

Original Research Article

Post-soviet changes in nitrogen and phosphorus stoichiometry in two large non-stratified lakes and the impact on phytoplankton



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ABSTRACT

The post-soviet period in Eastern Europe brought about fast changes in economy, land use, and environmental protection, whereas legacy effects of the previous era of heavy contamination continued emerging in the status of water bodies. In this paper, we analysed the post-soviet (since 1992) changes in catchment nutrient loadings and stoichiometry of nitrogen (N) and phosphorus (P) in two large non-stratified lakes in Estonia – Võrtsjärv and Peipsi. The drastic reduction in the application of P-fertilisers and P discharges with wastewaters since the early 1990s reduced P loadings and increased N/P loading ratio into both lakes. However, it was hard to find clear evidence of reduced in-lake nutrient concentrations and improved water quality. In both lakes, water transparency constantly decreased and phytoplankton biomass increased. Over the years, the difference in N/P ratio between the two lakes became smaller while the large differences in the cyanobacterial community composition remained. Although common thresholds in nutrient ratios favouring N₂-fixing species could be revealed in both lakes, the phytoplankton in Võrtsjärv, strongly dominated by *Limnothrix* spp., remained mostly light-limited and the relationship with N/P stoichiometry was indirect. Random Forest analysis indicated an important role of light limitation in both lakes. Constantly lower levels of N in the deeper Lake Peipsi favoured N₂-fixing species, which, as a paradox, became P-limited. As climate warming reinforces eutrophication phenomena in lakes by increasing internal nutrient loading and favouring bloom-forming cyanobacteria, more stringent measures would be needed to further limit nutrient loads (especially that of P) to lakes through improved wastewater treatment and increased efficiency of fertiliser application. © 2020 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

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1. Introduction

Long-term changes in nutrient loading and stoichiometry in water bodies reflect changes in agricultural practices and wastewater treatment. Since the early 1990s, many European lakes have experienced a decrease in nutrient loading and a considerable improvement of ecological status (Jeppesen et al., 2005). In contrast, changes in nutrient loading and stoichiometry in the former socialist countries have differed from those in Western Europe. Long-term efforts in Western Europe improved wastewater treatment and reduced phosphorus (P) loading (Kronvang et al., 2005; Jeppesen et al., 2007) while in post-socialist countries, a major nitrogen (N) decline in the 1990s, caused by reduced fertilizer use after the collapse of extensive agriculture, was a characteristic feature (Juhna and Klavins 2001). In Estonia, the application of both N- and P-fertilisers decreased sharply already in the 1980s as a sign of economic instability. According to Järvet et al. (2002), the rate of fertilization was the most important factor determining N runoff from the catchment. In response to the political and economic changes, also the consumption of P fertilizers in Eastern Europe fell sharply (Schoumans et al., 2015). While improved wastewater treatment at point sources is expected to have a fast effect on nutrient loading (Wu and Chen 2013), changes in fertilizer use affect nutrient runoff and in-lake concentrations with a time lag depending on soil saturation level and other retention processes within the catchment (Pärn et al., 2012), legacy stores in lake sediment (Jarvie et al., 2013), and hydraulic retention time of lakes (Søndergaard et al., 2013).

Changes in absolute nutrient loading and stoichiometry caused by these socio-economic transformations affected phytoplankton abundance, species composition and domination structure. For example, in Lake Müggelsee (former German Democratic Republic) where N concentrations responded immediately but P concentrations with a 20-year lag to loading reduction, the formerly dominant Oscillatoriales species disappeared, but the mean biovolume of the N₂-fixing cyanobacteria remained constant, however, were not able to compensate for the N-deficit (Shatwell and Köhler 2019). It was different in another polymictic lake, Lake Balaton (Hungary), where establishing N control by loading reduction was less successful and the total amount of nitrogen fixed by heterocystous cyanobacteria during summer months was more than three times higher than the external load by inflows. (Horváth et al., 2013). Several authors (e.g. Schindler 1977; Smith et al., 1995; Huisman and Hulot 2005; Vrede et al., 2009) have shown that low N/P ratios at generally high nutrient supply support the development of cyanobacteria, and especially N₂-fixing species, many of which form water blooms. Still, several studies have found P alone (Håkanson et al., 2007; Arvola et al., 2011), N alone (Downing et al., 2001; Dolman et al., 2012) or a combination of N and water temperature (Beaulieu et al., 2013) to be better predictors for cyanobacteria biomass than the N/P ratio. In polymictic lakes, synergistic mechanisms may catalyze cyanobacterial blooms once a certain threshold in warming is reached (Bartosiewicz et al., 2019). The question of increasing cyanobacterial dominance of weakly stratified eutrophic north temperate lakes is further blurred by frequent seasonal occurrence of light limitation episodes when neither N nor P are strictly limiting (Aldridge et al., 1995; Burger et al., 2007).

As an example, the present paper reanalyzes long term changes in N and P catchment loadings and stoichiometry in two large non-stratified lakes, Võrtsjärv and Peipsi, at a 25-year distance from the socio-economic change in Estonia in 1991. Lake Peipsi is the 4th largest lake of Europe shared between Estonia and Russia, and Lake Võrtsjärv – a large lake in Estonia located within the Lake Peipsi catchment (Fig. 1). Our prior studies (T. Nõges et al., 2007, 2008, 2010) covering the period 1985–2007, revealed that after the collapse of the Soviet Union in 1991, N loading to the lakes decreased faster than P loading, and the N/P ratio decreased. In both lakes, cyanobacterial blooms ceased during heavy N loading in the 1980s and intensified in Peipsi in the 1990s when N loading decreased (Nõges et al., 2007), causing pronounced summer fish-kills (Kangur et al., 2005). After Estonia became independent and joined the EU in 2004, more attention and funding was allocated to the improvement of sewage treatment. Agricultural activities that saw a major drawback in the 1990s, largely recovered thanks to new supportive measures from the EU, although the use of fertilizers remained far below past peak levels (Statistics Estonia: <http://andmebaas.stat.ee/Index.aspx?lang=en&DataSetCode=PM065>). Both of these developments were expected to have an impact on the nutrient stoichiometry of Peipsi and Võrtsjärv with implications on the ecosystem of these lakes, notably for phytoplankton and cyanobacteria. We thus hypothesized that:

1. Improved sewage treatment has further reduced P discharges and in-lake concentrations in both lakes. In contrast, N loading and in-lake concentrations have returned to increase due to rising agricultural activities.
2. Within the phytoplankton community, we expected to observe a reduction in the total biomass and proportion of bloom-forming cyanobacteria as a result of the assumed increase in N/P ratios of both the riverine discharge and in-lake concentrations.
3. We anticipated that mechanisms controlling phytoplankton composition and abundance in individual lakes differ partly from the effects of variables explaining most of the variance in the two-lake system together.

