# Temporal changes in nutrients in a deep oligomictic lake: the role of external loads versus climate change

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## ABSTRACT

The impact of climate change on stratification and mixing patterns has important effects on nutrient availability and plankton dynamics in deep lakes. We demonstrate this in a long-term study of Lake Maggiore, a deep oligomictic lake located in the subalpine lake district in Northern Italy. Studies on physical, chemical and biological features of the lake have been performed continuously since the 1980s. The lake recovered from eutrophication in response to a reduction of catchment nutrient loads and reached a stable oligotrophic status by the end of the 1990s, with average total phosphorus concentrations in the water column around 10 µg $L^{-1}$. However, both reactive and total phosphorus have slightly increased since 2010, leading to a shift in the lake trophic state towards mesotrophy. The increase in phosphorus has been limited to the hypolimnetic layers, concentrations being fairly stable or decreasing in the epilimnion. Reactive silica also progressively increased in the hypolimnion, while nitrate and total nitrogen concentrations have steadily decreased in both deep and surface layers, especially in the summer period. These changes were assessed in relation to catchment loads, atmospheric deposition and climate-related variations in stratification and mixing patterns and in nutrient retention. Long-term changes in primary production, represented by chlorophyll levels, and biovolume of the main algal groups were also considered. During the eutrophication period and until the 1990s, in-lake phosphorus concentrations were tightly related to external loads; successively, phosphorus and its vertical distribution up the water column became more controlled by internal processes, in particular by stratification and mixing regime. An increase of thermal stability and a reduced frequency and intensity of deep mixing events has fostered oxygen depletion and phosphorus and silica accumulation in the hypolimnion. Another consequence of reduced deep mixing events has been a reduction in nutrient replenishment of the upper layers at spring mixing. External loads are still the main driver of change for nitrogen compounds: the decrease in the atmospheric load of nitrogen that occurred in the Lake Maggiore area over the last decade, as an effect of reduced nitrogen emissions, has caused decreasing concentration of inorganic nitrogen in the lake. However, the phytoplankton community changes observed might also play a role in nitrogen dynamics, particularly in the nitrate minima observed during summer in recent years.

## INTRODUCTION

In this paper, we analysed long-term data on nutrient concentrations and related variables monitored on a monthly basis and at different depths in Lake Maggiore, a deep oligomictic lake belonging to the deep subalpine lake district in Northern Italy. Lake Maggiore was affected by eutrophication in the 1970s and 1980s, then it started a recovery process thanks to the reduction of external phosphorus loads and it reached a stable oligotrophic status by the end of the 1990s (Ruggiu *et al.*, 1998; Salmaso and Mosello, 2010). The main aims of the long-term data analyses we have performed were: i) to assess long-term trends and change points of algal nutrients (nitrogen (N), phosphorus (P), silica (Si)) over a 35-year period, with a focus on the oligotrophication phase of the lake; ii) to evaluate the role of external drivers (catchment loads, atmospheric deposition) *versus* internal processes, *i.e.* mixing regime and phytoplankton dynamics, in the observed nutrient variations.

Nutrients are key elements in lake functioning and play a fundamental role in the overall environmental state of lakes. Lake primary production is indeed limited by nutrient availability. Phosphorus in particular is the limiting factor for the majority of lakes and regulates phytoplankton abundance (Dillon and Rigler, 1974). Nitrogen usually occurs in much higher concentrations than phosphorus and therefore, even if required by primary producers in higher amount than phosphorus, nitrogen is more rarely a limiting factor in lakes (Elser *et al.*, 2007). However, recent findings suggest that both N and P may be limiting in the same lake in different periods and depths (Søndergaard *et al.*, 2017; Maberly *et al.*, 2020). Silica is usually much less considered than N and P in studies regarding

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nutrient limitation and lake eutrophication; however, silica has an essential role for the development of specific phytoplankton groups such as diatoms (Kilham, 1971; Egge and Aksnes, 1992) and it has been demonstrated that lakes can have a relevant role in Si fluxes and cycling (Scibona *et al.*, 2019).

Both N and P are involved in eutrophication processes: their excessive inputs enhance lake productivity, with cascading effects on the trophic levels and their interactions (Hutchinson, 1973). As a consequence, management of eutrophication strongly relies on the regulation of nutrients in lakes, both as absolute concentrations and relative ratios (Carvalho *et al.*, 2013). Nutrient dynamics in lakes are firstly dependent on external inputs: catchment loads, both from point and diffuse sources, are the primary cause of eutrophication, with phosphorus inputs playing a dominant role (Schindler *et al.*, 2008). In the case of nitrogen, atmospheric deposition has proved to be a relevant input source to lakes in areas subject to high pollutant deposition due to both local emissions and long-range transport (Bergström and Jansson, 2006). Once entered into lakes, nutrients are subject to uptake by primary producers but also to sedimentation of the particulate bound forms and biologically mediated transformation processes such as denitrification, nitrification, dissimilatory nitrate reduction to ammonium (DNRA) and anaerobic ammonium oxidation (anammox) (Søndergaard, 2007; Nizzoli *et al.*, 2018). In deep lakes, the homogenisation of the water column and the redistribution of nutrients are strongly dependent on the stratification and mixing regime. In particular, oligomictic lakes, where full overturn does not occur every year, are not subject to a regular homogenisation of nutrient concentrations along the water column, this process only occurring at the end of very cold and windy winters (Goldman and Jassby, 1990; Ambrosetti and Barbanti, 1999).

Climate change may affect nutrient concentrations in lakes by interacting with both input pathways and internal processes: precipitation amount and intensity drive the rates and ratios at which nutrients are exported from catchments to lakes (Hayes *et al.*, 2015), with an important contribution to nutrient flushing played by heavy precipitation events (Morabito *et al.*, 2018). Droughts also affect nutrient cycling by increasing the retention times of both water and nutrients, possibly favouring microbial transformation processes such as denitrification (David *et al.*, 2006). Nutrient concentrations and distribution within a lake are also driven by internal processes which are in turn affected by climate: examples are changes in the thermal structure/mixing regime and in primary productivity (Adrian *et al.*, 2009; Jeppesen *et al.*, 2005; Radbourne *et al.*, 2019; Salmaso *et al.*, 2020).

Nutrient levels, usually as annual average obtained from quarterly or more frequent samplings, are included in the chemical and physico-chemical elements required for the assessment of the ecological status of water bodies under the Water Framework Directive (WFD; EC, 2000). Also the evaluation of the long-term evolution of the trophic state of a lake (eutrophication/oligotrophication) is usually based on yearly data: seasonal patterns, phenology and vertical distribution of nutrients along the water column are less considered, also because frequent data in space and time are required for these evaluations.

An in-depth investigation of the spatial and temporal patterns of nutrients is relevant for a proper assessment of the overall ecological status of a lake and its evolution in response to both external (catchment loads) and internal drivers (biological processes, mixing regime). These evaluations are particularly important for deep lakes, where nutrient availability for primary producers depends on the replenishment to the upper layers occurring at spring overturn (Goldman and Jassby, 1990). Climate warming, enhancing lake stratification and decreasing frequency and depth of mixing events, may also affect the spring nutrient enrichment and consequently phytoplankton biomass and seasonal succession (Salmaso *et al.*, 2018).

Recent studies highlighted how, after reaching a stable trophic status, climatic drivers became prominent in Lake Maggiore in shaping phytoplankton species fluctuations and the pelagic food web as a whole (Morabito *et al.*, 2012; Tanentzap *et al.*, 2020). Furthermore, extreme precipitation events and sharp oscillations of the lake level proved to be relevant for phytoplankton blooms (Callieri *et al.*, 2014; Morabito *et al.*, 2018).

## METHODS

### Lake Maggiore

Lake Maggiore is a deep subalpine lake located between Italy (Piedmont and Lombardy regions) and Switzerland (Canton Ticino) (Fig. 1, Tab. 1). The lake, with a surface of 212.5 km$^2$, lies mostly in Italy (80% of the total surface), while the watershed (about 6600 km$^2$) is shared almost equally between the two countries. Lake Maggiore drains the upstream part of the watershed of River Ticino, which represents the main inflow of the lake together with rivers Toce, Tresa and Maggia. River Ticino is also the only emissary of the lake. Lake levels are controlled by the Miorina Dam to ordinarily allow for a 0.5 m difference between summer and winter (Fenocchi *et al.*, 2017).

Due to its morphological characteristics and climatic conditions, Lake Maggiore does not reach a full thermal and chemical homogenization every year: this only occurs following exceptionally cold and windy winters. For this reason, Lake Maggiore is classified as holo-oligomictic (Ambrosetti and Barbanti, 1999). The last complete overturn of Lake Maggiore occurred in 2005-2006; complete mixing in terms of homeothermy have been recorded also in 1991, 1999 and 2012 (Fenocchi *et al.*, 2018), but the latter was not accompanied by a full chemical homogenization of the water column.

The northernmost part of Lake Maggiore watershed is occupied by the Alps and is scarcely populated. Most of the population (about 670,000 inhabitants) lives in the subalpine area in the southern part of the catchment, where the main industrial activities are also located. The morphological characteristics of the area do not permit extensive agriculture (Mosello *et al.*, 2001). Due to its location North of the Po plain in Northern Italy, one of the most densely inhabited and urbanized regions of Europe, the area of Lake Maggiore has been subject to high deposition of atmospheric pollutants, especially nitrogen compounds (Mosello *et al.,* 2001). Nitrogen budget calculated for the whole Lake Maggiore watershed showed that atmospheric deposition is a relevant source of N, contributing up to 80% of the total input to the lake (Rogora *et al.*, 2006).

Together with lakes Orta, Como, Garda and Iseo, Lake Maggiore belongs to the "Southern Alpine Lake" site (LTER_EU_IT_008) of the Italian and European LTER (Long-Term Ecological Research) networks. Several syn-

