time as that of Cyanobacteria (Fig. A3). Cyanobacteria increased in proportion in the community after 2010 and represented >30 % of the community in 2021 (Fig. 1). Functionally, the main morpho-functional groups (MFG) were filamentous Conjugatophytes (10b, 24 % of the total biovolume), large pennate diatoms (6b, 18 % of the total biovolume), large centric diatoms (6a, 13 % of the total biovolume) and thin filamentous Cyanobacteria (5b, 11 % of the total biovolume).

Phytoplankton biovolume varied among seasons (F = 14.8; p-value < 0.001), with maximal values in autumn and spring. The mean annual phytoplankton biovolume over the studied period was 2609 ± 905 mm^3 m^{-3}, and no significant trend appeared from 1998 to 2021 (Fig. A3). However, the total phytoplankton biovolume increased over the years in summer (p-value = 0.003) and winter (p-value = 0.002). Phytoplankton diversity (Shannon index) showed a significant decreasing trend from 1998 to 2021 (F = 5.8; p-value = 0.01, Fig. A4). Phytoplankton diversity varied among seasons (F = 4.8; p-value < 0.001), with maximal values in summer.

3.2. Long-term trends in environmental drivers

The volume-weighted values of temperature (Fig. 2) for the whole studied period varied significantly with depth (F = 504.5, p-value < 0.001), with higher temperature in the upper water column (12.3 ± 3.9 °C for the 0–20 m layer) than in the deep layers (6.7 ± 0.3 °C from 50 m to the bottom), affecting the lake stability (Fig. 3). The temperature increased significantly over the years (F = 16.9, p-value < 0.001). Indeed, the rate of temperature increase in the water column in the period between 1993 and 2021 ranged between 0.01 and 0.04 °C y^{-1}, with an average of 0.024 °C y^{-1} ($\tau = 0.36$, p-value = 0.007). After the complete mixing events of 2005 and 2006 (i.e., during the period between 2007 and 2021), the rate of temperature increase in the water column was more elevated and reached an average of 0.06 °C y^{-1} ($\tau = 0.64$, p-value = 0.001). Focusing on the last decade (from 2011 to 2021), the rate of temperature increase was higher and reached 0.08 °C y^{-1} ($\tau = 0.63$, p-value = 0.008).

DSi increased significantly with depth (F = 1944.6, p-value < 0.001) and increased significantly over the years (F = 97.4, p-value < 0.001). The increase in DSi over the years varied between depths (F = 8.5, p-value < 0.001), and DSi increased more over the years in the bottom layers. The average DSi concentrations for the whole water column were relatively stable between 1993 and 2004, and then increased progressively after the complete mixing events (2005 and 2006) (Fig. 2).

TN significantly changed over depth (F = 237.7, p-value < 0.001), but not over the years (F = 3.0, p-value = 0.08). However, the effect of the years on TN concentration varied between depths (F = 48.9, p-value
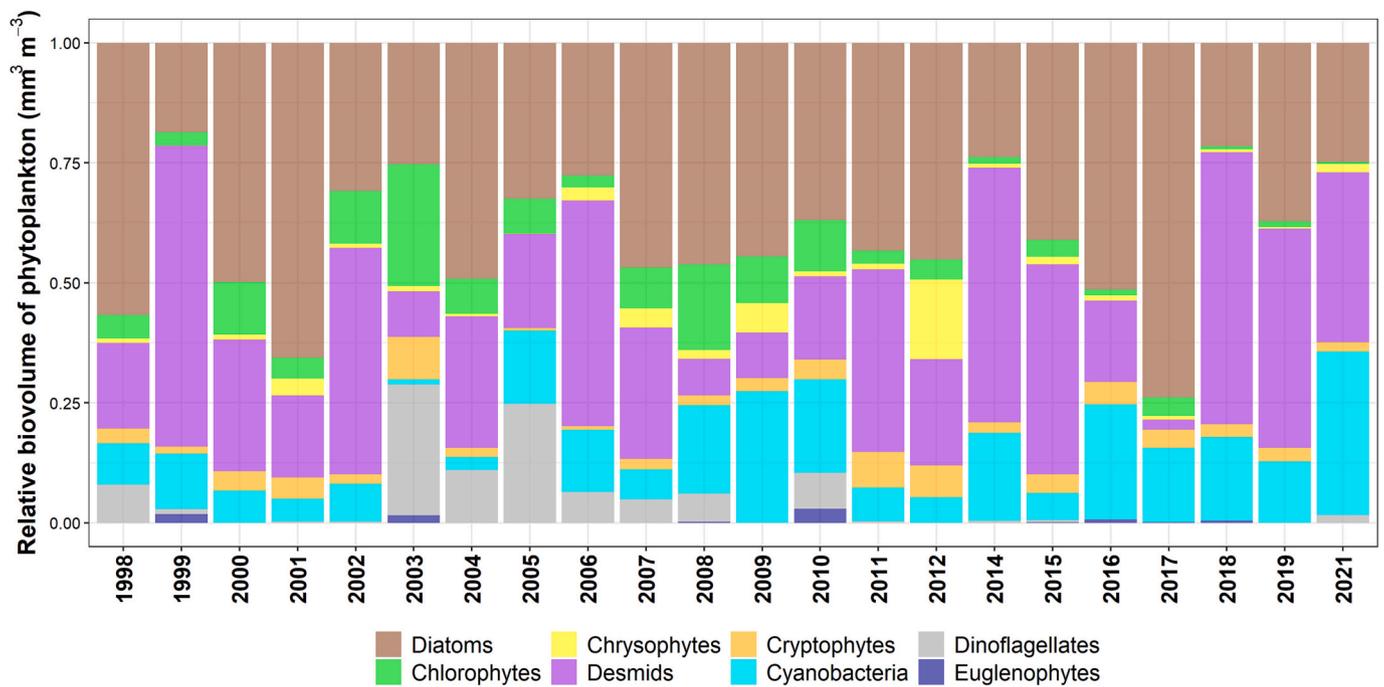
Fig. 1. Annual dynamics of the main taxonomical groups of phytoplankton in Lake Iseo from 1998 to 2021 (2013 and 2020 are missing).