2. Material and methods

2.1. Study site description

Lake Võrtsjärv (270 km², mean depth 2.8 m) is the largest lake located entirely within the borders of Estonia, while its watershed (3374 km²) forms part of the transboundary catchment (47,800 km²) of Lake Peipsi-Pihkva (Chudsko-Pskovskoe in



Fig. 1. Catchment of Lake Peipsi-Pihkva (including the subcatchment of Lake Võrtsjärv).

Russian) (3555 km², mean depth 7.1 m) (Fig. 1). Peipsi is the northernmost and largest basin of this partitioned lake. More than half (58%) of the total catchment belongs to Russia, 34% to Estonia, 7% to Latvia, and <1% to Byelorussia. The lake is shared between Russia (56%) and Estonia (44%). The Võrtsjärv catchment forms 21% of the Estonian part of the Peipsi catchment (16,323 km²). Võrtsjärv and Lake Pihkva, the southern basin connected to Peipsi through a narrow strait called Lämmijärv, act as “settling ponds” for Peipsi retaining a large part of the catchment nutrient loading. More detailed information on the lakes is given by Nõges and Nõges (2012) for Võrtsjärv and by Kangur et al. (2012) for Peipsi.

The magnitude of nutrient loads and sources has been uncertain for a long time due to poor data availability on the Russian side of the basin (Vassiljev and Stålnacke 2003). Recently a couple of papers based on GIS-modelling (Piirimäe et al., 2015) and analysis of 10-year data of riverine nutrient loads (Blank et al., 2017) has thrown some light upon the source apportionment and trends in nutrient loads in the two parts of the drainage basin. Comparison of two five-year sub-periods (2001–2005 and 2007–2011) by Blank et al. (2017) showed that there was a decrease in the total P (TP) loading from both parts of the catchment being more obvious (24%) in the River Emajõgi, the main inflow from Estonian side, than in the River Velikaya (13%). However, the total N (TN) load from the Russian side decreased significantly (20%) while it remained high and even showed a slight increase (4%) from the Estonian side in the second sub-period. In total, the TP loading to Lake Peipsi-Pihkva decreased by 19% while the TN loading by only 8%. Phytoplankton biomass including that of cyanobacteria decreased in response to the reduced nutrient content in the lake water and both lake parts showed a modest improvement in the ecological status. The slightly diverging loading dynamics in the two sub-basins is likely due to the different source apportionment. As Piirimäe et al. (2015) showed, based on modelling of the period 2006–2010, more than 90% of the P load on the Estonian side came from diffuse sources while point sources were still predominant in the P load from the Russian side. In the present paper, we analysed the nutrient loading data only from the Estonian part of the Peipsi catchment (including the Võrtsjärv catchment) and nutrient concentrations in Võrtsjärv and the Estonian part of Peipsi.

Despite the good connection between the two lakes via the River Emajõgi, their phytoplankton communities differ largely. Võrtsjärv is permanently dominated by thin filamentous cyanobacteria from the genus *Limnothrix* that only occasionally were found in Peipsi. Regular blooms of *Dolichospermum lemmermannii* (Richter) P. Wacklin, L. Hoffmann & J. Komárek described in Võrtsjärv already in the 1910s (Mühlen and Schneider 1920), disappeared in the 1970s and the common potentially bloom forming genera such as *Microcystis*, *Aphanizomenon* and *Dolichospermum* are represented in low numbers. *Gloeotrichia echinulata* P.G. Richter forming blooms in Peipsi was never found in Võrtsjärv. Besides nutrient loading, cyanobacterial dominance is supported by the warming trend (T. Nõges et al., 2010) and atmospheric stilling in the area that has increased the number of stratified days in these polymictic lakes (Woolway et al. 2017, 2019; Janatian et al., 2020).

2.2. Variable description

Herein, we used data on N and P mineral fertiliser application and N and P discharge with Estonian wastewaters for the 1987–2016 time period, obtained from the Statistics Estonia database (<http://pub.stat.ee/px-web.2001/dialog/statfile1.asp> last accessed on April 15, 2020).

Data on riverine N and P loading to Võrtsjärv and Peipsi from the Estonian part of the catchment for the 1992–2016 period was obtained from the Estonian Environment Agency (<http://www.keskkonnaagentuur.ee/et/eesmargid-tegevused/vesi/pinnavesi/jogede-arakande-koormused> last accessed on April 15, 2020). Loading calculations were based on monthly measured nutrient concentrations and daily water discharge data for 5 Võrtsjärv tributaries and 5 Peipsi tributaries.

Daily data on water level and water temperature (WT), as well as precipitation data from the nearest to the lakes meteorological stations (Tõravere for Võrtsjärv and Tiirikoja for Peipsi), were obtained from Estonian Environment Agency. We transformed the absolute water level data to lake specific water levels (WL) by adding the daily water level anomaly (difference from long-term mean) to the long-term mean depths of the lakes.

Monthly May to October data on TN, TP, dissolved inorganic nitrogen (DIN), soluble reactive phosphorus (SRP), Secchi depth (SD), chlorophyll-*a* concentrations (Chl) and phytoplankton in Võrtsjärv and the Estonian part of Peipsi for the period of 1992–2017 were collected under the Estonian State Monitoring Program (<http://seire.keskkonnainfo.ee/> last accessed on April 24, 2018). Samples were taken from pelagic sampling points, three in Võrtsjärv and four in Peipsi. Quantitative phytoplankton samples were fixed with Lugol's iodine and counted under an inverted microscope using the Utermöhl (1958) method (for details, see P. Nõges et al., 2010). We analysed the following phytoplankton variables: total biomass (B), percentages of cyanobacteria and diatoms in total biomass (CY%, BAC%), biomasses of cyanobacterial genera *Gloeotrichia* (G), *Dolichospermum* (D), *Aphanizomenon* (A) and *Microcystis* (M) and their combinations G + D + A for nitrogen fixing species (GDA) and G + D + A + M for bloom-forming species (GDAM).