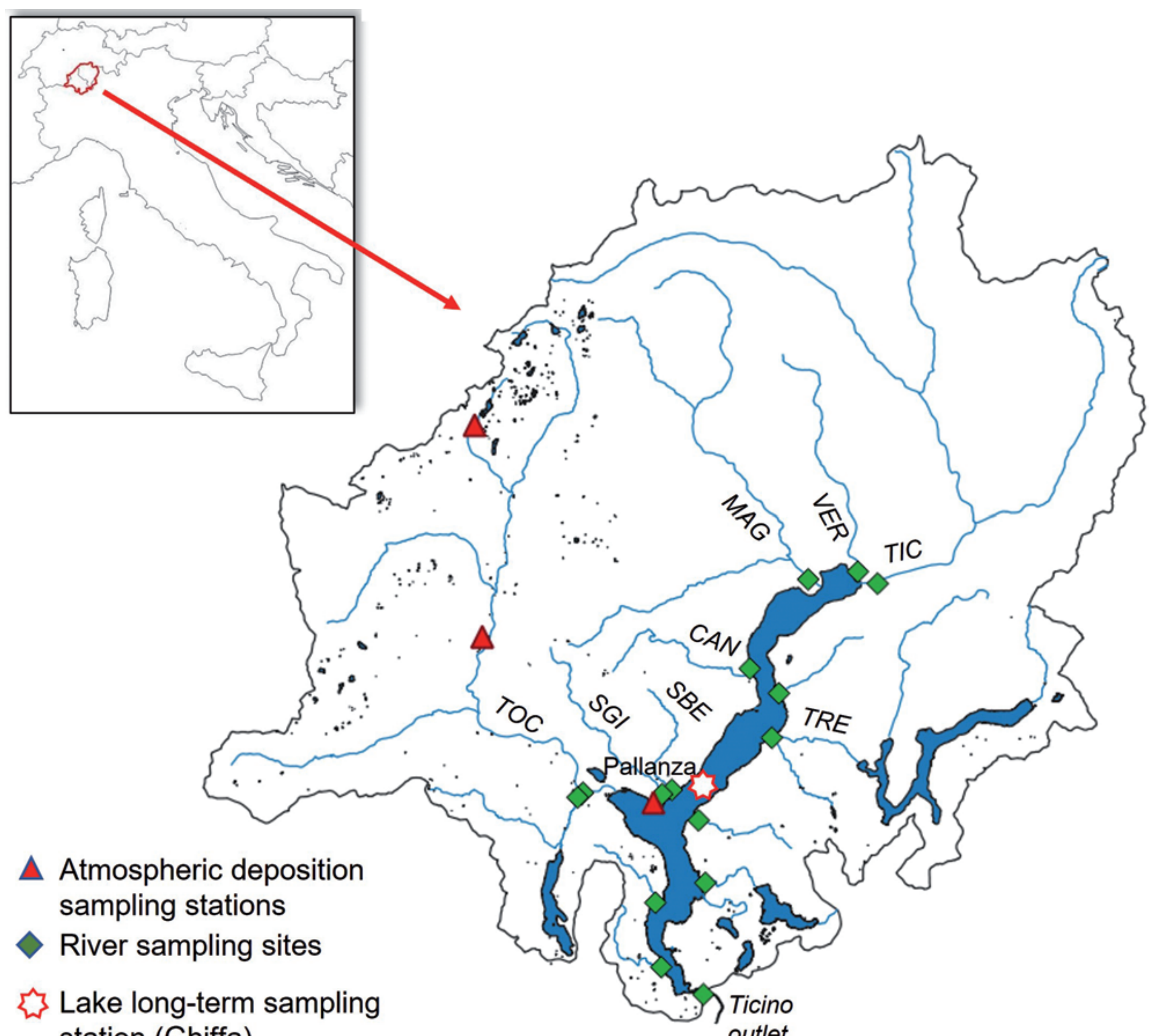


**Fig. 1.** Location of Lake Maggiore in Italy and Lake Maggiore watershed with the location of the sampling sites and stations considered in this study. TIC, River Ticino; VER, River Verzasca; MAG, River Maggia; CAN, River Cannobino; SBE, River San Bernardino; SGI, River San Giovanni; TOC, River Toce; TRE, River Tresa.

optic studies have been performed on the deep subalpine lakes together, considering their physical, chemical and biological features and assessing their response to various natural and anthropogenic drivers (for a synthesis, see Salmaso *et al.*, 2020). Studies on Lake Maggiore, in particular, have been performed continuously since the 1980s in the framework of the limnological campaigns funded by the International Commission for the Protection of Italian-Swiss Waters (CIPAIS; www.cipais.org). These campaigns have been regularly performed on a monthly basis at the Ghiffa site (deepest point of the lake, 370 m depth; Fig. 1).

## Data and methods

Water temperature has been measured at discrete depths with mercury-filled thermometers until 2010; successively, thermal profiles started to be recorded with a multi-parameter probe (Idronaut CTD 304). Water samples for chemical analyses have been collected at the following depths: 0, 5, 10, 20, 30, 50, 100, 150, 200, 250, 300, 360 m. Within the same monitoring program, monthly samplings and chemical analyses of the main tributaries (13 rivers) and of the lake outflow (River Ticino) have been also performed. Chemical analyses of both lake and river samples have been run at the water chemistry laboratory of the CNR Water Research Institute (CNR IRSA, previously CNR ISE) in Verbania Pallanza, using standard methods for freshwater samples (APHA AWWA WEF, 2012; APAT IRSA-CNR, 2003). Regular quality assurance/quality controls procedures are adopted in the laboratory to assure the data quality and the comparability of the data through time, including the use of control charts and the participation to national and international intercomparisons. Details on the analytical methods and the QA/QC procedures adopted in the laboratory can be found at http://www.idrolab.irsa.cnr.it/.

Integrated samples for chlorophyll-a analysis and phytoplankton biovolume and density were also collected on a monthly basis through the 0-20 m layer (Morabito *et al.*, 2012). Chl-a was measured by spectrophotometric technique in the period 1984-2009 (APAT IRSA-CNR, 2003), while from 2010 measurements were taken using a vertical profiling instrument (FluoroProbe, BBE Moldaenke) after a careful check of the comparability of the two methods.

**Tab. 1.** Main morphometric and hydrological characteristics of Lake Maggiore and its watershed.

| | | |
|---|---|---|
| Mean watershed altitude | m asl | 1270 |
| Mean lake altitude | m asl | 194 |
| Watershed area (lake included) | km$^2$ | 6599 |
| Lake area | km$^2$ | 212.5 |
| Mean depth | m | 177 |
| Maximum depth | m | 370 |
| Max width | km | 10.0 |
| Mean width | km | 3.9 |
| Shoreline length | km | 170 |
| Volume | km$^3$ | 37.5 |
| Mean outflow discharge (1978-2018) | m$^3$ s$^{-1}$ | 280.5 |
| Theoretical renewal time | yrs | 4.2 |

The annual loads of P and N to Lake Maggiore have been evaluated by regular monitoring of the main tributaries. Annual loads were calculated on the basis of monthly concentrations and daily river discharge data according to Sonzogni *et al.* (1978). The inputs from the areas not drained by the tributaries (*e.g.*, shoreline area; 11% of the total catchment area) were estimated attributing them the average areal coefficient obtained from the drained areas, as described in Rogora *et al.* (2019). The output from the lake was calculated on an annual basis by the concentration and discharge data available for the River Ticino outlet and % retention in the lake estimated as: (input-output)/input * 100.

Due to the relevant role of atmospheric deposition as a source of pollutants in the Lake Maggiore area, long-term monitoring of precipitation chemistry has been performed since the 1980s in several stations covering the whole watershed (Rogora *et al.*, 2016). Presently three monitoring stations are still active in the Italian part of the watershed; in this study we used the data of the Pallanza wet-only station (Fig. 1), located on the lake shore, at 198 m asl, covering the period 1984-2019. In Pallanza an automatic weather station has also been operating since 1951. Monthly average air temperature over 1981-2019 were used in this study to assess trends.

We considered annual average values or values at spring overturn in the period 1984-2019 to identify the main phases in the long-term change of nutrient concentrations, nutrient ratios and lake trophic status. To this aim, Chl-a concentrations and phytoplankton biovolume were used as proxy of primary productivity. An in-depth investigation of the temporal changes in water temperature, dissolved oxygen (DO) and nutrients was done using monthly data collected over the period 1988-2019 along the water column (12 depths). In total, about 4500 measurements were available for each variable. For Chl-a and phytoplankton biovolume only one measurement was available for each sampling date, corresponding to the integrated sample 0-20 m, for a total of 384 measurements.

Trends and change points were then tested using monthly data distinguished between epilimnetic (volume-weighted average for the layer 0-25 m) and hypolimnetic (25-370 m) zones. Seasonal decomposition by loess smoothing (SDL) was used to decompose the time series into the trend and seasonal component (Zuur *et al.*, 2007). Trend significance was evaluated using the Mann-Kendall Test (MKT) or the Seasonal Kendall Test (SKT) for monthly data and trend slopes calculated with the Sen's method (Hirsch *et al.*, 1982; Marchetto *et al.*, 2013). Change points in the monthly time series of epilimnetic and hypolimnetic average values were estimated as change in mean and variance using the Binary Segmentation method (Scott and Knott, 1974). Statistical analyses were performed using RStudio, version 1.3.1073, packages "rkt" and "changepoint". The vertical distribution of nutrients along the water column and the seasonal patterns of epilimnetic Chl-a and biovolume were inspected by interpolating the monthly data by Kriging (Surfer software).

The metadata and the chemical dataset used in the present paper are available in DEIMS-SDR (Dynamic Ecological Information Management System - Site and dataset registry - at Lake Maggiore ID: https://deims.org/f30007c4-8a6e-4f11-ab87-569db54638fe).

## RESULTS

### Catchment loads and atmospheric deposition

The annual catchment loads of total nitrogen and total phosphorus to Lake Maggiore both decreased significantly since the 1980s (Fig. 2; MKT $p<0.001$ for both P and N loads). The decrease was more regular in the case of P loads, which passed from 350-400 t P $y^{-1}$ in the first

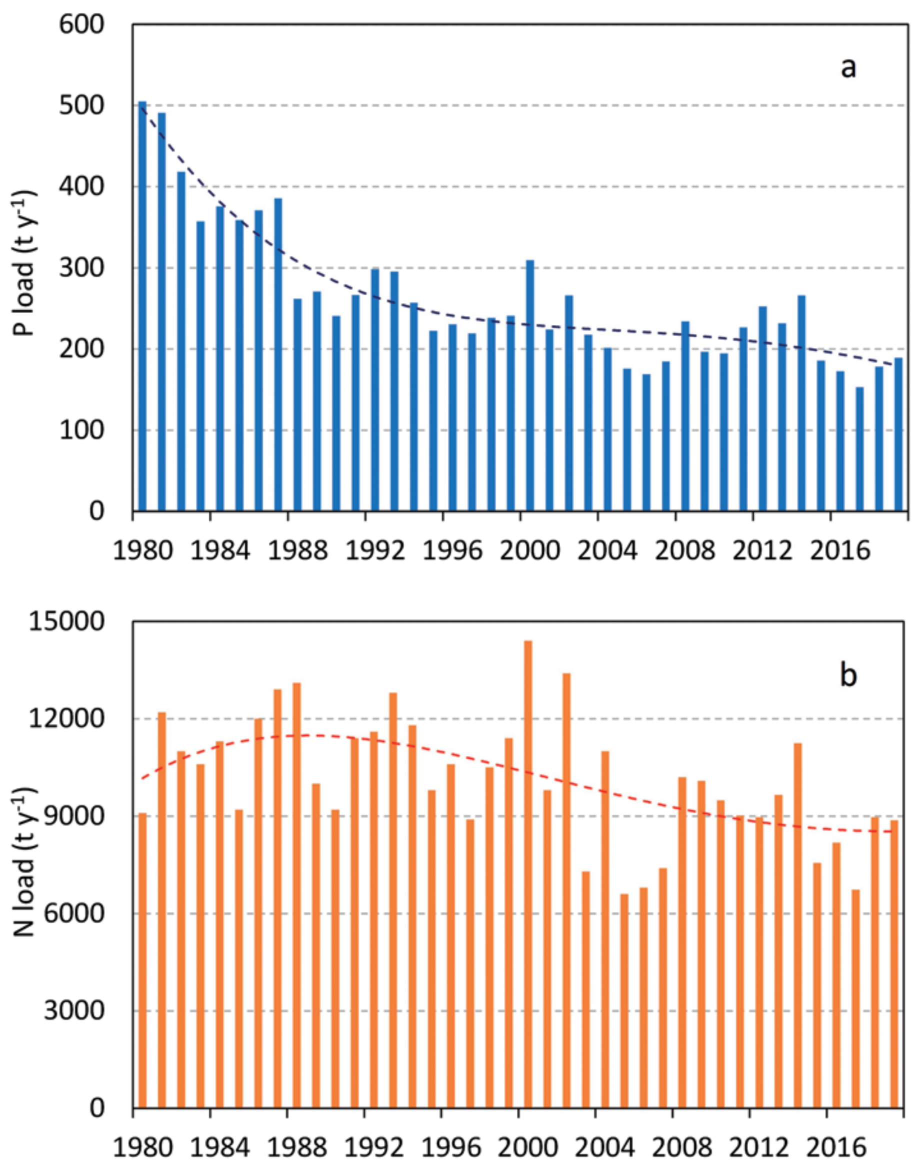


**Fig. 2.** Annual catchment loads of total P (a) and total N (b) to Lake Maggiore calculated from the monthly monitoring of the main tributaries.

half of the 1980s to the recent values below 200 t P $y^{-1}$. Nitrogen loads showed a higher interannual variability, with values ranging from about 6,500 to more than 14,000 t N $y^{-1}$. Despite the pronounced interannual changes, a tendency towards lower N loads can be seen since 2005-2006, when average loads moved from around 11,000 t N $y^{-1}$ to 8,500 t N $y^{-1}$ (Fig. 2).