< 0.001) and TN concentrations increased over the years in the upper layers. TN concentrations were higher in the upper layers, except during the complete mixing event in 2005, where TN concentrations reached higher values at the bottom (Fig. 2). Higher TN concentrations in the upper layers than in the bottom were reached again after 2010. TN concentrations in the upper layers were relatively stable between 1993 and 2004 (870.4 ± 62 µg L^{-1}), and then increased in 2007 after the complete mixing events. TN concentrations in the upper layers remained high from 2007 to 2021 (955 ± 111 µg L^{-1}). After the complete mixing events, maximum annual TN concentrations in the upper layers were reached in 2011 (1032 µg L^{-1}).

SRP concentrations increased significantly with depth (F = 4166.5, p-value < 0.001) and over the years (F = 849.8, p-value < 0.001). The increase in SRP over the years varied among depths (F = 100.6, p-value < 0.001): At the bottom, SRP concentrations were relatively stable between 1993 and 2004, and then they were drastically higher after the complete mixing events, i.e., from 2007 to 2021 (Fig. 2). In the bottom layers, higher annual values exceeded 100 µg L^{-1}. In the upper layers, maximum SRP concentrations were reached in 2005 during the complete mixing events, (66.5 µg L^{-1} in March) with an annual average of 17 µg L^{-1}.

Finally, the DSi:TP ratio decreased significantly with depth (F = 388.7, *p*-value < 0.001) and over the years (F = 22.9, p-value < 0.001). Moreover, the DSi:TP ratio increased over the years in the upper layers (F = 2.6, p-value = 0.008).

During the 22 years, complete water mixing occurred only twice, in 2005 and 2006 (Fig. 3). Extended vertical mixing (i.e., to depths of 150 m or more) no longer occurred after 2006. In particular, during the period from 2007 to 2021, several years were characterized by mixing depths reaching <20 % of the water column, corresponding to 36 % of the lake volume (e.g. 2007, 2008, 2015, 2016, and 2019). Schmidt's stability values during winter–spring turnovers showed strong interannual variability, generally reaching higher values when the mixing depth was low (Figs. 3 and A5). However, deep mixing is not always observed at low stability values, since other factors, such as wind, must trigger convective movements.

3.3. Effect of climate and nutrients on phytoplankton

Cumulative monthly precipitation was negatively and/or weakly correlated with TP (r = −0.14, p-value = 0.034) and TN (r = 0.09, p-value = 0.18) concentrations in the upper water column, resulting in the exclusion of precipitation from the explanatory variables of nutrient changes in the epilimnion. Since the water temperature in the upper water column and Schmidt's stability had a very strong correlation (r = 0.98, p-value < 0.001), the mixing depth was chosen as the stratification variable in the analyses.

We used a SEM to disentangle the complex network of direct and indirect effects of temperature and nutrients on phytoplankton biovolume, richness, and evenness. The sample size used for the final model was n = 212, with df = 21. The value of cfi was 1.000 indicating a very good fit of the model (threshold > 0.90). The other statistical parameters of the model were also very good, with srmr value of 0.029 (threshold < 0.08) and rmsea value of 0.000 (threshold < 0.08). The final model resulting from the backward stepwise selection showed that phytoplankton richness was not influenced by the environmental drivers (Fig. 4). The path coefficients of the model showed that phytoplankton were more influenced by temperature than by nutrients. The water temperature in the upper water column was strongly negatively correlated with the mixing depth (β = −0.70), which means stronger stratification at higher temperatures. The mixing depth was positively correlated with the SRP (β = 0.78) and DSi (β = 0.23) concentrations in the upper layers and negatively correlated with the DSi:TP ratio (β = −0.46). Among nutrients, only the DSi:TP ratio influenced phytoplankton and was negatively correlated with phytoplankton biovolume (β = −0.30). The DSi:TP ratio was positively correlated with phytoplankton evenness, but the effect was weak (β = 0.18). Water temperature in the upper water column was positively correlated with phytoplankton biovolume (β = 0.37) but negatively correlated with phytoplankton evenness (β = −0.33). TN concentrations in the upper layers were not regulated by mixing depth and TN concentrations did not influence phytoplankton biovolume and evenness.