Under water light conditions were characterized by the relative light intensity in the mixed layer $I_{\text{mix}\%}$ compared to surface irradiance I_0 . We used the equation by Riley (1957) for mixed layer irradiance:

$$I_{\text{mix}} = I_0 \cdot [1 - \exp(-K_d \cdot z)] / K_d \cdot z \quad (1)$$

where K_d is the diffuse attenuation coefficient and z the depth of the mixed layer. Taking I_0 as 100% and expressing (K_d) through its relationship with Secchi depth (SD) in Võrtsjärv (Reinart and Nõges 2004):

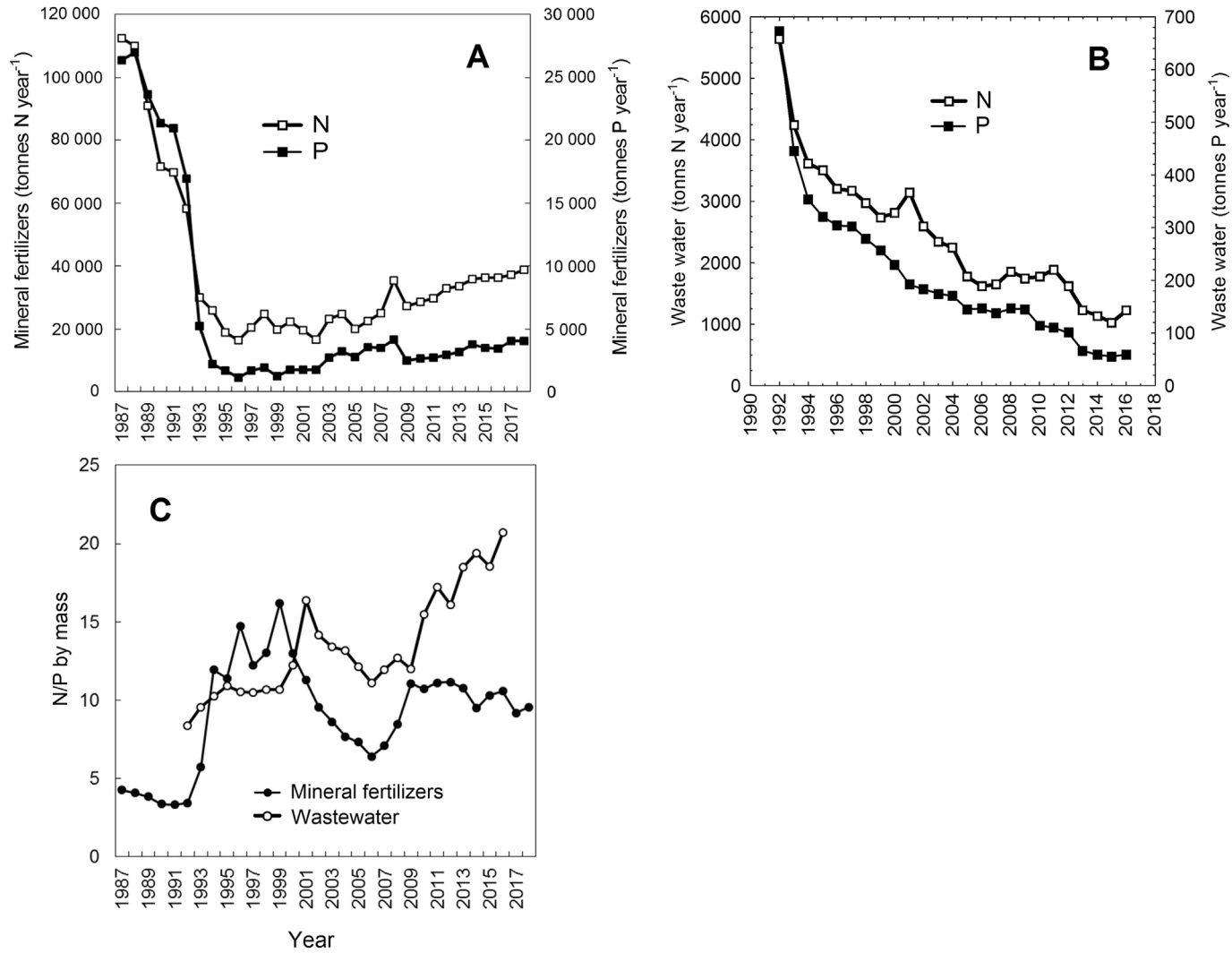


Fig. 2. Changes in the application of N and P fertilisers (A), N and P discharge with wastewaters (B), and the N/P mass ratio in applied fertilisers and discharged wastewaters (C) in Estonia in 1992–2016.