The overall P budget, calculated on annual basis by considering the total P input and the P export through the lake outflow (Ticino River), showed that in-lake P retention has slightly decreased since the 1980s (MKT $p<0.05$) but remained fairly stable (between 50 and 60%) in the last decade.

The long-term trend of inorganic N deposition at the Pallanza wet-only monitoring station is shown in Fig. 3. A shift towards lower values can be seen around 2005, both for reduced (N-$NH_4$) and oxidized (N-$NO_3$) nitrogen deposition. The total inorganic N deposition (N-$NH_4$+N-$NO_3$) passed from 25-35 kg N $ha^{-1}$ $y^{-1}$ in the 1980s and 1990s to less than 20 kg N $ha^{-1}$ $y^{-1}$ in recent years.

The long-term data of N-$NO_3$ concentrations in the main tributaries of Lake Maggiore (contributing altogether to more than 80% of the total inflow to the lake) showed positive trends in the 1980s and 1990s (Fig. 4). Then, since around 2004, N-$NO_3$ concentrations started to decrease in all rivers, except for River Tresa, the outlet of Lake Lugano, as nutrient dynamics in this river are strongly affected by those of the lake.

## Meteo-climatic drivers, stability and mixing

Air temperature trends in Lake Maggiore area were evaluated from data of the Pallanza weather station (Fig. 1): both minimum (Tmin) and maximum (Tmax) air temperature increased significantly over the last 4 decades ($p<0.001$), with the steepest trends for Tmax in spring and summer (0.09 and 0.10°C $y^{-1}$). Winter data showed an aggrading trend in the very last few years (2014-2019) (Fig. 1S). Water temperature also increased significantly between 1988-2009 (Fig. 2S), more steeply in the epilimnion, while hypolimnetic temperatures increased afterwards (Tab. 2), with a change point in 2014 (Tab. 3). The increasing rate over the whole period (1988-2019) was 0.021 and 0.011°C $y^{-1}$, with an overall change of 0.66 and 0.34°C in the epilimnion and hypolimnion, respectively. The trend analysis at seasonal level revealed that epilimnetic water warmed significantly only in winter and autumn (Tab. 1S). In particular, the minimum water temperature which is usually recorded in February tended to be higher in recent years (Tab. 1S; Fig. 2S), possibly as a response to the steep positive trend in air temperature: since 2014, the epilimnetic water temperature in February was steadily above 7°C with respect to the values of the previous period, mostly between 6.5 and 6.8°C (higher values were recorded in 2007 and 2008 as an effect of the complete mixing of 2005-2006).

Since 2006, Lake Maggiore underwent partial mixings in the late winter period (between late February and early

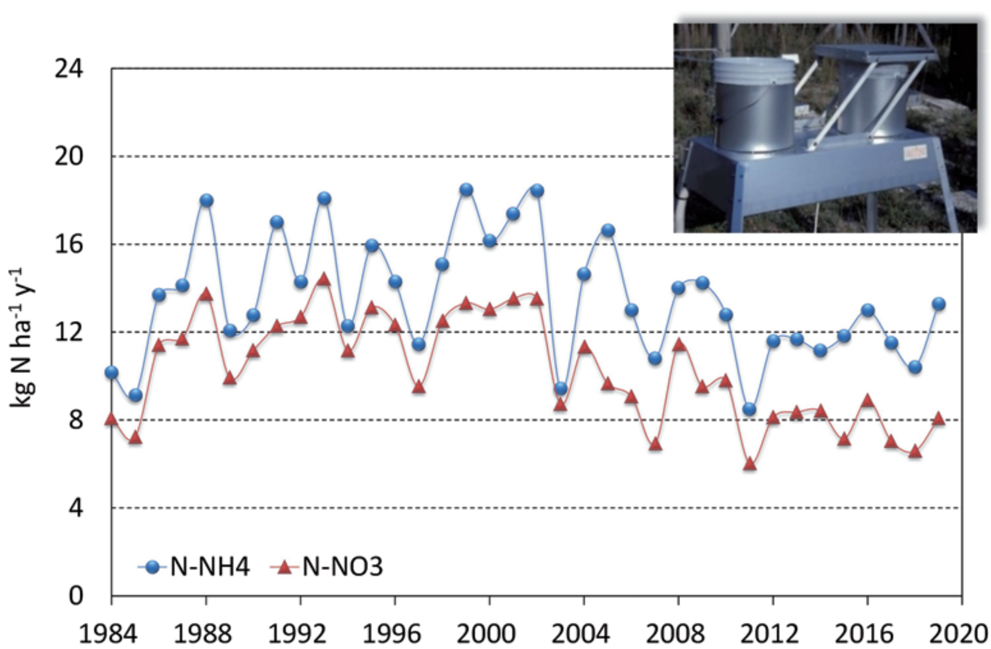


**Fig. 3.** Long-term trend (1984-2019) of inorganic N deposition (N-$NH_4$ and N-$NO_3$) in the atmospheric deposition sampling station of Pallanza.

**Tab. 2.** Results of the Seasonal Kendall Test (SKT) applied to monthly blocks of data representative of the epilimnion (0-25 m) and hypolimnion (25-370 m) of the lake.

| | Temp. | $O_2$ | $N\text{-}NO_3$ | $N\text{-}NH_4$ | TN | RP | TP | Si |
|---|---|---|---|---|---|---|---|---|
| **1988-2009** | | | | | | | | |
| **Epilimnion** | | | | | | | | |
| p | *** | *** | *** | ** | n.s. | n.s. | *** | n.s. |
| B | 0.038 | -0.026 | 2.734 | -0.120 | -0.001 | 0.009 | -0.097 | 0.005 |
| **Hypolimnion** | | | | | | | | |
| p | * | *** | *** | *** | *** | n.s. | *** | *** |
| B | 0.005 | 0.026 | 3.837 | -0.080 | 0.002 | -0.006 | -0.098 | 0.022 |
| **2010-2019** | | | | | | | | |
| **Epilimnion** | | | | | | | | |
| p | n.s. | n.s. | *** | n.s. | *** | n.s. | ** | n.s. |
| B | 0.010 | -0.013 | -7.899 | 0.000 | -0.010 | 0.000 | -0.084 | 0.005 |
| **Hypolimnion** | | | | | | | | |
| p | *** | *** | *** | *** | *** | *** | *** | *** |
| B | 0.037 | -0.170 | -8.305 | 0.140 | -0.010 | 0.270 | 0.278 | 0.024 |

*p, significance level; ***$p<0.001$; **$p<0.01$; *$p<0.05$; red, positive trends; blue, negative trends; n.s., not significant; trend slope (B) units: Temp, °C $y^{-1}$, $O_2$, mg $L^{-1}$ $y^{-1}$, TN, Si mg $L^{-1}$ $y^{-1}$, $N\text{-}NO_3$, $N\text{-}NH_4$, RP, TP, µg $L^{-1}$ $y^{-1}$.*

**Tab. 3.** Change points identified in the time series of monthly epilimnetic and hypolimnetic data. Only significant change points ($p<0.05$) are reported. For Chl-a and biovolume, integrated data over 0-20 m were used.

| | Temp. | $O_2$ | $N\text{-}NO_3$ | $N\text{-}NH_4$ | TN | RP | TP | Si | Chl-a | Biovolume |
|---|---|---|---|---|---|---|---|---|---|---|
| **Epilimnion** | - | - | 2010 | 1991 | 2009 | 2006 | 2006 | - | 2011<br>2014 | - |
| **Hypolimnion** | 1993<br>2014 | 1999<br>2011<br>2015 | 2000<br>2011<br>2016 | 1991 | 1990<br>2015 | 1991<br>2011 | 1990<br>2011 | 2000<br>2014 | | |

*-, no change points.*

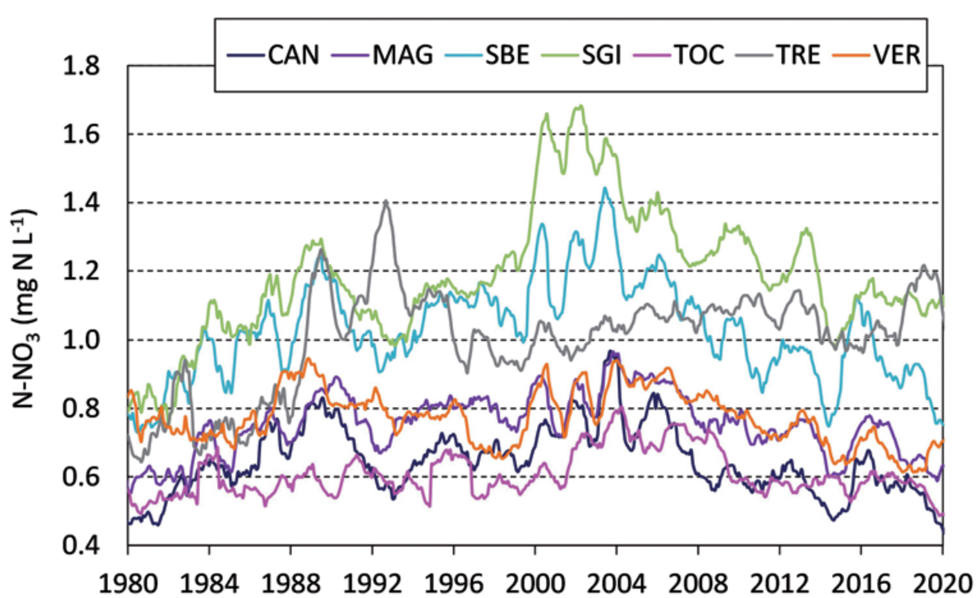


**Fig. 4.** Monthly concentrations (12-point running average) of $N\text{-}NO_3$ in the main tributaries of Lake Maggiore between 1980-2019. TIC, River Ticino; VER, River Verzasca; MAG, River Maggia; CAN, River Cannobino; SBE, River San Bernardino; SGI, River San Giovanni; TOC, River Toce; TRE, River Tresa.

March). The depth of the late winter circulation calculated on the basis of the vertical profiles of temperature, conductivity and dissolved oxygen showed a tendency towards less and less deep mixing in recent years, with the mixing depth mostly below 100 m (Fig. 5). The annual average values of the Schmidt stability, calculated from the monthly profiles of water temperature, increased in time (MKT $p<0.001$), confirming the aggrading resistance of the water column to mixing (Fig. 5).