NMDS illustrated the changes in phytoplankton community composition during the two sampling periods, together with the ordination of environmental parameters (Fig. 5). A progressive transition in
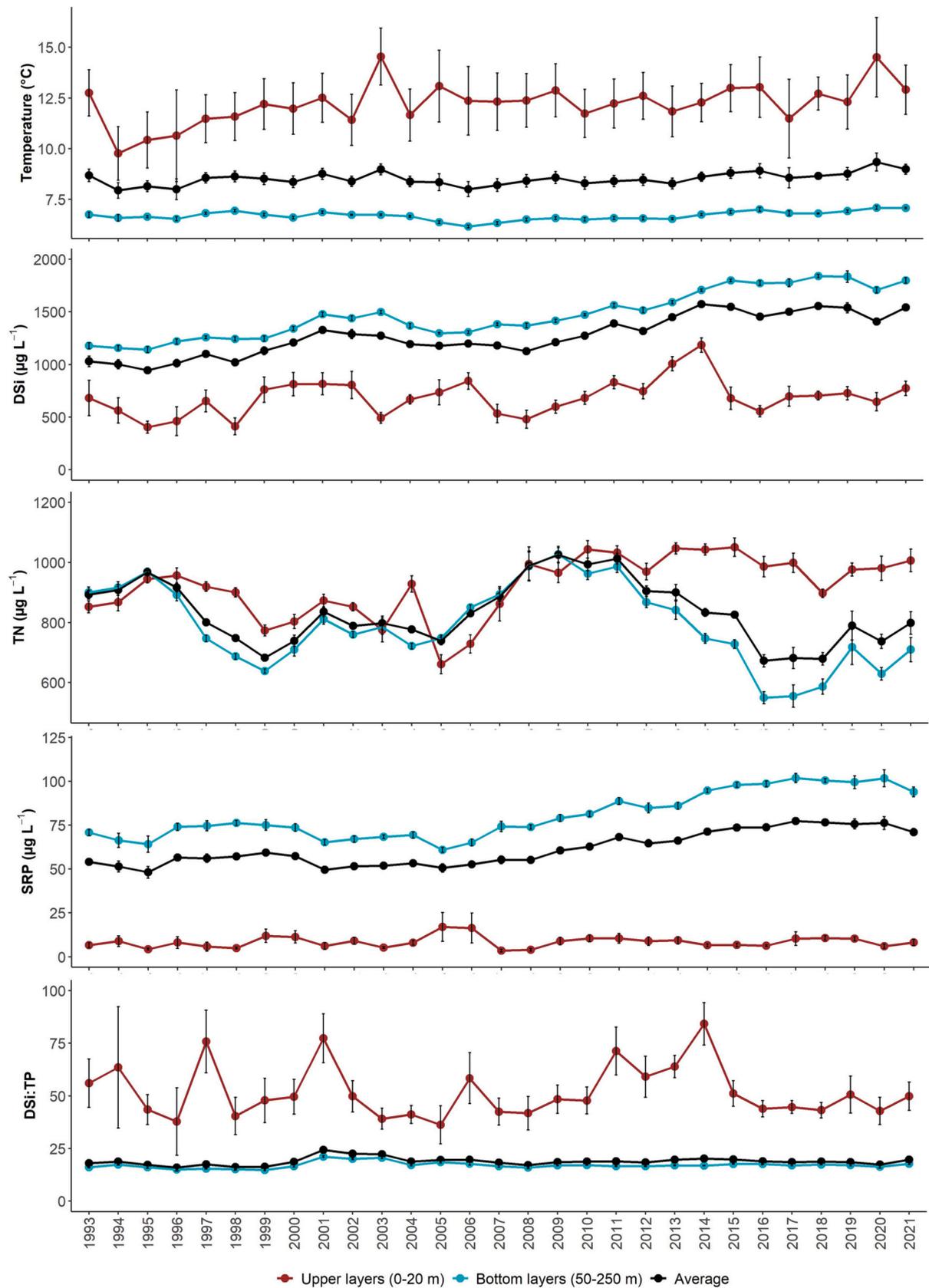
Fig. 2. Temporal variations of volume-weighted environmental variables in Lake Iseo between 1993 and 2021. SRP: soluble reactive phosphorus; DSi:TP: ratio of dissolved silica to total phosphorus. TN: total nitrogen. Black