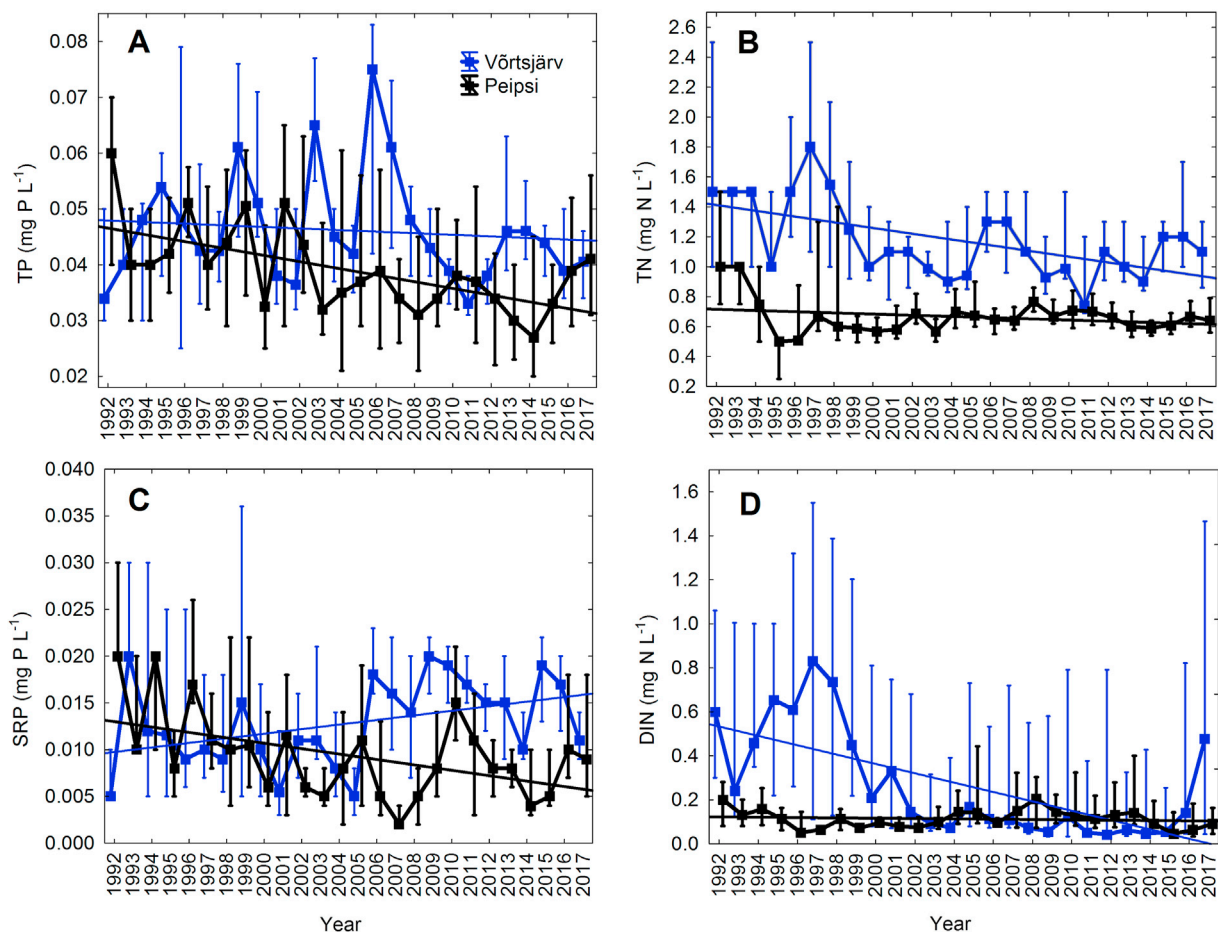


Fig. 3. Median (squares) and quartile (whiskers) concentrations of nutrients in lakes Vörtsjärv and Peipsi in 1992–2017. A – total P (TP); B – total N (TN); C – soluble reactive P (SRP); D – dissolved inorganic N (DIN).

$$K_d = 1.95 SD^{-0.74} \quad (R^2 = 0.89; p < 0.001) \quad (2)$$

and Peipsi (Alikas and Kratzer, 2017)

$$K_d = 4.48 SD^{-1.22} \quad (R^2 = 0.81; p < 0.001) \quad (3)$$

and taking the depth of the mixed layer (z) equal to WL (considering fully mixed conditions in both shallow lakes). SD was measured at each phytoplankton sampling occasion by Secchi disk.

2.3. Data analysis

Pearson correlations were run and time sequence graphs produced by STATISTICA 64 Version 13 (Dell. Inc. 1984–2016). Data on precipitation and nutrient loading were detrended by first difference before estimating correlations. Trends were assessed based on the slope factor and statistical significance of the linear fit to data. Differences between median values were analysed using the Kruskal-Wallis test.

To detect collinearity among the variables, Variance Inflation Factor (VIF) test was used in R package ‘usdm’ with the threshold set at 10 (Miles 2014) and variables which exceeded the threshold value were excluded from further analyses. We tested the significance of constraints via means of 999 permutations under the reduced model. The R-language function `anova.cca` in the `vegan` library was used (Oksanen et al., 2009).

Implementing important procedures in R for Random Forest (“randomForest” package) supplies two reliable measures for each explanatory variable. We used the first measure (%IncMSE), which is related to the mean decrease in model accuracy. It