### Temporal and spatial patterns of nutrient concentrations and related variables

Between 1988-2019, the lake was affected by a significant increase of water temperature, more pronounced in the epilimnion (0.04°C $y^{-1}$; Tab. 2). Dissolved oxygen (DO) increased in the hypolimnion, due to the occurrence of some complete or deep mixing episodes over this period (*e.g.*, 1991, 1999, 2006). On the other hand, oxygen decreased in surface waters, mainly as an effect of the decreasing occurrence of summer maxima (oversaturation) during the oligotrophication phase (Tab. 2). In the last decade (Tab. 2), oxygen stabilised in surface waters but significantly decreased in the deep layers (-0.17 mg $L^{-1}$ $y^{-1}$), which were also affected by a significant temperature increase (0.04°C $y^{-1}$). No significant change points were identified in water temperature and dissolved oxygen (DO) in the surface layer, while changes occurred in 1993 and 2014 for temperature and in 1999, 2011 and 2015 for DO in the hypolimnion (Tab. 3).

As regards TP, the analysis showed a decoupling of trends between surface and deep water in the last decade (Fig. 6), where TP decreased and increased respectively (-0.08 and +0.28 µg $L^{-1}$ $y^{-1}$). No significant trends were detected for epilimnetic RP in both periods (Tab. 2). It must be emphasized that RP concentrations in surface waters are very low and, in most cases, close to the limit of the detection (LOD) of the method used (2 or 3 µg P $L^{-1}$ according to the analytical range used) (Fig. 2S). As a consequence, long-term changes can be hardly detected. Significant change points in RP and TP epilimnetic concentrations occurred in 2006, while changes in the hypolimnetic values were in 1990-1991 and 2011.

TP concentrations increased in deep water particularly below 200 m (Fig. 7), where TP progressively accumulated and reached concentrations of 25-30 µg $L^{-1}$ in recent years. These concentrations were similar to those recorded at the end of the 1980s-early 1990s. Data clearly show the effect of full overturns occurred in 1991, 1999 and 2006, followed by an almost complete homogenization of TP along the water column. Between 1999 and 2007, replenishment of TP in the surface layers occurred in spring, probably as an effect of quite deep circulations. However, this pattern became more irregular since 2008, with more and more evident depletion of TP occurring in the epilimnion (average concentration of 6 µg $L^{-1}$). The pattern of RP (Fig. 2S) was the same, with concentrations in the deep layer (below 200 m) approaching 16-18 µg $L^{-1}$ and values of 2-4 µg $L^{-1}$ in the surface layer.

Silica behaved similarly to P as regards the accumulation in the hypolimnetic layers where concentrations significantly increased (+0.22 mg $L^{-1}$ $y^{-1}$), especially in the last decade (Tab. 2b). Indeed, below 200 m, Si concentrations approached 2.0 mg $L^{-1}$ in recent years (Fig. 6c). However, silica remained fairly stable in the surface layers, as indicated by the lack of a significant trend (Tab. 2) and of change points in surface water, while shifts oc-

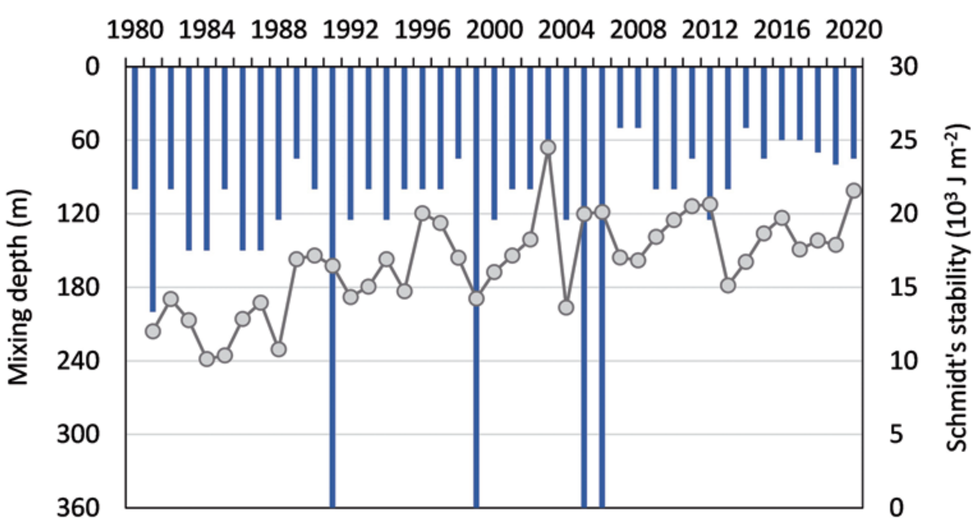


**Fig. 5.** Mixing depth at spring turnover (blue bars) in Lake Maggiore and annual average values of the Schmidt's stability (grey dots).

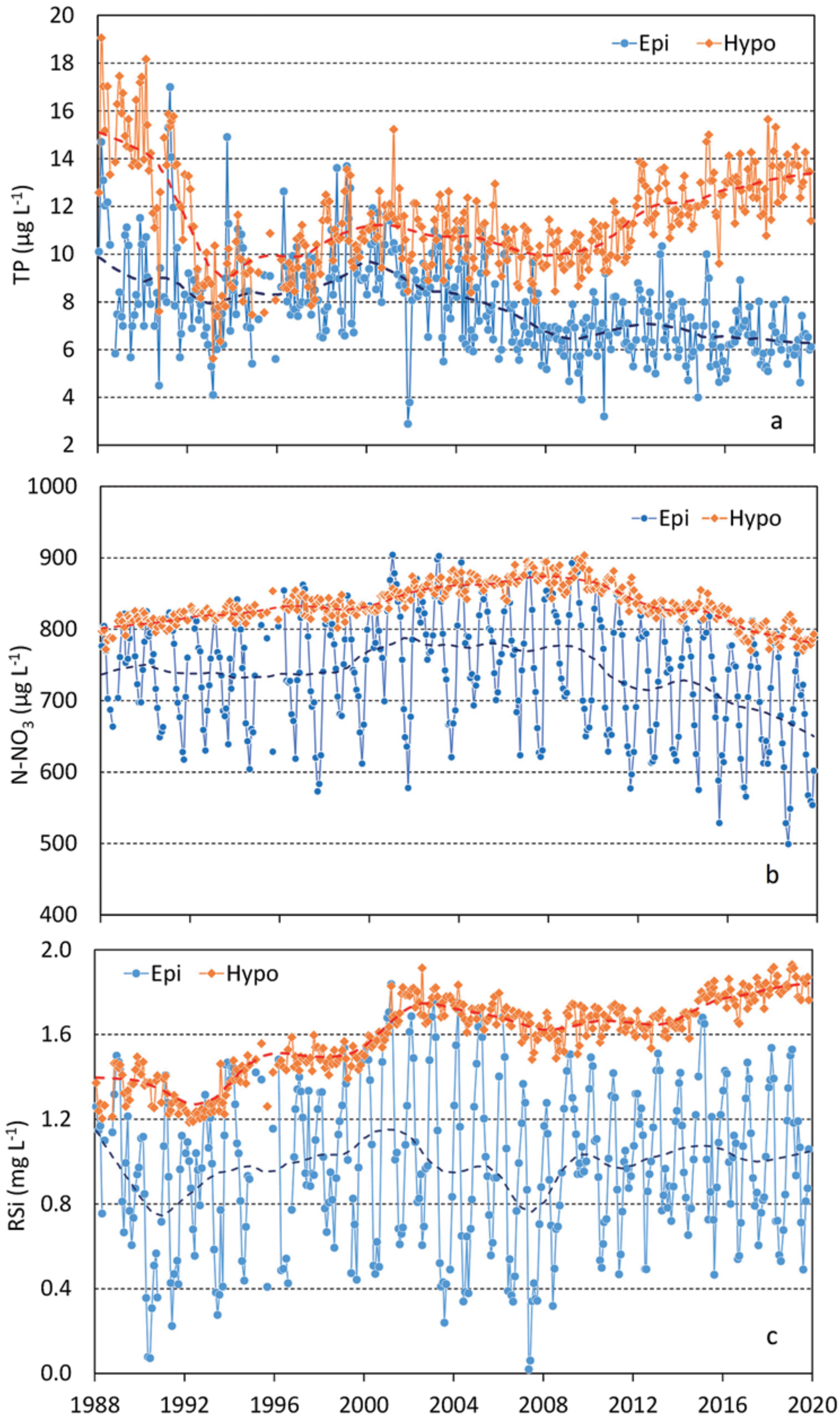


**Fig. 6.** Monthly epilimnetic and hypolimnetic concentrations of (a) total phosphorus (TP), (b) N-$NO_3$ and (c) Reactive Silica (Rsi) in Lake Maggiore between 1988-2019. The dashed lines are the trend components obtained by SDL.

curred in the hypolimnion in 2000 and 2014 (Tab. 3). A distinct seasonal pattern can be seen in silica concentrations, with minima in late spring and summer and maxima in early spring (March), as an effect of diatoms seasonal successions. Between 1988 and 2009 epilimnetic silica significantly increased in early spring (Tab. 1S), with annual maxima above 1.6 mg $L^{-1}$ between 2001 and 2006 (Fig. 6c), while no trends were detected afterwards.

N-$NO_3$ increased significantly, both in the epilimnion and the hypolimnion, between 1988-2019, then it decreased significantly in both layers (-8 µg $L^{-1}$ $y^{-1}$; Tab. 2; Fig. 6b). Change points in epilimnetic N-$NO_3$ and TN occurred in 2009-2010, while in the hypolimnion 2015-2016 was a significant change point (Tab. 3). The depletion of N-$NO_3$ in the epilimnion was particularly evident since 2010 when concentrations fell below 500 µg $L^{-1}$ in the summer period.

N-$NH_4$ is present in very low concentrations in Lake Maggiore, mostly below 20 and 5 µg $L^{-1}$ $y^{-1}$ in epilimnetic and hypolimnetic waters, respectively. Therefore, even though trends were significant, changes in terms of concentrations were negligible (about ±0.1 µg $L^{-1}$ $y^{-1}$).