line represents the annual average for the whole water column, red line represents the annual average for the upper layers (0–20 m) and blue line represents the annual average for the bottom layers (50–250 m). Errors bars represent standard-errors.
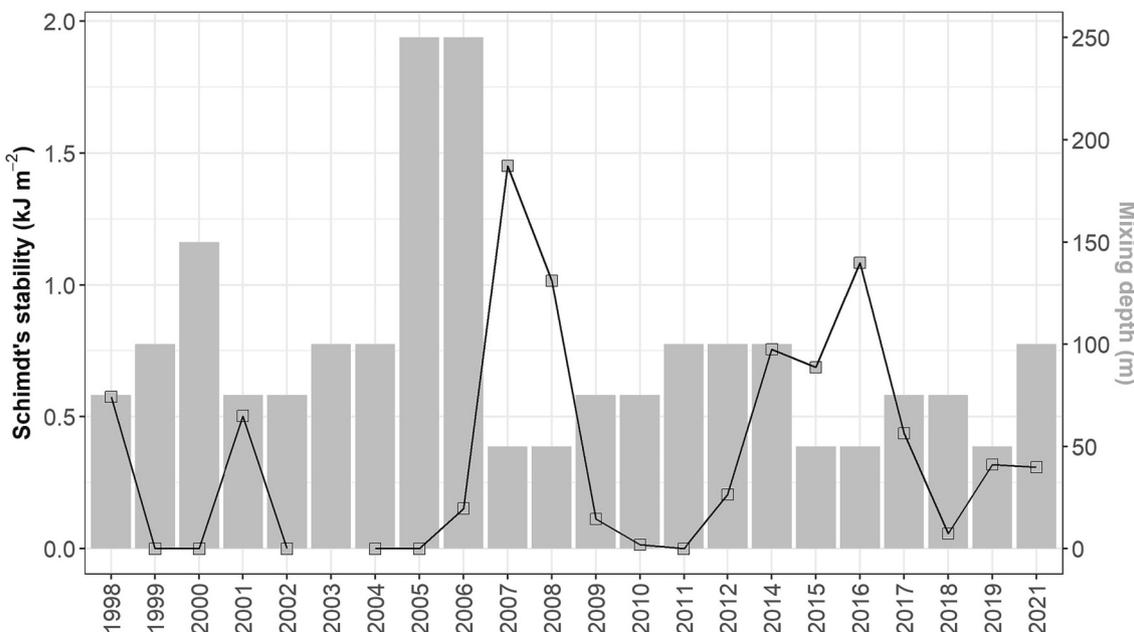
Fig. 3. Maximum mixing depth and Schmidt's stability at winter-spring turnover in the period 1998–2021 in Lake Iseo.
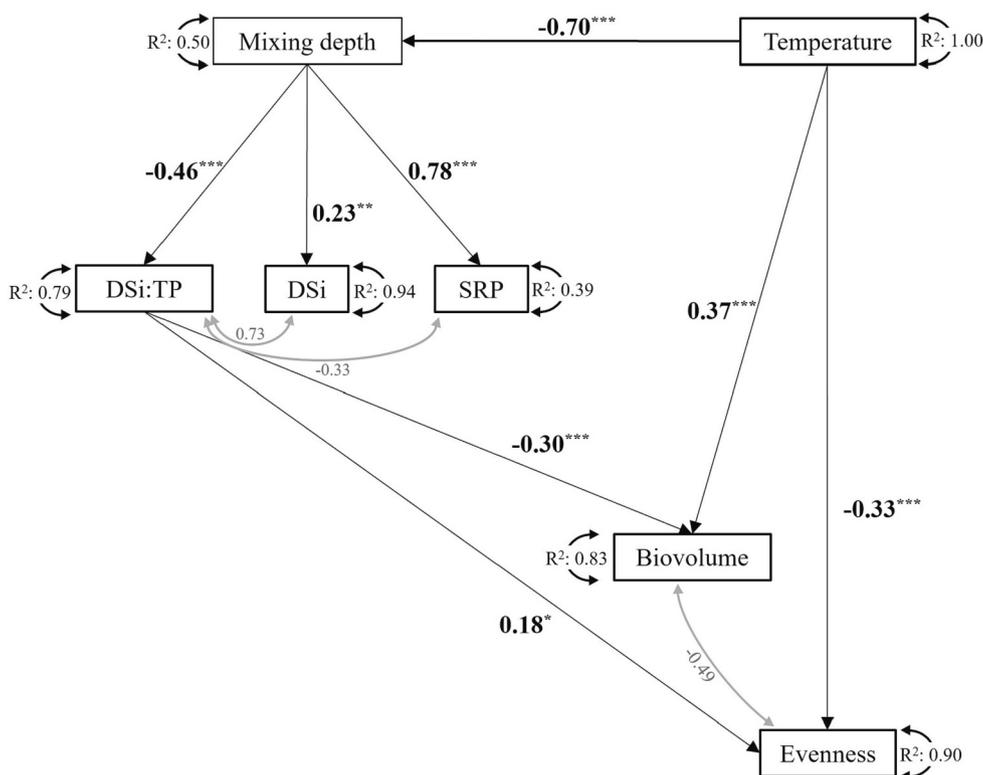