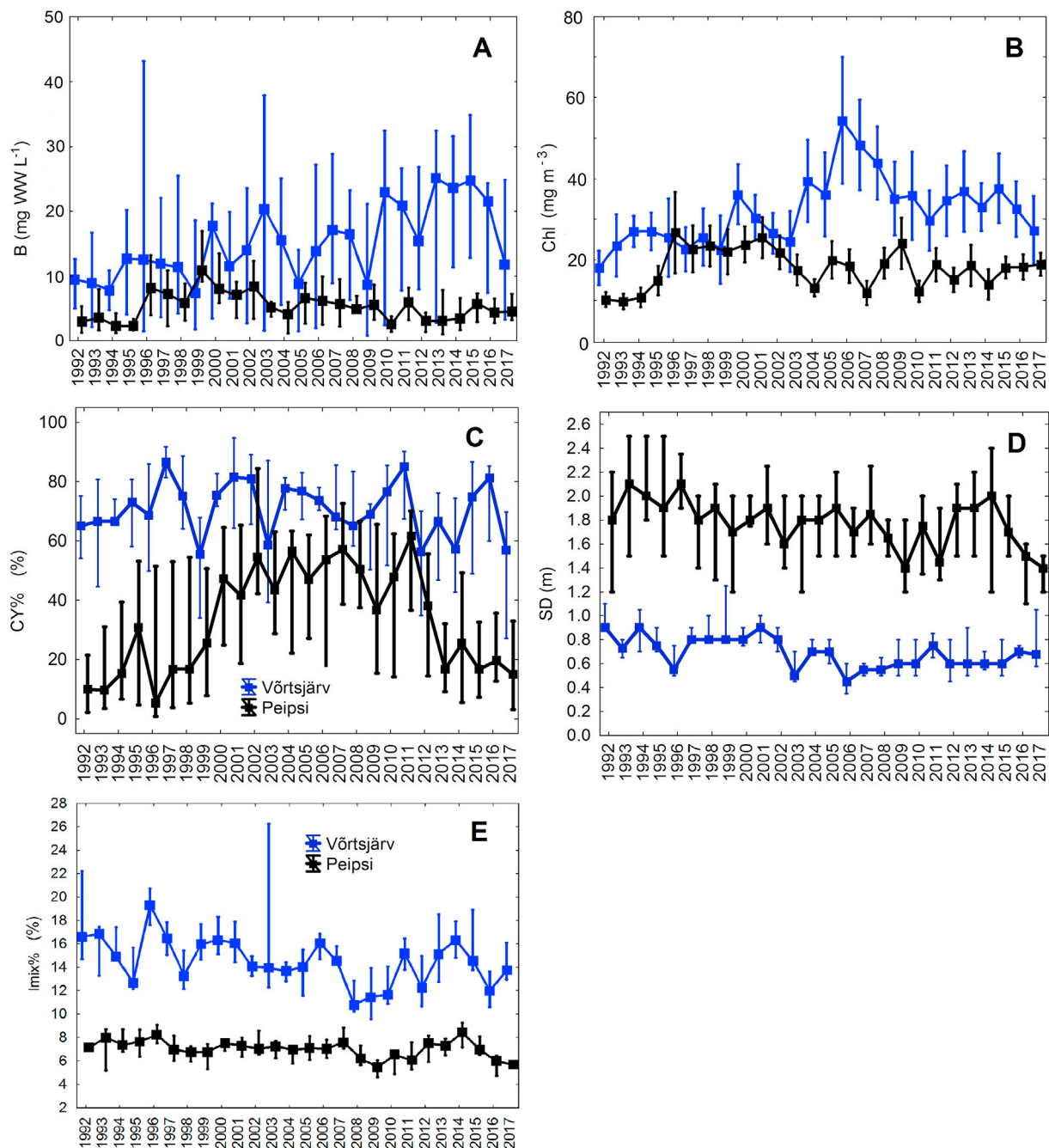


Fig. 4. May–October median values (squares) and quartiles (whiskers) of A – total phytoplankton biomass (B); B – chlorophyll *a* concentration (Chl); C – share of cyanobacteria in total biomass (CY%); D – Secchi depth (SD); and E – light intensity in the mixed layer relative to surface irradiance ($I_{mix\%}$) in lakes Vörtsjärv and Peipsi in 1992–2017.

shows how the model's prediction power gets worse if we drop the high ranked variables from the model (Dewi and Chen, 2019). Three strongest explanatory variables revealed by the RF analysis were included in generalized additive models (GAM function of the mgcv library in R), to explain the behaviour of phytoplankton parameters in the whole system and in the two lakes separately.

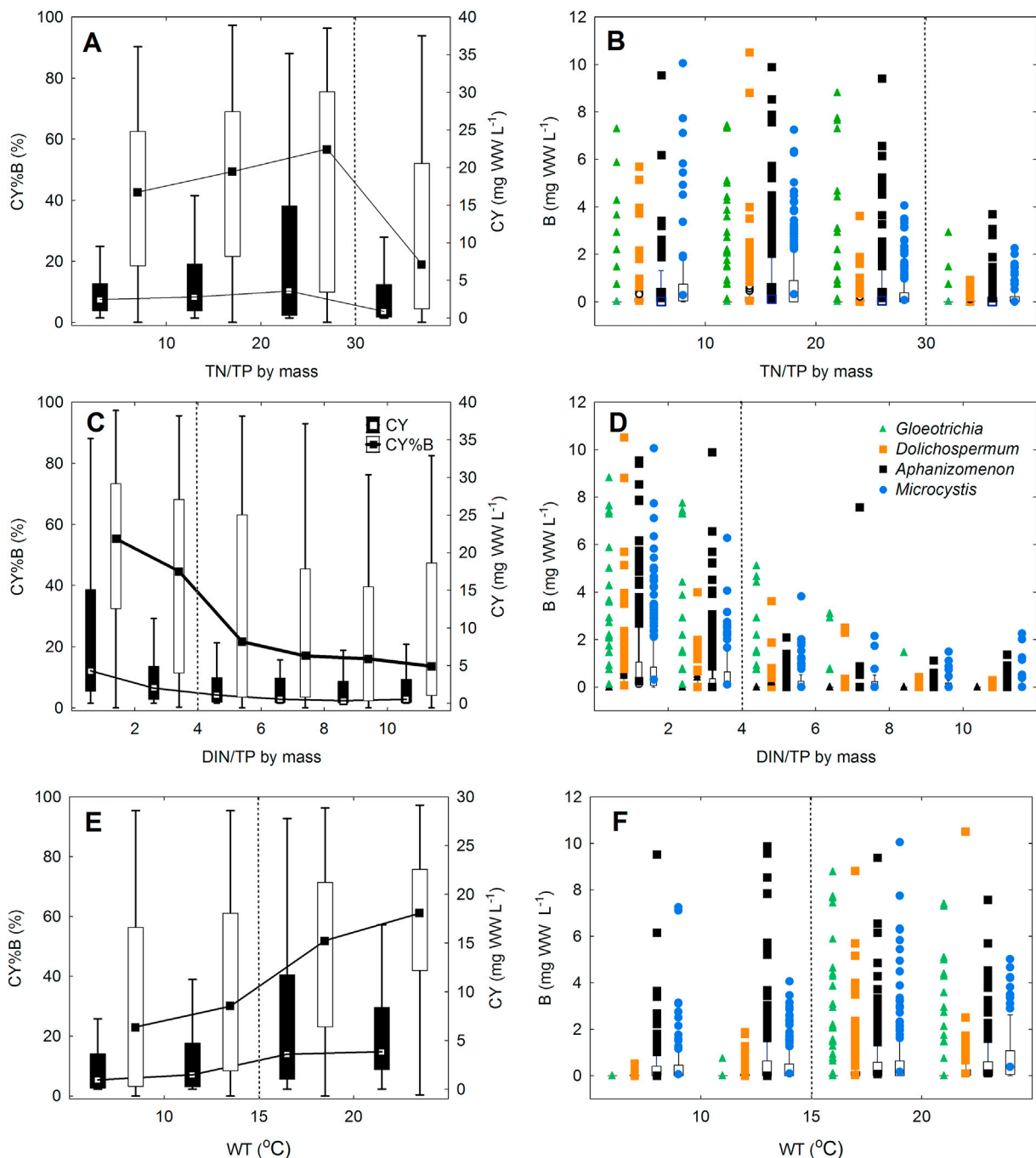


Fig. 5. Dependence of cyanobacteria on nutrient ratios and water temperature over May–October in lakes Võrtsjärv and Peipsi in 1992–2017. Biomass of cyanobacteria (CY), their share in the total phytoplankton biomass (CY%), and biomasses of bloom-forming cyanobacteria vs TN/TP mass ratio (A & B); vs DIN/TP mass ratio (C & D); and vs water temperature (E & F). Critical TN/TP = 30, critical DIN/TP = 4, and critical Temp = 15 °C are indicated by vertical lines. Abbreviations same as in Fig. 3. Box & whiskers plots show median, quartiles and non-outlier ranges.