During 1988-2009, Chl-a and phytoplankton biovolume significantly decreased; successively the trends of these variables levelled off (Tab. 4). Both Cyanobacteria

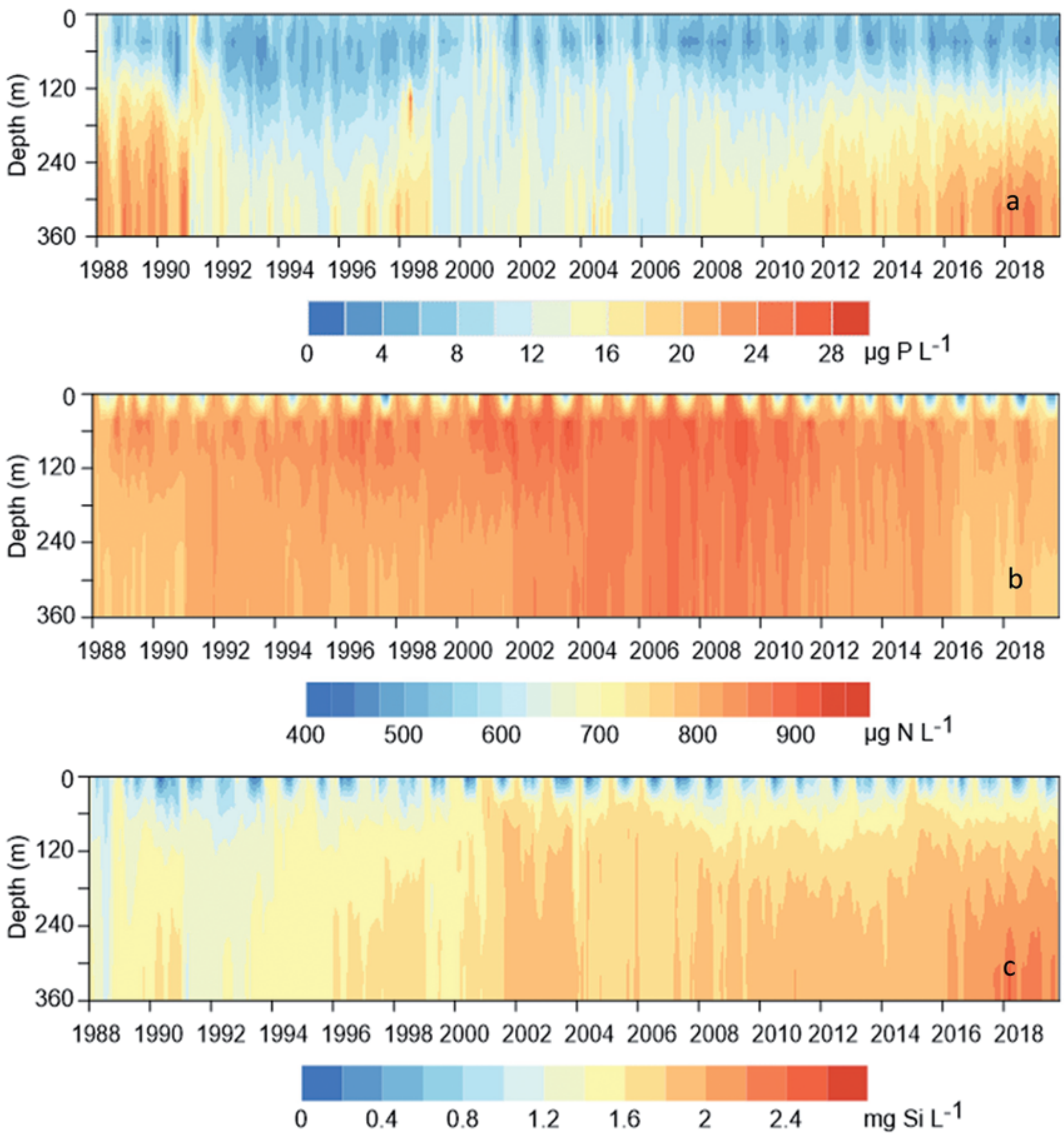


**Fig. 7.** Temporal change in the vertical distribution of TP (a), N-$NO_3$(b) and Si (c) in Lake Maggiore. Plots obtained by interpolation (Kriging) of monthly data gathered at 12 depths along the water column between 1988-2019.

and Chlorophyta decreased significantly, while a slight increase occurred in the biovolume of Chrysophyta in both periods. Chl-a concentrations slightly increased in the last decade, with change points in 2011 and 2014 (Tab. 4), while no significant changes were found in the total phytoplankton biovolume (Tab. 3).

## DISCUSSION

### Trophic evolution of the lake

Summarizing the temporal evolution of TP and TN in Lake Maggiore since 1984, three main phases can be identified (Fig. 8): i) from 1984 to the early 1990s, characterized by the start of the oligotrophication (TP decrease) and the beginning of the TN increasing phase; ii) from 1990 to 2009, during which TP remained fairly stable (9-11 µg P L$^{-1}$) while TN increased; iii) 2010 onwards, characterized by a slight increase of TP (13-14 µg P L$^{-1}$) and a drop of TN levels (Fig. 2). The N:P ratio significantly changed during these three periods: it passed from about 50-60 (molar ratio) in the eutrophication period to 200-250 in the oligotrophication phase; then it decreased in the most recent years due to the contemporary increase of TP and decrease of TN reaching the present value of 150. Despite these changes, conditions of N limitation or N and P co-limitation never occurred in Lake Maggiore, when considering concentrations of both the total forms of N and P or the readily bioavailable forms RP (soluble reactive P) and DIN (dissolved inorganic N). Even when considering seasonal nutrient minima, DIN never went below 0.1 mg N L$^{-1}$, a threshold possibly indicating N limitation (Maberly *et al.*, 2020).

Beside TP, Chl-a values and phytoplankton biomass, as a proxy of lake productivity, also confirmed the oligotrophication process of the lake: they both decreased

**Tab. 4.** Results of the Seasonal Kendall Test (SKT) applied to monthly blocks of data of Chl-a and biovolume (integrated data 0-20 m).

| | Chl-a | Biovol. | Cyano | Diato | Chryso | Crypto | Dino | Chloro |
|---|---|---|---|---|---|---|---|---|
| **1988-2009** | | | | | | | | |
| **0-20 m** | | | | | | | | |
| p | *** | *** | *** | n.s. | * | n.s. | * | *** |
| B | -0.047 | -46.79 | -7.39 | 0.29 | 0.33 | -0.08 | -0.74 | -0.57 |
| **2010-2019** | | | | | | | | |
| **0-20 m** | | | | | | | | |
| p | * | n.s. | *** | n.s. | * | n.s. | n.s. | *** |
| B | 0.07 | -17.41 | -3.97 | -7.71 | 1.56 | -0.99 | -1.32 | -0.98 |

*p, significance level; ***p<0.001; **p<0.01; *p<0.05; red, positive trends; blue, negative trends; n.s., not significant; trend slope (B) units: Chl µg L$^{-1}$ y$^{-1}$. Biovol. cm$^{3}$ m$^{-3}$ y$^{-1}$.*

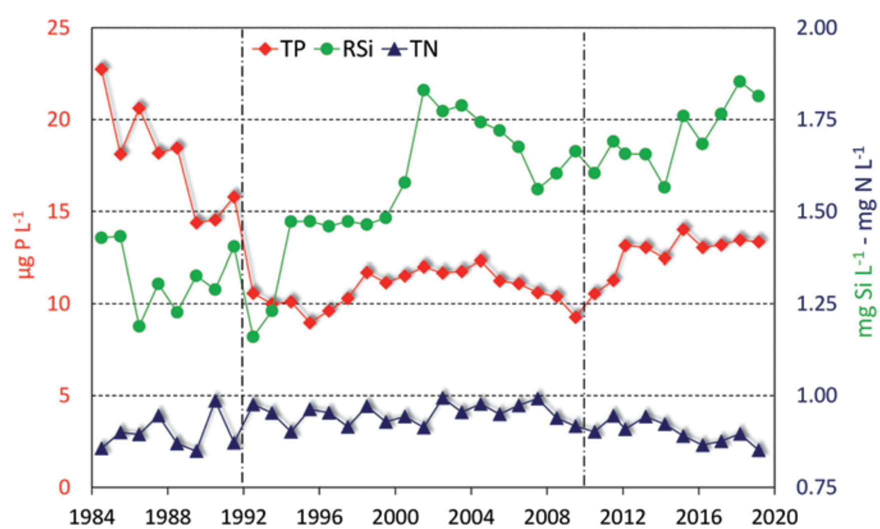


**Fig. 8.** Temporal evolution of total phosphorus (TP), total nitrogen (TN) and reactive silica (RSi) in Lake Maggiore between 1984-2019 (volume weighted average values along the water column at spring overturn).

from the mid-1980s until 2005-2006, with annual mean Chl-a concentration declining from 4.5-5.5 µg $L^{-1}$ to 2.0-3.0 µg $L^{-1}$ (Fig. 9). However, since 2007 strong fluctuations of Chl-a values were observed, with values close or above 4.0 µg $L^{-1}$ in some years (*e.g.*, 2011, 2016 and 2017). Also phytoplankton biovolume has shown marked interannual changes in the last decade, from minima of 0.6-0.7 $cm^3$ $m^{-3}$ up to 2.0 $cm^3$ $m^{-3}$ due to some algal blooms *e.g.* in 2011 and 2016 (Tapolczai *et al.*, 2015, Bresciani *et al.*, 2018).

The analyses of nutrient time series confirmed a change around 2010 affecting both absolute values (*e.g.*, increasing phosphorus, decreasing nitrogen concentrations) and distribution along the water column (decoupling between epilimnetic and hypolimnetic concentrations of P and Si). On the basis of the present levels of TP, Lake Maggiore falls into the category of mesotrophy according to OECD (1982). Further, the recent increase of TP and Chl-a could be interpreted as a sign of decreasing water quality and a deviation from policy targets such as those fixed by the WFD. It was therefore important to focus on the main drivers of the observed changes in nutrients and on the relative importance of external loads versus internal processes.

## External and internal drivers of the long-term evolution of nutrients concentrations

The gradual decrease of P inputs to Lake Maggiore since the 1980s was due to several interventions, both in the Italian and Swiss part of the lake watershed, including the establishment of water treatment plants and the reduction of total phosphorus in detergents (Morabito *et al.*, 2012). With respect to P, N loads decreased at a lesser extent and were highly affected by precipitation amount, being atmospheric deposition the main N source in the area of Lake Maggiore (Rogora *et al.*, 2006).

As the P load was fairly stable in the last decade, between 150 and 250 t P $y^{-1}$, external loads couldn't have contributed to the observed recent increase of in-lake P levels. The average P concentrations in the lake were significantly related to the external inputs (expressed as the average TP concentrations in inflowing waters) in the 1980s and 1990s *i.e.*, during the oligotrophication phase (linear regression: $R^2 = 0.79$, $p< 0.001$); since the 2000s, the relationship was not evident (Fig. 10). These results indicate that present P concentrations in Lake Maggiore are only partially controlled by external inputs. The slight recent increase of the TP as average values along the water column (Fig. 8) depends on the increasing concentrations in the deep hypolimnion (Figs. 6a and 7a).

Increasing heat content and thermal stability, promoted by increasing water temperature (Tab. 1S), coupled with a tendency towards decreasing wind speed in winter months (Ambrosetti and Barbanti, 1999; Ambrosetti *et al.*, 2010; Fenocchi *et al.*, 2018) are causing less and less deep mixing at spring turnover. During the last decade, mixing in Lake Maggiore rarely extended beyond 30% of the water column (or 50% of lake volume) (Rogora *et al.*, 2018). As described in details in Fenocchi *et al.* (2018), the duration of complete-mixing events, the mixing depth in the years without full turnover and the strength and duration of stratification affect the exchange of substances between top and bottom layers, explaining the oxygen depletion in the deep waters of Lake Maggiore (Rogora *et al.*, 2018) and the progressive accumulation of P and Si observed in the present study.

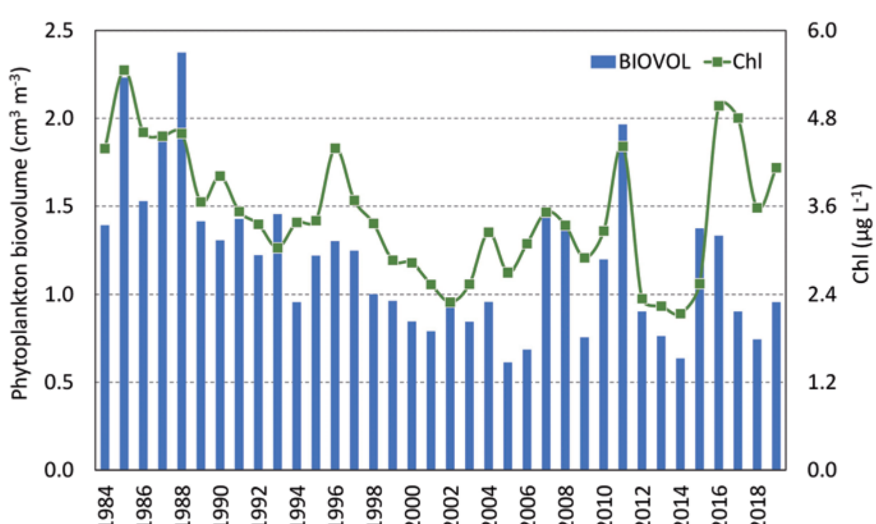


**Fig. 9.** Annual average values (from monthly data) of total phytoplankton biovolume and Chl-a concentrations in Lake Maggiore since 1984.