Fig. 4. Final structural equation model explaining direct and indirect effects of temperature and nutrient concentrations on log-transformed phytoplankton bio-volume and phytoplankton evenness. The stratification effect is represented by mixing depth. The numbers on arrows correspond to the standardized path co-efficients (β) and R^2 are the R-squared values. Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.'. Statistical parameters of the model were the following: n = 212; df = 21; cfi = 1.000; srmr = 0.029; rmsea = 0.000. SRP: soluble reactive phosphorus; DSi: dissolved silica; TP: total phosphorus.

community composition occurred over the years, mainly related to a change in TN concentrations in the upper layers. Dinophyceae were more present during the period 1998–2010 and then almost disappeared from the community. Water temperature in the upper layers ($r^2 = 0.36$; p-value < 0.001), TN ($r^2 = 0.12$; p-value = 0.001), and DSi:TP ($r^2 = 0.07$; p-value = 0.02) appeared to be key explanatory variables

structuring the phytoplankton community. Among the diatoms, Cosci-nodiscophyceae were associated with a low DSi:TP ratio, whereas Bacillariophyceae and Mediophyceae were correlated with a higher DSi:TP ratio. Coccolithophyceae were also associated with a high DSi:TP ratio. Cyanophyceae seemed not to be structured by any environmental variables; however, ordination on MFG groups showed that higher
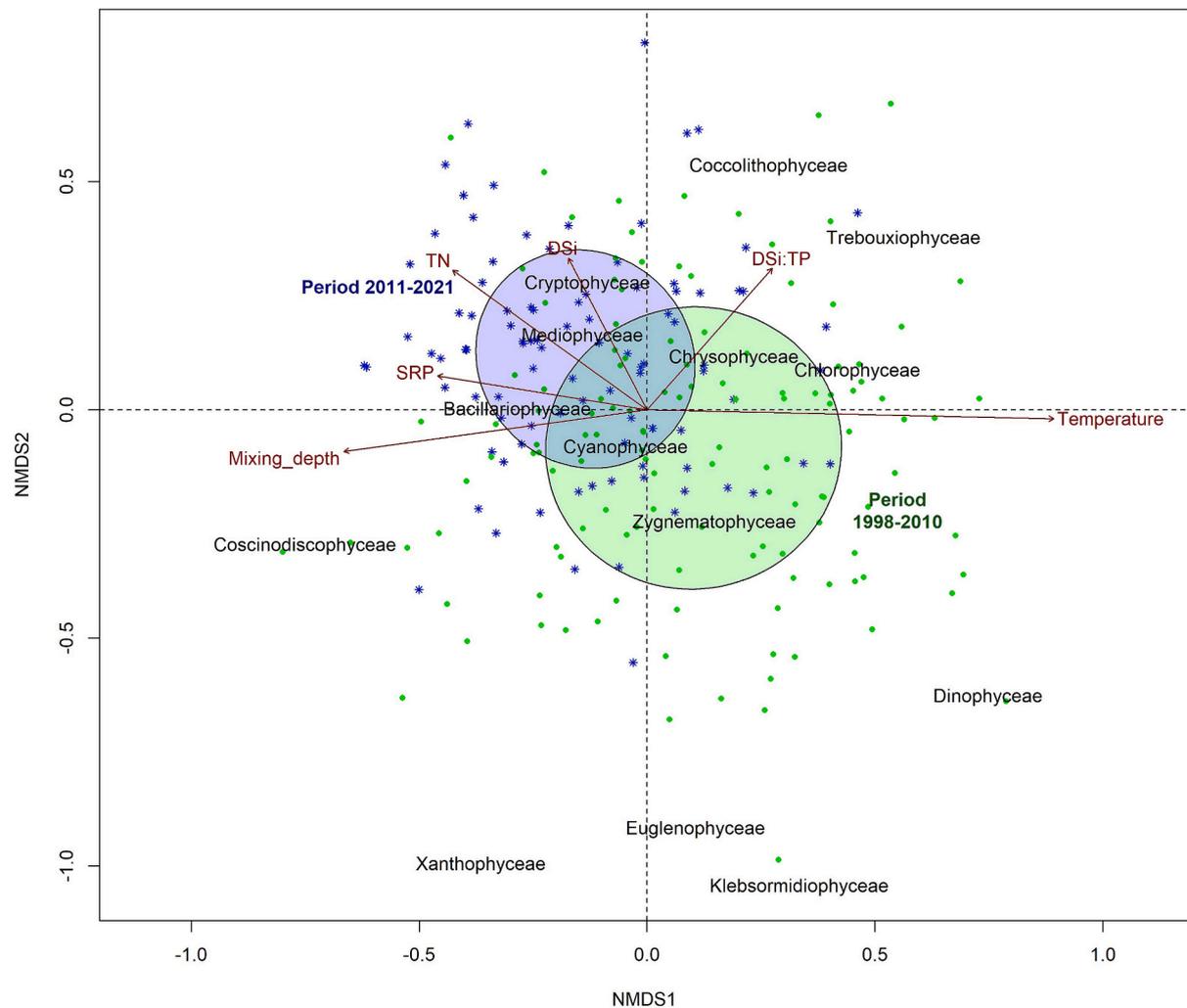
Fig. 5. NMDS ordination of samples based on biovolume of taxonomic groups in Lake Iseo for 22 years (2013 and 2020 are missing). Ellipses separate the phytoplankton community in two distinct periods [1998–2010, green ellipse] and [2011–2021, blue ellipse]. The stress value was of 0.194. SRP: soluble reactive phosphorus; TN: total nitrogen; DSi: dissolved silica; TP: total phosphorus. Stratification dynamics is represented by mixing depth.

temperature drove the biovolume of large vacuolated Chroococcales (5b), filamentous Chlorophytes (10a), large Dinophytes (1b), small colonial Chroococcales (5d) and Nostocales (5e) to a lesser extent (Fig. A6). Finally, large centric diatoms (6a) were associated with a high mixing depth.