3. Results

3.1. Trends in N and P loading from Estonian agriculture and wastewaters

The decreasing trend in mineral fertilizer application since its peak in the mid-1980s, continued well into the mid-1990s, resulting in a total 7-fold decrease in the use of N-fertilizers and 24-fold decrease in the use of P-fertilizers by the year 1996

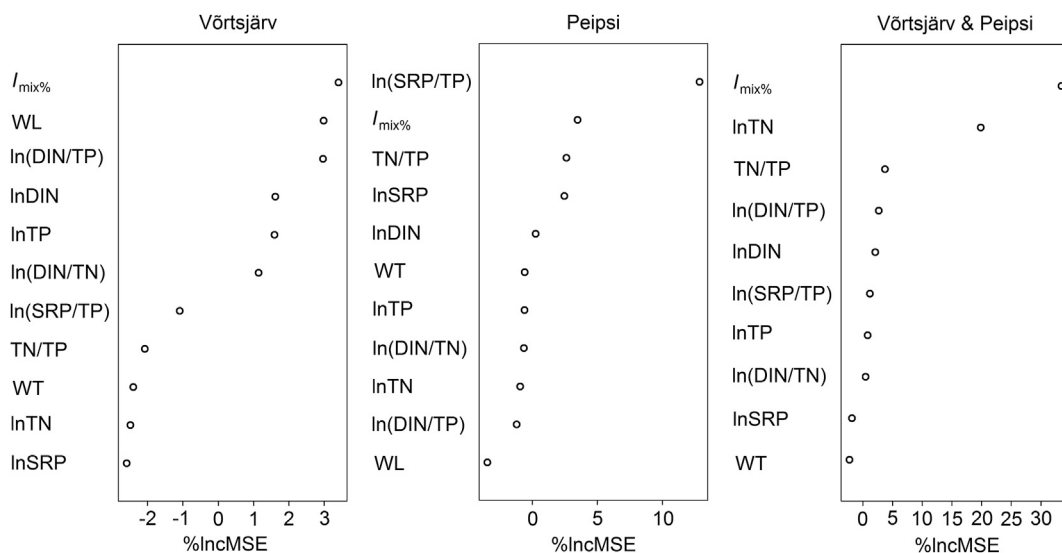


Fig. 6. Random Forest ranking of environmental factors determining chlorophyll concentration in lakes.

(Fig. 2A). Together with the recovery and restructuring of agriculture in the independent Republic of Estonia, however, the application of fertilizers started to slightly increase again, notably so from the early 2000s onwards when Estonia joined the EU and the agricultural subsidies enabled wider use of agrochemicals (Fig. 2A). The decrease in N and P discharge in wastewaters was smoother and has continued up to the present (Fig. 2B). Compared with 1992 values, the present N/P ratio in both fertiliser application and wastewater discharges is more than twofold higher (Fig. 2C).

3.2. Trends in N and P riverine loadings and in-lake concentrations

Besides strong fluctuations in nutrient loadings attributable to variable amount of precipitation (see Table S1 in Supplement 1), a decreasing trend occurred in TP loading to both lakes (here and onward only the Estonian part of the Peipsi catchment considered) (Suppl. Fig. S1A). TN loading to Peipsi increased while no significant trend was found for Vörtsjärv (Suppl. Fig. S1B). Decreasing P loads at increasing or steady N loads led to a substantial increase in the TN/TP loading ratio to both lakes while the increase was faster in Peipsi (Suppl. Fig. S1C).

The in-lake concentrations of TP and TN were lower during the last decade than before (Fig. 3A and B). In mid-2000s, TP in Vörtsjärv was much higher than in Peipsi but by the end of the study period, the concentrations almost equalised at about 40 µg/L in both lakes. TN concentrations in Vörtsjärv have remained about twice as high as those measured in Peipsi. Over the study period, average SRP values decreased in Peipsi but increased in Vörtsjärv (Fig. 3C) while DIN decreased in both lakes (Fig. 3D).

In 1992, the median May–October TN/TP (mass ratio) was 35 in Vörtsjärv and 19 in Peipsi (Suppl. Fig. S2A), while the corresponding DIN/TP mass ratios were 8.4 and 3.3 (Suppl. Fig. S2B). By the 2000s, both ratios decreased substantially in Vörtsjärv and the difference between the two lakes became smaller. Since the 2000s, the DIN/TP ratio in Peipsi surpassed that of Vörtsjärv (Suppl. Fig. S2B).

Median surface water temperature in May–October was by 0.6 °C higher in Vörtsjärv than in Peipsi but in neither lakes had a significant trend (Suppl. Fig. S2C). Still the 25% quartiles representing the temperatures in the three colder months (May,

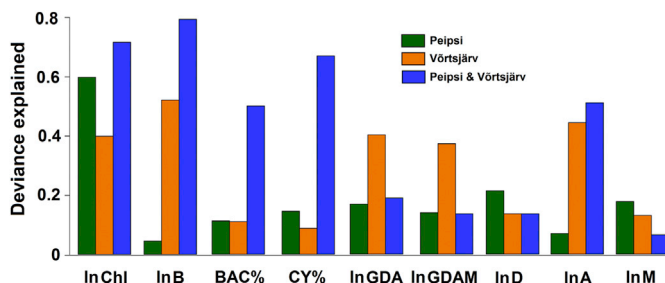


Fig. 7. Explained deviance in phytoplankton variables by GAM models using the top three explanatory variables revealed by Random Forest analysis (see Fig. 6).

September, October) increased significantly ($p < 0.05$) by $0.6\text{ }^{\circ}\text{C}$ per decade in both lakes. As the two lakes are hydrologically connected, their water levels had similar dynamics and amplitude (Suppl. Fig. S2D) but given the more than two-fold difference in mean depths, had a stronger relative impact on lake depth and volume in Võrtsjärv.

3.3. Phytoplankton trends and light conditions in Peipsi and Võrtsjärv

Average May–October phytoplankton biomass and Chl were constantly higher in Võrtsjärv than in Peipsi whereas the values increased over time in Võrtsjärv expanding the discrepancy (Fig. 4A and B). The median share of cyanobacteria in phytoplankton biomass (CY%) exceeded almost permanently 60% in Võrtsjärv but reached close to this value in Peipsi only temporarily in the 2000s remaining around 20% for the rest of the time. (Fig. 4C). Remarkably, the biomasses of *Dolichospermum*, *Aphanizomenon* and *Microcystis* were rather similar in both Peipsi and Võrtsjärv (Suppl. Fig. S3) although surface scums were only very rarely observed in Võrtsjärv but occurred in Peipsi almost every summer. The Secchi depth (SD), initially more than twofold higher in Peipsi than in Võrtsjärv, declined in both lakes but as the decline was faster in Peipsi, the water transparency has been slightly converging over the years (Fig. 4D). Despite lower water transparency in Võrtsjärv, the relative light intensity in the mixed layer ($14.9 \pm 3.1\%$ of the surface irradiance) was more than double of that in the deeper Peipsi ($6.9 \pm 1.4\%$) (Fig. 4E).