The present dynamics of P in Lake Maggiore appear to be less dependent on external loads, but regulated by internal processes, particularly by stratification and mixing patterns. The amount of TP transported from the hypolimnion to the surface layers by convective winter mixing is decreasing, causing the progressive accumulation in the deep layers. Further, the lack of exchange between surface and deep waters prevents the replenishment of phosphorus to the upper layers, as demonstrated by the slight decrease of TP in epilimnion in the recent period (Tab. 2; Fig. 6a).

Nitrogen showed a distinct pattern with respect to P, with a significant decrease both in the epilimnetic and hypolimnetic layers since 2010 (Figs. 6b and 7b; Tab. 2). Previous studies have shown how atmospheric deposition of N compounds on the lake and its watershed is the main source of N to Lake Maggiore (Rogora *et al.*, 2006). The relevance of deposition as a N source to the lake is also confirmed by the significant relationship between the annual precipitation amount over the lake watershed and the calculated total N load to the lake (Fig. 3S), with the highest loads (above 12000 t N $y^{-1}$) recorded in years with higher than average precipitation (*e.g.*, 1993, 2000, 2002). However, precipitation over the Lake Maggiore catchment did not change significantly in time, as shown by the time series data of the Pallanza meteorological station (Saidi *et al.*, 2013). Therefore, changes in precipitation and water inflows to Lake Maggiore do not explain the decreasing N load of the recent period (Fig. 2b).

Lake Maggiore is located northward of the Po plain, one of the most densely populated and industrialized area of Europe. The lake watershed has proved to be highly impacted by the deposition of N compounds, ranging between 15 and 30 kg $ha^{-1}$ $y^{-1}$ as the sum of reduced (N-$NH_4$) and oxidized N (N-$NO_3$). N-$NH_4$ and N-$NO_3$ almost equally contributed to the total inorganic N deposition, with an aggrading importance of N-$NH_4$ in time (Rogora *et al.*, 2016). The high N atmospheric loads affected freshwater ecosystems causing in some cases N saturation of soil catchments and increasing export of $NO_3$ to lakes and rivers (Rogora *et al.*, 2012). Starting from the second half the 2000s, as a consequence of policies for air emission reduction, a widespread decrease of N deposition began, both in the alpine and in the lowland part of the Lake Maggiore watershed (Rogora *et al.*, 2016). This decrease in N atmospheric inputs caused the decreasing concentrations in the main tributaries (Fig. 4) and consequently the diminishing N loads to Lake Maggiore since around 2005 (Fig. 2b). The response of the lake became evident after 2010, when TN started to decrease with a delay of a few years with respect to the main changes in atmospheric deposition. The annual N budget showed a slight decrease of % retention (MKT $p<0.05$) since the 1980s, and varied between 5 and 45% of the incoming load in the last decade. In contrast to P, N is decreasing significantly both in surface and deep layers (Figs. 6b and 7b). Diminishing external inputs certainly played the dominant role in the decrease of N in the lake, especially in the epilimnion. However, a contribution to the decrease of $NO_3$ may also come from the denitrification of $NO_3$, both present in the water column and produced by nitrification: the progressive isolation of the deep layers may indeed promote its consumption in the hypolimnion (Viaroli *et al.*, 2018). Furthermore, denitrification is a temperature dependent process which may be fostered in a warmer climate, even though with wide differences between systems (Veraart *et al.*, 2011).

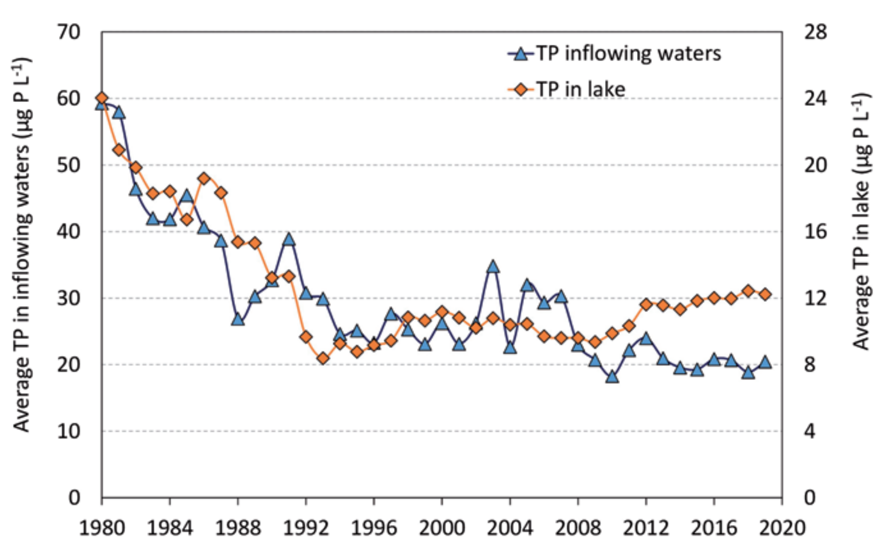


**Fig. 10.** Annual average TP concentrations in inflowing waters and in the lake between 1980-2019.

In the case of silica, two contrasting climate-related effects drive epilimnetic concentrations - the lack of spring replenishment due to the limited mixing of the water column and high runoff consequent to heavy precipitation events which could mobilize silicates and increase the export from the catchment to the lake (Morabito *et al.*, 2012; 2018). Silica concentrations in Lake Maggiore have indeed proved to be dependent on runoff consequent to high rainfall events (Morabito *et al.*, 2018), with some peaks in silica concentrations recorded in autumn, when major floods in Lake Maggiore occurred (*e.g.*, 2000, 2002, 2014). Because the frequency of extreme meteorological events has been shown to increase in the study area (Saidi *et al.*, 2013), the latter process could partly counteract the lack of deep mixing and nutrient distribution along the water column and explain the absence of a significant trend in epilimnetic silica (Tab. 2; Tab. 1S; Fig. 6c). Seasonal succession and long-term evolution of diatoms also play a role in the pattern of epilimnetic silica; however, as discussed below, the biovolume of diatoms did not change in time in Lake Maggiore (Fig. 11).

Other studies already showed how variability in stratification and mixing patterns has a substantial role in shaping nutrient cycling in lakes, including their entrapment in bottom water and their availability in epilimnion (North *et al.*, 2014; Yang *et al.*, 2016; Schwefel *et al.*, 2019). This in turn affects phytoplankton community composition and seasonal succession (Salmaso *et al.*, 2018), with cascading effects on the whole lake food web (Verburg *et al.*, 2003; Leoni *et al.*, 2014; Cohen *et al.*, 2016). Further, eventual complete mixing events in lakes which have been subject to many years of progressive nutrient accumulation and oxygen depletion in the hypolimnion, could have detrimental effects on the entire lake ecosystem (Holzner *et al.*, 2009; Jane *et al.*, 2021). Storm events also cause important changes in lake physical and chemical environments, possibly restructuring phytoplankton communities and their dynamics as well as nutrient cycling (Stockwell *et al.*, 2020).

The aggrading concentration of TP, as average values along the water column, in Lake Maggiore is an example of climate-induced change in lake trophic status. Similar effects have been described for the deep subalpine lakes Garda (Salmaso *et al.*, 2018) and Lugano (Lepori *et al.*, 2018) as well for other deep lakes North of the Alps (Yankova *et al.*, 2017; Schwefel *et al.*, 2019). These results overall are extremely important for water quality objectives: restoration targets should be revised taking into account the influence of climate-related drivers such as mixing depth and stability (Lepori and Capelli, 2021).

A further threat to lake water quality related to climate change is the progressive depletion of deep-water oxygen, which is affecting lakes worldwide (Jane *et al.*, 2021). In Lake Maggiore deep water oxygen levels are still far from being critical, despite a recent sharp decline of concentrations (Rogora *et al.*, 2018), so that a contribution to nutrient levels from internal loadings (sediment release) can be excluded at the moment. However, modelling of Lake Maggiore under climate change scenarios showed that anoxia would occur in the deep hypolimnion and P accumulation will be strengthened unless global greenhouse

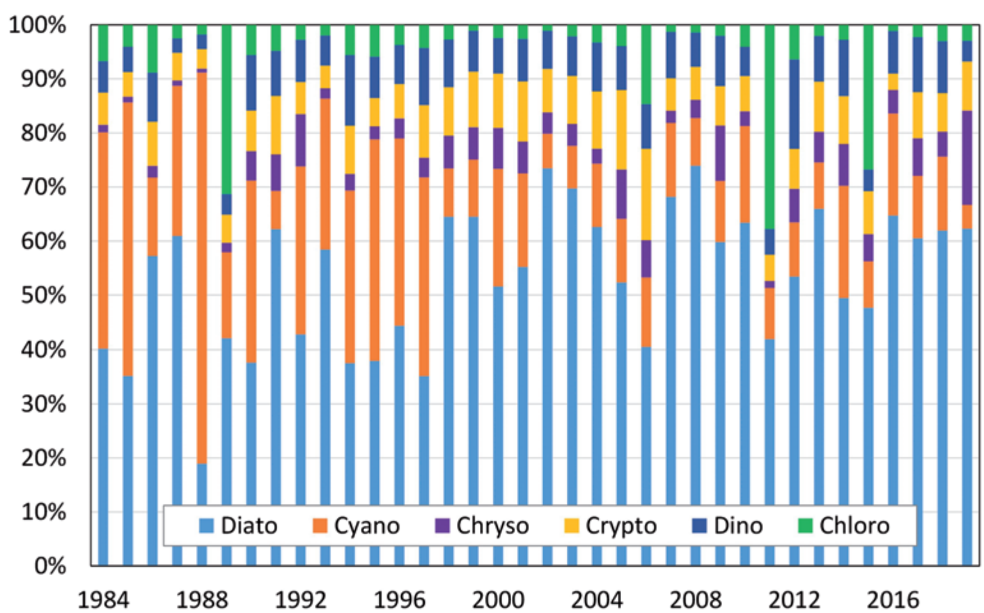


**Fig. 11.** Proportion of phytoplankton groups as a percentage of the total biovolume in Lake Maggiore.

gas emission starts to be reduced immediately (Fenocchi *et al.*, 2020). Even under the present situation, nutrient cycling within the lake, especially P and Si, appears to be strongly regulated by stratification and mixing patterns and meteo-climatic drivers, with overall effects which may partly counteract the efforts to reduce external loads and recover the lake from eutrophication.

In addition to catchment and atmospheric loads and change in the mixing regime, phytoplankton seasonal succession and interannual change may play an important role in nutrient variations. This role may be particularly important in the case of N compounds: N-$NO_3$ and consequently total N decreased at all depths, with steeper and more significant trends in the epilimnetic layer in spring and summer (Tab. 1S) and pronounced summer minima in recent years (*e.g.*, 2011, 2015, 2018) (Fig. 6b).