4. Discussion

4.1. Trends in temperature and nutrients in Lake Iseo

Deep lakes are particularly exposed to the double threat posed by climate change through increased temperature and its effect on nutrient cycling (Jenny et al., 2020). However, the complex implication of these two factors on lake water quality and ecosystem functioning remains largely unresolved. The combination of these factors is expected to result in unforeseen and nonlinear ecological responses, potentially leading to more severe impacts on aquatic ecosystems than currently observed (Woolway et al., 2022).

Global studies have shown a coherent increase in lake temperature trends (e.g., Schneider and Hook, 2010; Woolway et al., 2020). For instance, O'Reilly et al. (2015) synthesizing in situ and satellite-derived lake data, showed a mean global increase of 0.34 °C decade^{-1}. However, the spatial heterogeneity of these temperature dynamics suggests that lake characteristics strongly influence the effects of climate change on

individual lakes (O'Reilly et al., 2015; Pareeth et al., 2017). Therefore, studying single lakes in detail becomes crucial to understanding the specific factors that mediate their responses to changing environmental conditions. This approach allows for a more comprehensive grasp of the complexities of lake ecosystems and aids in developing targeted conservation and management strategies. Our results showed that the average rate of temperature increase in the water column of Lake Iseo between 1993 and 2021 was 0.24 °C decade^{-1}. This is very close to the values reported globally for other waterbodies (O'Reilly et al., 2015). Moreover, this trend is concordant with the results of Salmaso and Mosello (2010), who observed a range of temperature increases between 0.01 and 0.03 °C y^{-1} between the 1970s and 2005/2009 in this lake. According to Rogora et al. (2018), a synchronous pattern of increasing water temperature has been evident since 2006 in several DSLs, after the sudden cooling of the water column caused by the last complete mixing events of 2005 and 2006 which affected these lakes. However, in Lake Iseo, the rate of water temperature increase accelerated considerably in the most recent years. For example, the rate of water temperature increase was 0.06 °C y^{-1} over the period 2007–2021 after the last complete mixing events induced by cooling of atmospheric temperature during harsh and windy winters, and was particularly strong during the last decade, reaching an average of 0.08 °C y^{-1} between 2011 and 2021. Large-scale climate change scenarios for temperate lakes have projected a rate of temperature increase of approximately 0.10–0.11 °C decade^{-1}

(Shatwell et al., 2019). For Lake Iseo, climate change scenarios (2012–2050) have predicted an overall average increase in lake-water temperature of 0.012 °C y^{-1}, and reinforced Schmidt's thermal stability of the water column in winter (Valerio et al., 2015). Our new findings demonstrated that future warming scenarios should be greater than previously anticipated given the increased rise in water temperature recorded over the past ten years in Lake Iseo.

This temperature rise is expected to have a significant influence on the thermal dynamics of the lake and the flow of nutrients, directly affecting phytoplankton growth (Jane et al., 2021). Earlier investigations in DSLs have demonstrated a strong temporal coherence among variables related to temperature, particularly winter air temperatures and spring surface water temperatures, along with spring epilimnetic phosphorus levels (Salmaso et al., 2014). Notably, the extent of vertical mixing plays a crucial role in redistributing nutrients throughout the water column, with this effect being more important in meso-eutrophic lakes, such as Lake Iseo, which is characterized by more pronounced vertical chemical gradients (Leoni et al., 2018; Salmaso et al., 2014). Our findings indicate that the phosphorus concentration is increasing in the bottom layers of Lake Iseo, exceeding 100 μg P L^{-1} in 2020. This phosphorus internal loading could have serious consequences for planktonic organisms, particularly in scenarios where complete mixing occurs in the future that will make the nutrients directly available in the epilimnion to phytoplankton (Woolway and Merchant, 2019). It has been shown that phosphorus concentrations >30–50 μg L^{-1} are directly related to the dominance of Cyanobacteria and diatoms in the phytoplankton community (Salmaso and Tolotti, 2021). In our study, SRP in the upper water column reached >60 μg L^{-1} during the last complete mixing events. Thus, during complete turnover, phosphorus concentrations in Lake Iseo should come closer to those found in more eutrophic lakes, which could impact phytoplankton species at the community level.

Concerning nitrogen, our results showed that TN concentrations increased in the upper layers of Lake Iseo after the last complete mixing and remained elevated. The increase in TN concentrations in the upper layers was mainly caused by nitrate, whereas ammonium increased more in the bottom layers (data not shown), which seems typically related to the meromictic condition of the lake (Scibona et al., 2022). Other DSLs have also recently experienced an increase in nitrogen concentrations, mainly attributed to atmospheric depositions of NO$_3$-N and NH$_4$-N, as intensive agriculture is practically nonexistent in the catchments of these lakes (Salmaso et al., 2007). Indeed, even if atmospheric nitrogen has decreased in Europe in recent years (Ackerman et al., 2019), high loads of oxidized nitrogen (NO$_2$, nitric acid, and nitrate-containing particles) were detected in 2020 in northern Italy. This load is a consequence of the deposition of both oxidized and reduced compounds emitted by combustion processes (Fagerli et al., 2022).