3.4. Relationships of the N/P ratio and water temperature with cyanobacterial blooms

Analysing two lakes together, we found that CY% and the total biomass of bloom-forming cyanobacteria *Gloeotrichia*, *Dolichospermum*, *Aphanizomenon* and *Microcystis* (GDAM) were considerably higher at TN/TP mass ratio <30 (Fig. 5 A&B) and at DIN/TP mass ratio <4 (Fig. 5 C&D) than at higher values (Kruskal-Wallis test, $p < 0.00001$). Water temperature over $15\text{ }^{\circ}\text{C}$ considerably enhanced CY% and maximum biomass values of bloom-forming species (Fig. 5 E&F, Kruskal-Wallis test, $p < 0.00001$).

3.5. The RF and GAM model results

To rank the importance of variables explaining phytoplankton dynamics in the lakes, we focused in the RF analysis on Chl as the most common phytoplankton abundance parameter (Fig. 6). $I_{\text{mix}\%}$ was the only among top two ranking explanatory variables for Chl that was common for both lakes separately but also together. In the shallower Võrtsjärv, $I_{\text{mix}\%}$ was followed by the specific water level (WL) and two variables, DIN/TP and DIN, describing nitrogen availability. In Peipsi, three variables related to phosphorus availability (SRP/TP, TN/TP, SRP) occurred among the top 4. If the two lakes were analysed together, the effect of WL was so overwhelming that we excluded it from the graph. The following variables by importance were $I_{\text{mix}\%}$, TN, and TN/TP.

To analyse the effect of the leading factors on the other phytoplankton variables, we applied the GAM model for the top three explanatory variables based on RF results. As the RF ranking was based on Chl, the GAM models for this variable were the strongest explaining between 40 and 71% of the total deviance (Fig. 7, Supplement 2). In Võrtsjärv, the GAM model revealed a significant role of WL also on the total biomass, biomass of *Aphanizomenon* and variables including it (GDA, GDAM). Beside the model for Chl, none of the models were significant in Lake Peipsi. In GAM models for two lakes together, more than 50% of the deviance was explained for the general abundance metrics (Chl, B), the share of the major phytoplankton groups (BAC%, CY%), and for *Aphanizomenon* biomass (Fig. 7). Among explanatory variables, $I_{\text{mix}\%}$ was significant for total biomass, CY %, and *Aphanizomenon* biomass, while TN/TP for *Dolichospermum* biomass and the sum of bloom forming cyanobacteria (GDAM) (see the p-values in Supplement 2).

4. Discussion

We anticipated that improved sewage treatment has further reduced P discharges and in-lake concentrations in both lakes, while N loading and in-lake concentrations have returned to increase due to rising agricultural activities. The drastic reduction in the application of P-fertilisers in Estonia in the early 1990s and the continuing decline in P discharges with wastewaters (Fig. 2A and B) was clearly reflected in P load to both lakes going down over the study period (Suppl. Fig. S1A). The scarce information available for the Russian part of the catchment (Piirimäe et al., 2015; Blank et al., 2017) still suggests that rather similar decreasing trends in nutrient loads have taken place also there. Although the use of P-fertilizers has slightly gone up, the loading followed rather the decreasing trend of P discharged with wastewaters. In N loads to Peipsi (from the Estonian catchment), a major drop from more than 16,000 tonnes to less than 8000 tonnes was observed within just two years from 1990 to 1992 (Nöges et al., 2004, not covered by the present study). Since 1996, the application of N-fertilizers increased again that, despite declining loads with wastewaters, turned N loading to the lakes to an increase. The leading role of agricultural non-point pollution in N loading is confirmed also by the fact that most of the interannual variability of riverine N loading over large areas is explained by precipitation (Sinha and Michalak 2016). The TN/TP loading ratio has been clearly increasing, more strongly for Peipsi than Võrtsjärv (Suppl. Fig. S1C).

Despite decreasing P and increasing N/P in the loadings that, according to the stoichiometric concept (Smith et al., 1995), ought to reduce the risk of cyanobacterial blooms, we failed to detect a clear nutrient reduction and water quality

improvement in the lakes. In Peipsi, this could partly be explained by the large uncertainty of loadings from the Russian portion of the catchment. However, there was no clear response to the reduced loadings to Võrtsjärv either, which has an entirely Estonian catchment with reliable loading data available. This shows the strong inertia of natural systems to changing pressures where the responses are often delayed and follow the trajectories of hysteresis (Scheffer et al., 2001; Scheffer and Carpenter 2003). The inertia could be caused by large legacy P pools stored in lake sediments during the decades of peak loading which, if released, could nullify the expected concentration decreases from loading reduction. The observed increase in water temperature may increase internal loading from the sediment and P recycling rate (Carpenter 2003; Genkai-Kato and Carpenter 2005). Cyanobacteria having higher temperature optima benefit disproportionately from lake warming (Paerl and Huisman 2009). As climate warming reinforces the symptoms of eutrophication (Moss et al., 2011), water quality might not improve in a warmer world despite the efforts to improve the efficiency of wastewater treatment and the use of fertilisers, and to reduce nutrient discharges to water bodies.

Inertia could be observed also regarding nitrogen where the increased N loading to Peipsi was not reflected in the in-lake TN concentrations that, after a period of elevated values in the 1990s, remained rather stable thereafter. Changes in inorganic nutrients, especially the elevated SRP concentrations in Võrtsjärv since 2006 at decreasing TP concentrations, and a concurrent drop in DIN concentrations is likely a hint of phytoplankton switching from P limitation to N limitation in this lake. In Peipsi, instead, this period was characterised by elevated DIN levels giving evidence of weakening N limitation.