Both Chl-a and phytoplankton total biovolume decreased in Lake Maggiore between 1988-2009 (Tab. 4; Fig. 9). The long-term decline of phytoplankton biovolume was mainly due to the significant decrease of Cyanobacteria, since its contribution to the total biovolume decreased from 30-40% during the oligotrophication period to 10% in recent years (Fig. 11). Chlorophyta showed high abundance in some years (*e.g.*, 2011, 2015), due to blooms of the filamentous green alga *Mougeotia*, which has been recently reported to appear in large deep lakes of the peri-alpine region, postulated to be favoured by re-oligotrophication process and high water column stability (Tapolczai *et al.*, 2015). Further, *Mougeotia* possesses exceptional physiological plasticity being able to grow under a wide range of environmental conditions (Zohary *et al.*, 2019).

Diatoms did not change in terms of absolute biovolume, but their relative importance increased in time, reaching about 60% of the total biovolume in recent years (Fig. 11). Several field studies demonstrated that N forms are able to change the relative abundance of Cyanobacteria and diatoms (McCarthy *et al.*, 2009), and the relative contribution of Chrysophyta and dinoflagellates to total phytoplankton biomass (Blomqvist *et al.*, 1994; Poxleitner *et al.*, 2016). Various laboratory studies have observed differential utilization of N compounds by phytoplankton (Dortch, 1990; Levasseur *et al.*, 1993). A preference for $NH_4$ as the N source, for example, was noted in algal groups such as flagellates, green algae and Cyanobacteria (Blomqvist *et al.,* 1994; Dortch, 1990; Donald *et al.*, 2011; Domingues *et al.*, 2011), which have higher uptake affinities for this compound (Litchman *et al.,* 2007). However, growth rates may be higher under $NO_3$ supply, especially for a range of diatoms (Dortch, 1990; Litchman *et al.*, 2007, Glibert *et al.*, 2016).

The very recent years (2016-2019) were characterized by some peaks in Chl-a concentrations (Fig. 4S) and an increase in annual average values (Fig. 9), mostly due to diatom blooms in spring and early summer. A negative relationship, however not significant ($R^2$=0.27, p=0.12) was found between the maximum concentrations of Chl-a in spring months and the minima N-$NO_3$ concentrations in summer in the period 2010-2019 (Fig. 5S), suggesting a possible role of the spring diatom blooms in N depletion. Since diatoms exhibit high maximum nutrient uptake rates and are better adapted at utilizing nitrate (Litchman *et al.*, 2007), it might be plausible their contribution further exacerbates the nitrate minima during the summer period in the epilimnion of Lake Maggiore. However, to test this hypothesis and to focus in more details on the possible role of phytoplankton on nutrient dynamics, also in relation to both short and long-term climate induced changes, a better insight at species or functional groups level is required (Stockwell *et al.*, 2020).

## CONCLUSIONS

The analysis of long-term nutrient concentrations in Lake Maggiore over more than 30 years revealed slight but important changes: after recovering from eutrophication, the lake reached a stable oligotrophic status until around 2010, when a reversal in the trend of P and N occurred, respectively increasing and decreasing with respect to the previous period. The upward trend of phosphorus concentrations as average values along the water column, coupled with a slight increase of Chl-a concentrations in the very recent years, indicated a tendency towards mesotrophic conditions. However, when considering the nutrient distribution along the water column, it was evident that P is increasing in the deep layers only, while it remained stable or even decreased in the surface layers. This pattern was due to the lack of deep mixing events at spring turnover, fostering nutrients accumulation in the hypolimnion and hindering at the same time the replenishment to the trophogenic layers. The mixing depth and mixed volume of water at spring overturn in Lake Maggiore have been steadily declining in recent years due to the aggrading thermal stability of the water column. This situation, coupled with the deoxygenation of the deep waters, is one of the most evident effects of climate change on deep lakes and it is forecasted to continue in the near future, possibly counteracting the positive effects of interventions to reduce P catchment loads which has led to the oligotrophication of Lake Maggiore. If external loads are less important in driving the P levels in the lake, they are still relevant in the case of nitrogen, whose concentrations are strongly dependent on atmospheric deposition, representing the dominant vehicle of N to the lake. Climate drivers, in the form of increasing air and water temperatures and heavy rainfall events, have proved to be crucial in both the short- and long-term change in nutrient levels and distribution.

Nutrient concentrations, their temporal and spatial patterns, and their ratios are presently in an unstable condition in Lake Maggiore and their trends are expected to be more and more dependent on meteo-climatic drivers. Nutrients regulate fundamental processes in lakes and are key criteria for the evaluation of the quality of freshwater resources. For these reasons, monitoring the long-term evolution of nutrients, at a sufficiently high temporal and spatial frequency, is fundamental to a better comprehension of lake functioning and for a proper evaluation of changes in lake trophic and ecological status. Understanding the drivers of these changes is also needed to direct the management options for ensuring the quality of Lake Maggiore does not decline further.

## ACKNOWLEDGEMENTS

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## REFERENCES

Adrian R, O'Reilly CM, Zagarese H, Baines SB, Hessen DO, Keller W, Livingstone DM, Sommaruga R, Straile D, Van Donk E, Weyhenmeyer GA, Winder M, 2009. Lakes as sentinels of climate change. Limnol. Oceanogr. 54:2283-2297.

Ambrosetti W, Barbanti L, 1999. Deep water warming in lakes: an indicator of climatic change. J. Limnol. 58:1-9.

Ambrosetti W, Barbanti L, Carrara EA, 2010. Mechanisms of hypolimnion erosion in a deep lake (Lago Maggiore, N. Italy). J. Limnol. 69:3-14.

APAT, IRSA-CNR, 2003. [Metodi analitici per le acque].[in Italian]. APAT Manuali e Linee Guida 29; 2003.

APHA, AWWA, WEF, 2012. Standard Methods for the examination of water and wastewater. 22nd Edition. American Public Health Association, Washington.

Bergström A-K, Jansson M, 2006. Atmospheric nitrogen deposition has caused nitrogen enrichment and eutrophication of lakes in the northern hemisphere. Glob. Change Biol. 12:635-643.

Blomqvist P, Pettersson A, Hyenstrand P, 1994. Ammonium-nitrogen: a key regulatory factor causing dominance of non-nitrogen- fixing cyanobacteria in aquatic systems. Arch. Hydrobiol. 132:141-164.

Bresciani M, Cazzaniga I, Austoni M, Sforzi T, Buzzi F, Morabito G, Giardino C, 2018. Mapping phytoplankton blooms in deep subalpine lakes from Sentinel-2 and Landsat-8. Hydrobiologia 824:197-214.

Callieri C, Bertoni R, Contesini M, Bertoni F, 2014. Lake level fluctuations boost toxic cyanobacterial "oligotrophic blooms." PLoS One 9:e109526.

Carvalho L, McDonald C, de Hoyos C, Mischke U, Phillips G, Borics G, Poikane S, Skjelbred B, Solheim AL, Van Wichelen J, Cardoso AC, 2013. Sustaining recreational quality of European lakes: minimizing the health risks from algal blooms through phosphorus control. J. Appl. Ecol. 50:315-323.

Cohen AS, Gergurich EL, Kraemer BM, McGlue MM, McIntyre PB, Russell JM, Simmons JD, Swarzenski PW, 2016. Climate warming reduces fish production and benthic habitat in Lake Tanganyika, one of the most biodiverse freshwater ecosystems. P. Natl. Acad. Sci. USA 113:9563-9568.

David MB, Wall LG, Royer TV, Tank JL, 2006. Denitrification and the nitrogen budget of a reservoir in an agricultural landscape. Ecol. Appl. 16:2177-90.

Dillon PJ, Rigler FH, 1974. The phosphorus-chlorophyll relationship in lakes. Limnol. Oceanogr. 19:767-773.

Domingues RB, Barbosa AB, Sommer U, Galvao HM, 2011. Ammonium, nitrate and phytoplankton interactions in a freshwater tidal estuarine zone: potential effects of cultural eutrophication. Aquat. Sci. 73:331-343.

Donald DB, Bogard MJ, Finlay K, Leavitt PR, 2011. Comparative effects of urea, ammonium, and nitrate on phytoplankton abundance, community composition, and toxicity in hypereutrophic freshwaters. Limnol. Oceanogr. 56:2161-2175.

Dortch Q, 1990. The interaction between ammonium and nitrate uptake in phytoplankton. Marine Ecol. Prog. Ser. 61:83-201.

European Commission, 2000. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 Establishing a Framework for Community Action in the Field of Water Policy. Off. J. 2000: L327/1. European Commission, Brussels.

Egge JK, Aksnes DL, 1992. Silicate as regulating nutrient in phytoplankton competition. Mar. Ecol. Prog. Ser. 83:281-289.

Elser JJ, Bracken ME, Cleland EE, Gruner DS, Harpole WS, Hillebrand H, Ngai JT, Seabloom EW, Shurin JB, Smith JE, 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecol. Lett. 10:1135-1142.

Fenocchi A, Rogora M, Sibilla S, Dresti C, 2017. Relevance of inflows on the thermodynamic structure and on the modeling of a deep subalpine lake (Lake Maggiore, Northern Italy/Southern Switzerland). Limnologica 63:42-45.

Fenocchi A, Rogora M, Sibilla S, Ciampittiello M, Dresti C, 2018. Forecasting the evolution in the mixing regime of a deep subalpine lake under climate change scenarios through numerical modelling (Lake Maggiore, Northern Italy/Southern Switzerland). Clim. Dynam. 51:3521-3536.

Fenocchi A, Rogora M, Marchetto A, Sibilla S, Dresti C, 2020. Model simulations of the ecological dynamics induced by climate and nutrient load changes for deep subalpine Lake Maggiore (Italy/Switzerland). J. Limnol. 79:1963.

Glibert PM, Wilkerson FP, Dugdale RC, Raven JA, Dupont CL, Leavitt PR, Parker AE, Burkholder JM, 2016. Pluses and minuses of ammonium and nitrate uptake and assimilation by phytoplankton and implications for productivity and community composition, with emphasis on nitrogen enriched conditions. Limnol. Oceanogr. 61:165-197.

Goldman CR, Jassby A, 1990. Spring mixing depth as a determinant of annual primary production in lakes, p. 125-132. In: M.M. Tilzer and C. Serruya (eds.), Large lakes. Ecological structure and function. Springer, New York.

Hayes NM, Vanni MJ, Horgan MJ, Renwick WH, 2015. Climate and land use interactively affect lake phytoplankton nutrient limitation status. Ecology 96:392-402.

Hirsch RM, Slack JR, Smith RA, 1982. Techniques of trend analysis for monthly water quality data. Water Resour. Res. 18:107-121.

Holzner C, Aeschbach W, Simona M, Veronesi M, Imboden D, Kipfer R, 2009. Exceptional mixing events in meromictic Lake Lugano (Switzerland/Italy), studied using environmental tracers. Limnol. Oceanogr. 54:1113-1124.

Hutchinson GE, 1973. Eutrophication. Am. Sci. 61:269-279.