Interestingly, it seems that the physico-chemical modifications provoked by the last complete mixing events have affected the lake for consecutive years, at least until 2011. These results can be deduced from TN concentrations in the upper layers, which reached their peak in 2011. A similar pattern was observed for oxygen concentrations, which reached their minimum concentrations at the bottom in 2010–2011 (see Rogora et al., 2018). This pattern in oxygen and nitrogen concentrations highlights the "memory effect" of the complete turnover already pointed out in DSLs, as physico-chemical parameters remain affected by mixing during the consecutive years following complete mixing (Rogora et al., 2018).

In contrast to nitrogen, silica has a predominantly sedimentary cycle with a particulate fraction that tends to settle (Scibona et al., 2022). Indeed, dissolved silica is continuously assimilated by phytoplankton (mainly diatoms), and then settles with organisms' death, accumulating in the bottom layers and sediments (Hobbs et al., 2010). Therefore, the lake's evolution toward meromixis, by enhancing silica segregation in the bottom layers, has strong impacts on silica redistribution and

availability in different lake layers. Silica concentrations in the bottom of Lake Iseo followed the trend already observed by Leoni et al. (2019): Since the last complete mixing events in 2005 and 2006, silica concentrations in the hypolimnion have progressively increased. This enhanced silica segregation in the deeper water layers may cause nutrient disequilibrium in the epilimnion. Indeed, the fraction of silica and phosphorus retained within the lake (79 % and 75 %, respectively) is higher than that of TN (45 %), which likely unbalances the ratio among these three nutrients in the photic zone (Scibona et al., 2022). This disequilibrium in nitrogen, phosphorus, and silica ratios may produce unfavorable conditions for siliceous phytoplankton species.

Overall, our results underlined a degradation of the physicochemical status of Lake Iseo over the period 1993–2021, despite the reduction of phosphorus external loadings permitted by recent management programs. The main cause of lake degradation is the critical rate of temperature increase, which has recently accelerated during the last decade. The warming, directly related to the stratification strengthening, has had serious impacts on nutrient cycling, reflected by the overall increase and segregation of nitrogen, phosphorus, and silica concentrations in the bottom layers during the study period. These findings have important implications, especially in scenarios where complete mixing is expected to occur in the future.

4.2. Temperature and nutrient effects on phytoplankton community

Temperature increases and nutrient concentrations are the key drivers of changes in phytoplankton community structure and composition (Salmaso and Tolotti, 2021). Our results showed that phytoplankton biovolume and evenness were more influenced by the direct effect of temperature than by nutrient availability. It has been shown that the relative importance of temperature and nutrients on phytoplankton biovolume depends on the trophic status of lakes, and nutrients may play a larger role in oligotrophic lakes, while temperature should be more important in mesotrophic lakes (Rigosi et al., 2014; Leoni et al., 2018). Indeed, in low-nutrient lakes, phytoplankton limitation by nutrients may prevent its response to increasing water temperature, while the removal of nutrient limitation that occurs in more nutrient-rich systems increases the phytoplankton sensitivity to warming. Such results have already been observed by Brookes and Carey (2011), which demonstrated that Cyanobacteria respond to increased temperature only at higher nutrient levels.

The link between phytoplankton diversity and climate warming is not totally understood; however, important central tendencies for the effects of species loss have emerged in many studies (Hillebrand et al., 2008). Our study showed that the effect of temperature increase was positively related to phytoplankton biovolume but negatively related to phytoplankton evenness, with no effect on genus richness, in accordance with Hillebrand (2011). His research demonstrated that higher temperature led to a higher dominance of single/few species and that the higher dominance of these most productive species enhanced total community production (Hillebrand et al., 2012). It is likely that the changing abiotic conditions of Lake Iseo, especially after 2010, where the magnitude of change seemed to be more pronounced, promoted the dominance of a few phytoplankton genera that are more tolerant to these new conditions and that grow well at high temperatures.

The phytoplankton community composition shifted between the periods 1998–2010 and 2011–2021. This temporal shift in phytoplankton composition was mainly driven by changes in nitrogen concentrations. Dinoflagellates were essentially found during the period 1998–2010, while they were almost absent from the community after 2011. It has been demonstrated that dinoflagellate species need high nitrogen concentrations for growth, but can be outcompeted by Cyanobacteria if temperature and nutrient conditions are optimal (Recknagel et al., 2022). In our study, the peaks of dinoflagellates and Cyanobacteria seemed to occur generally during the same period of the year, likely indicating that these two groups use common nutrient

resources. We thus suggest that the increase in water temperature combined with higher nitrogen concentrations, observed after 2010, gave Cyanobacteria a competitive advantage over dinoflagellates. Additionally, it has been shown that some species of Cyanobacteria and dinoflagellates exert reciprocal, density-dependent allelopathic activity and that exudates from Cyanobacteria may inhibit the photosynthesis of dinoflagellate species (Gross, 2003). In Lake Iseo, the dominant Cyanobacteria is represented by *Planktothrix* spp., for which allelopathy has long been documented (Lenard and Poniewozik, 2022). NMDS analysis of phytoplankton MFG groups showed that large vacuoled Chroococcales (group 5b) were highly correlated with higher temperatures. Other cyanobacterial groups, such as small Chroococcales and Nostocales (groups 5d and 5e, respectively) were also correlated with higher temperature, but to a lesser extent. Thus, Cyanobacteria were favored by increasing water temperature and therefore might become more important under future warming scenarios, as has been suggested many times (Brasil et al., 2016; Salmaso and Tolotti, 2021).