Since first published (Redfield 1934, 1958), the so called 'Redfield ratio' has been considered to roughly characterise the assimilation ratio of N and P by phytoplankton (16:1 by atoms and 7:1 by mass) and thus to indicate the potential for nutrient limitation. The concept, though widely criticised throughout the past 85 years, is now applied with precaution considering its limitations. For example, as shown by Klausmeier et al. (2004), N/P tends to be lower during exponential growth of phytoplankton and higher during growth plateaus. Bergström (2010) suggested DIN/TP rather than TN/TP for indicating the limiting nutrient for phytoplankton because dissolved organic N compounds in TN are largely refractory while both dissolved inorganic and organic P forms in TP are bioavailable for phytoplankton. Their analysis on Scandinavian lakes showed that mass ratios TN/TP > 41 and DIN/TP > 3.4 indicated P limitation while TN/TP < 19 and DIN/TP < 1.5 N limitation. For German lakes, Dolman et al. (2016) found N limitation to be most likely at DIN < 100 µg/l and DIN/TP < 1.6 and P limitation at SRP < 10 µg/l and DIN/TP ≥ 1.6. Burson et al. (2018) showed in chemostat experiments with North Sea phytoplankton that increasing N and P loads at constant ratio shifts the competition from nutrients to light. Based on the analyses of 21 years of monitoring data in deep and shallow sites of Lake Champlain, Isles et al. (2017) provided a conceptual model showing that, with continuing climate trends, N/P ratio will decrease and blue-green dominance will increase both in deep and shallow lakes. Applying DIN/TP as a limitation indicator, Isles et al. (2018) showed a considerable strengthening of N limitation (from 32% to 62%) in Swedish headwater streams and nutrient-poor lakes over 25 years.

How does the concept of the importance of the N/P ratio fit with our data? The TN/TP in both lakes is now around 20 (slightly higher in Võrtsjärv and lower in Peipsi) and DIN/TP less than 2, which, according to Bergström (2010), are in the region in which clear N limitation appears. Our analysis shows a step increase of N₂-fixing cyanobacteria in Peipsi and Võrtsjärv already at TN/TP < 30 and DIN/TP < 4 (Fig. 5), which supports the hypothesis of the presence of N limitation in both lakes. In Peipsi Cyanobacteria are, indeed, dominated by N₂-fixing genera *Gloeotrichia*, *Dolichospermum*, *Aphanizomenon* and surface scum forming *Microcystis*, which is highly competitive for reduced inorganic N forms (Blomqvist et al., 1994; Scott and McCarthy, 2010). The period of elevated DIN/TP values in Peipsi (Suppl. Fig. S2B) saw a clear drop in *Gloeotrichia* extremes which likely lost its advantage in the conditions of weakening N limitation.

Still the question remains why we have not seen heavy cyanobacterial blooms in the more eutrophic Võrtsjärv despite even lower DIN/TP ratios than in Peipsi, while they almost regularly occur in the latter. In Võrtsjärv where the biomass and dominance of cyanobacteria is much higher than in Peipsi (Fig. 4), species of Oscillatoriales prevail, which neither form surface scums nor fix N₂. The dominant cyanobacteria in Võrtsjärv, *Limnothrix planktonica* and *L. redekei* (Nöges and Nöges 2012), can tolerate low light and survive in turbid waters (Reynolds 1994). Our recent study (Janatian et al., 2020) revealed even strengthening of light limitation of phytoplankton in this lake since the mid-1990s.

Although both lakes can be categorised as shallow, the RF analysis suggested that the major differences in the phytoplankton abundance (and likely also composition) were accounted for by the different mean depth modifying light conditions in the mixed layer, but also by different nitrogen availability. Peipsi receives waters that have passed through shallow well-mixed hypertrophic lakes, Lake Pihkva for waters from Russia and Võrtsjärv for Estonia, with high N-retention capacity (Nöges et al., 1998; Kangur and Möls 2008). N-retention by these basins guarantees constantly lower N-supply in Peipsi and has led to the development of a strong N₂-fixing community in this lake, which abundance is strongly controlled by SRP availability (Fig. 6).

The most frequent N₂-fixing species in Võrtsjärv, *Aphanizomenon skujae*, is usually occurring in low numbers (compared with the dominating *Limnothrix* spp.), but developed a high peak at the record lowest water levels in Võrtsjärv in 1996 when the light conditions temporarily improved (P. Nöges et al., 2010). Low water levels in Võrtsjärv diminish DIN/TP ratios as wind-induced sediment erosion supports denitrification by bringing nitrate-rich lake water into contact with exposed anoxic sediment layers, releasing N₂ from the lake and supporting anaerobic phosphorus leakage from sediment to water. Hence, the association of low DIN/TP ratio with more N₂-fixers in Võrtsjärv is not necessarily a causal effect of changed nutrient stoichiometry but rather two epiphenomena of changing water levels that affect phytoplankton through light conditions and nutrient stoichiometry through sediment mixing.

In assumingly nutrient-limited Peipsi we observe regular water blooms and a specific sequence of bloom-forming species with *Gloeotrichia* developing earlier in the season at lower nutrient concentrations followed by *Dolichospermum*, *Aphanizomenon* and *Microcystis* (Nõges et al., 2004). The research by Laugaste et al. (2013) confirmed this and showed that in Peipsi high water temperatures favour most strongly *Gloeotrichia* and high phosphorus concentrations support *Microcystis*.

Due to constantly stronger sediment disturbance in Võrtsjärv, its water contains large amounts of detrital particles that shade light and that is reflected in twice smaller Secchi depth and twice higher Chl compared to Peipsi (with the standard method, part of detrital pigments is accounted as Chl). Despite higher turbidity at low water levels, the average light availability in the mixed layer even improves as the dark zone becomes more restricted (Nõges and Järvet 1995). Water level fluctuations of similar range affect Võrtsjärv stronger as they comprise a higher percentage of the water column. Historically, phytoplankton in Võrtsjärv has been light-limited (P. Nõges et al., 2010) and in Peipsi nutrient-limited (Nõges et al., 1996). The present study, however, showed that temporary light limitation is likely also in Peipsi. Despite better water transparency in Peipsi, the calculated $I_{\text{mix}\%}$ was smaller than in Võrtsjärv and was ranked as the second important factor determining phytoplankton abundance in this lake.

Obviously, a retrospective study based on field measurements cannot give a conclusive answer to the question how important is the N/P stoichiometry in determining the community composition of cyanobacteria or the risk of water blooms. It is especially difficult to distinguish between the effects of single nutrients and their stoichiometric ratios. As climate warming reinforces eutrophication by increasing internal nutrient loading and favouring bloom-forming cyanobacteria, the main message for water managers would be to further limit nutrient loads (especially that of P) to lakes through improved wastewater treatment and increased efficiency of fertiliser application.

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.

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Appendix A. Supplementary data

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