Jane SF, Hansen GJA, Kraemer BM, Leavitt PR, Mincer JL, North RL, Pilla RM, Stetler JT, Williamson CE, Woolway RL, Arvola L, Chandra S, DeGasperi CL, Diemer L, Dunalska J, Erina O, Flaim G, Grossart H-P, Hambright KD, Hein C, Hejzlar J, Janus LL, Jenny J-P, Jones JR, Knoll LB, Leoni B, Mackay E, Matsuzaki S-I S, McBride C, Müller-Navarra DC, Paterson AM, Pierson D, Rogora M, Rusak JA, Sadro S, Saulnier-Talbot E, Schmid M, Sommaruga R, Thiery W, Verburg P, Weathers KC, Weyhenmeyer GA, Yokota K, Rose KC, 2021. Widespread deoxygenation of temperate lakes. Nature 594:66-70.

Jeppesen E, Søndergaard M, Jensen JP, Havens KE, Anneville O, Carvalho L, Coveney MF, Deneke R, Dokulil MT, Foy B, Gerdeaux D, Hampton SE, Hilt S, Kangur K, Köhler J, Lammens EH, Lauridsen TL, Manca M, Miracle MR, Moss B, Nõges P, Persson G, Phillips G, Portielje R, Romo S, Schelske CL, Straile D, Tatrai I, Willén E, Winder M, 2005. Lake responses to reduced nutrient loading - an analysis of contemporary long-term data from 35 case studies. Freshwater Biol. 50:1747-1771.

Kilham P, 1971. A hypothesis concerning silica and the freshwater planktonic diatoms. Limnol. Oceanogr. 16:10-1.

Leoni B, Garibaldi L, Gulati RD, 2014. How does interannual trophic variability caused by vertical water mixing affect reproduction and population density of the *Daphnia longispina* group in Lake Iseo, a deep stratified lake in Italy? Inland Waters 4:193-203.

Lepori F, Bartosiewicz M, Simona M, Veronesi M, 2018. Effects of winter weather and mixing regime on the restoration of a deep perialpine lake (Lake Lugano, Switzerland and Italy). Hydrobiologia 824:229-242.

Lepori F, Capelli C, 2021. Effects of phosphorus control on primary productivity and deep-water oxygenation: insights from Lake Lugano (Switzerland and Italy). Hydrobiologia 848:613-629.

Levasseur M, Thompson PA, Harrison PJ, 1993. Physiological acclimation of marine phytoplankton to different nitrogen sources. J. Phycol. 29:587-595.

Litchman E, Klausmeier C, Schofield O, Falkowski P, 2007. The role of functional traits and trade-offs in structuring phytoplankton communities: scaling from cellular to ecosystem level. Ecol. Lett. 10:1170-1181.

Maberly SC, Pitt JA, Davies PS, Carvalho L, 2020. Nitrogen and phosphorus limitation and the management of small productive lakes. Inland Waters 10:159-172.

Marchetto A, Rogora M, Arisci S, 2013 Trend analysis of atmospheric deposition data: a comparison of statistical approaches. Atmos. Environ. 64:95-102.

McCarthy M, James R, Chen Y, East T, Gardner W, 2009. Nutrient ratios and phytoplankton community structure in the large, shallow, eutrophic, subtropical lakes Okeechobee (Florida, USA) and Taihu (China). Limnology 10: 215-227.

Morabito G, Oggioni A, Austoni M, 2012. Resource ratio and human impact: How diatom assemblages in Lake Maggiore responded to oligotrophication and climatic variability. Hydrobiologia 698:47-60.

Morabito G, Rogora M, Austoni M, Ciampittiello M, 2018. Could the extreme meteorological events in Lake Maggiore watershed determine a climate-driven eutrophication process? Hydrobiologia 824:163-175.

Mosello R, Calderoni A, Marchetto A, Brizzio MC, Rogora M, Passera S, GA Tartari, 2001. Nitrogen budget of Lago Maggiore: the relative importance of atmospheric deposition and catchment sources. J. Limnol. 60:27-40.

Nizzoli D, Bartoli M, Azzoni R, Longhi D, Castaldelli G, Viaroli P, 2018. Denitrification in a meromictic lake and its relevance to nitrogen flows within a moderately impacted forested catchment. Biogeochemistry 137:143-161.

North RP, North RL, Livingstone DM, Köster O, Kipfer R, 2014. Long-term changes in hypoxia and soluble reactive phosphorus in the hypolimnion of a large temperate lake: consequences of a climate regime shift. Glob. Change Biol. 20:811-823.

OECD, 1982. Eutrophication of waters. Monitoring, assessment and control. OECD, Paris: 154 pp.

Poxleitner M, Trommer G, Lorenz P, Stibor H, 2016. The effect of increased nitrogen load on phytoplankton in a phosphorus-limited lake. Freshwater Biol. 61:1966-1980.

Radbourne AD, Elliott JA, Maberly SC, Ryves DB, Anderson NJ, 2019. The impacts of changing nutrient load and climate on a deep, eutrophic, monomictic lake. Freshwater Biol. 64:1169-1182.

Rogora M, Arisci S, Marchetto A, 2012. The role of nitrogen deposition in the recent nitrate decline in lakes and rivers in Northern Italy. Sci. Total Environ. 417-418:214-223.

Rogora M, Buzzi F, Dresti C, Leoni B, Patelli M, Lepori F, Mosello R, Salmaso N, 2018. Climatic effects on vertical mixing and deep-water oxygen content in the subalpine lakes in Italy. Hydrobiologia 824:33-50.

Rogora M, Colombo L, Marchetto A, Mosello R, Steingruber S, 2016. Temporal and spatial patterns in the chemistry of wet deposition in Southern Alps. Atmos. Envir. 146:44-54.

Rogora M, Giacomotti P, Mosello R, Orrù A, Tartari GA, 2019. [Evoluzione stagionale e a lungo termine delle caratteristiche chimiche del Lago Maggiore e bilancio dei nutrienti a lago (azoto e fosforo)], p. 31-54. In: CNR Istituto di Ricerca sulle Acque, Sede di Verbania (eds.), [Ricerche sull'evoluzione del Lago Maggiore. Aspetti limnologici. Programma triennale 2016-2018. Campagna 2018 e rapporto triennale 2016-18].[Book in Italian]. Commissione Internazionale per la protezione delle acque italo-svizzere.

Rogora M, Mosello R, Calderoni A, Barbieri A, 2006. Nitrogen budget of a subalpine lake in North-Western Italy: the role of atmospheric input in the upward trend of nitrogen concentrations. Verh. Internat. Verein. Limnol. 29:2027-2030.

Ruggiu D, Morabito G, Panzani P, Pugnetti A, 1998. Trends and relations among basic phytoplankton characteristics in the course of the long-term oligotrophication of Lake Maggiore (Italy). Hydrobiologia 369:243-257.

Saidi H, Ciampittiello M, Dresti C, Ghiglieri G, 2013. The climatic characteristics of extreme precipitations for short-term intervals in the watershed of Lake Maggiore. Theor. Appl. Climatol. 113:1-15.

Salmaso N, Boscaini A, Capelli C, Cerasino L, 2018. Ongoing ecological shifts in a large lake are driven by climate change and eutrophication: evidences from a three decades study in Lake Garda. Hydrobiologia 824:177-195.

Salmaso N, Buzzi F, Capelli C, Cerasino L, Leoni B, Lepori F, Rogora M, 2020. Responses to local and global stressors in the large southern perialpine lakes: Present status and challenges for research and management. J. Great Lakes Res. 46:752-766.

Salmaso N, Mosello R, 2010. Limnological research in the deep southern subalpine lakes: synthesis, directions and perspectives. Adv. Oceanogr. Limnol. 1:29-66.

Schindler DW, Hecky RE, Findlay DL, Stainton MP, Parker BR, Paterson MJ, Beaty KG, Lyng M, Kasian SEM, 2008. Eutrophication of lakes cannot be controlled by reducing nitrogen input: Results of a 37-year whole-ecosystem experiment. P. Natl. Acad. Sci. USA 105:11254-11258.

Schwefel R, Müller B, Boisgontier H, Wüest A, 2019. Global warming affects nutrient upwelling in deep lakes. Aquat. Sci. 81:50.

Scibona A, Nizzoli D, Cristini D, Longhi D, Bolpagni R, Viaroli P, 2019. Silica storage, fluxes, and nutrient stoichiometry in different benthic primary producer communities in the littoral zone of a deep subalpine lake (Lake Iseo, Italy). Water 11:2140.

Scott AJ, Knott M, 1974. A cluster analysis method for grouping means in the analysis of variance. Biometrics 30:507-512.

Søndergaard M, 2007. Nutrient dynamics in lakes - with emphasis on phosphorus, sediment and lake restorations. DSc Dissertation, University of Aarhus, Denmark.

Søndergaard M, Lauridsen TL, Johansson LS, Jeppesen E, 2017. Nitrogen or phosphorus limitation in lakes and its impact on phytoplankton biomass and submerged macrophyte cover. Hydrobiologia 795:35-48.

Sonzogni WC, Monteith TJ, Bach WN, Hughes VG, 1978. United States Great Lakes tributary loadings. International Reference Group on Great Lakes Pollution from Land Use Activities. International Joint Commission: 187 pp.

Stockwell JD, Doubek JP, Adrian R, Anneville O, Carvalho L, De Senerpont Domis LN, Dur G, Frassl MA, Grossart HP, Ibelings BW, Lajeunesse MJ, Lewandowska AM, Llames ME, Matsuzaki SS, Nodine ER, Nõges P, Patil VP, Pomati F, Rinke K, Rudstam LG, Rusak JA, Salmaso N, Seltmann CT, Straile D, Thackeray SJ, Thiery W, Urrutia-Cordero P, Venail P, Verburg P, Woolway IR, Zohary T, Andersen MR, Bhattacharya R, Hejzlar J, Janatian N, Kpodonu ATNK, Williamson TJ, Wilson H L, 2020. Storm impacts on phytoplankton community dynamics in lakes. Glob. Change Biol. 26:2756- 2784.

Tanentzap AJ, Morabito G, Volta P, Rogora M, Yan ND, Manca M, 2020. Climate warming restructures an aquatic food web over 28 years. Glob. Change Biol. 26:6852- 6866.

Tapolczai K, Anneville O, Padisák J, Salmaso N, Morabito G, Zohary T, Tadonléké RD, Rimet F, 2015. Occurrence and mass development of *Mougeotia* spp. (Zygnemataceae) in large, deep lakes. Hydrobiologia 745:17-29.

Veraart AJ, de Klein JJM, Scheffer M. 2011. Warming can boost denitrification disproportionately due to altered oxygen dynamics. PLoS One 6:e18508.

Verburg P, Hecky RE, Kling H, 2003. Ecological consequences of a century of warming in Lake Tanganyika. Science 301:505-507.

Viaroli P, Azzoni R, Bartoli M, Iacumin Paola, Longhi D, Mosello R, Rogora M, Rossetti G, Salmaso N, Nizzoli D, 2018. Persistence of meromixis and its effects on redox conditions and trophic status in Lake Idro (Southern Alps, Italy). Hydrobiologia 824:51-69.

Yang Y, Colom W, Pierson D, Pettersson K, 2016. Water column stability and summer phytoplankton dynamics in a temperate lake (Lake Erken, Sweden). Inland Waters 6:4, 499-508.

Yankova, Y, Neuenschwander S, Köster O, Posch T, 2017. Abrupt stop of deep water turnover with lake warming: drastic consequences for algal primary producers. Sci. Rep. 7:13770.

Zohary T, Alster A, Hadas O, Obertegger U. 2019. There to stay: invasive filamentous green alga Mougeotia in Lake Kinneret, Israel. Hydrobiologia 831:87-100.

Zuur AF, Ieno EN, Smith GM, 2007. Analysing ecological data. Springer, New York: 672 pp.