Finally, we observed an interesting effect of the DSi:TP ratio on both phytoplankton biovolume and community composition. The SEM showed that the DSi:TP ratio was regulated by the dynamics of stratification: The ratio decreased when the mixing depth increased. This result can be explained by the higher positive effect of mixing on SRP concentrations in the upper layers ($R^2 = 0.78$) compared to DSi concentrations ($R^2 = 0.23$), which in turn should be linked to the sedimentary cycle of silica (Scibona et al., 2022). A smaller DSi:TP ratio promoted several diatom taxa, namely, Coscinodiscophyceae, which were also functionally associated with a high mixing depth (group 6a, large centric). In our study, large centric diatoms were represented by the *Aulacoseira* and *Melosira* genera. Previous studies have already shown that large centric diatoms such as *Aulacoseira* sp. are controlled by the thermal and nutrient stratification of lakes, typically blooming during periods of enhanced mixing (Pedraza Garzon and Saros, 2022; Reynolds, 2006). The dense, heavily silicified cells are suspended by mixing, but as stratification develops, the cells sink rapidly to the lake bottom.

Our NMDS results showed that the other classes of diatoms were also regulated by changes in the DSi:TP ratio. As for large centrics, small pennates (group7b, *Achnanthes* spp.) were found at a lower DSi:TP ratio. Large pennate Bacillariophyceae (group 6b, *Fragilaria* spp.) and small centric Mediophyceae (group 7a, *Cyclotella* spp.) were observed at a higher DSi:TP ratio. It has been reported that the DSi:P ratio is a key variable regulating the growth of different groups of diatoms in freshwater (Tolotti et al., 2007). Our results confirm that Fragilariaceae grow well at higher DSi:TP ratios. However, small centric diatoms (especially the genus *Cyclotella*) are traditionally found at low DSi:TP ratios, needing relatively high P concentrations to grow (Yankova et al., 2017), which was not the case in our study. This can be explained by the high phosphorus concentrations in Lake Iseo, ensuring that neither phosphorus nor silica reach limiting concentrations for this group of algae. Interestingly, the group of Coccolithophyceae was highly related to a high DSi:TP ratio. In Lake Iseo, Coccolithophyceae were mainly represented by the genus *Chrysochromulina*, which belongs to the order Prymnesiales (Haptophyta). Although haptophytes are predominantely calcified, Prymnesiales are estimated to have diverged from coccolithophores approximately 280 Myr ago (Liu et al., 2010), resulting in the presence of diatom-like silicon transporters (SITs) in both silicifying and calcifying coccolithophores (Durak et al., 2016). The silica requirements of these taxa for calcification may have a major influence on their competitive interactions with diatoms and other siliceous phytoplankton.

In Lake Iseo, a large proportion of the phytoplankton community is composed of diatoms. Thus, we can assume that the indirect effect of climate on phytoplankton biovolume and diversity, via the change in the DSi:TP ratio, is linked to a change in diatom dominance. Given the observed tendency of increasing stratification and increasing DSi:TP ratio in Lake Iseo, the contribution of large pennate Fragilariaceae and

small centric Mediophyceae to the phytoplankton community should increase in the future.

5. Conclusions

Our study disentangled the direct and indirect effects of climate change on phytoplankton ecology in a deep mesotrophic subalpine lake, L. Iseo. The collection of an extensive, multifaceted long-term dataset, encompassing not only chemical and physical parameters but also the biological community and hydrodynamic characteristics, allowed us to perform a powerful interpretative modeling, which led to disentangle the pathways by which temperature and nutrient variations could regulate the lacustrine phytoplankton community. Across the studied period, warming in the upper water column led to an increase in the total phytoplankton biovolume and to a loss of phytoplankton diversity. This diversity loss was caused by the decrease in phytoplankton evenness, likely provoked by the increased dominance of a few taxa. Vertical lake mixing, which is influenced by climate, plays a central role in distributing nutrients throughout the water column. Moreover, not all nutrients carry equal significance in shaping the community development and structure; rather, it appears that changes in specific nutrients and their stoichiometric ratios play a pivotal role in determining the composition and reorganization of the phytoplankton community.

Long-term research, monitoring, and modeling play a crucial role in providing essential scientific support for the ecosystem-based management of resources, activities, and services and for developing mitigation strategies. In this regard, our research suggested a pathway analysis that allowed us to effectively describe specific ecosystem dynamics, providing a replicable model that can be applied in other lakes with different trophic levels. Moreover, our results could help in building more complex future scenarios linked to the interaction of several anthropogenic stressors on lakes.

CRediT authorship contribution statement

Flavia Dory drafted the article and have made a substantial contribution to the analysis and data interpretation. Barbara Leoni contributed substantially to data acquisition, to the design of the article and revised the article critically for important intellectual content. Veronica Nava contributed actively to data acquisition, analysis, and revised the article critically for important intellectual content. Morena Spreafico, Valentina Orlandi, Valentina Soler contributed substantially to data acquisition and laboratory analysis and All co-authors have seen, read and approved this manuscript in the submitted form.

Declaration of competing interest

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

Data availability

Data will be made available on request.

Acknowledgments

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.167566.

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