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Article

Nutrient control to prevent the occurrence of cyanobacterial blooms in a eutrophic lake in southern Sweden, used for drinking water supply

Jing Li [1], * Lars-Anders Hansson [2] and Kenneth M. Persson [3]

[1,*] Correspondence; Water Resources Engineering, Lund University, jing.li@tvrl.lth.se; Tel:+46 46 222 94 70
[2] Aquatic ecology, Lund University; lars-anders.hansson@biol.lu.se
[3] Water Resources Engineering, Lund University; kenneth_m.persson@tvrl.lth.se

Abstract: Control of nutrients, mainly nitrogen (N) and phosphorus (P), plays a significant role in preventing cyanobacterial blooms (harmful algal blooms (HABs)). This study aimed at evaluating changes in the risk of the occurrence of cyanobacterial blooms and advancing the understanding of how N and P affect the growth of cyanobacteria in a eutrophic lake, Lake Vombsjön, in southern Sweden. Statistical analysis was used to demonstrate the pattern of cyanobacterial blooms, that the highest content present in September and the later that algal blooms occur, the more likely it is a cyanobacterial bloom as cyanobacteria became dominating in October and November (90%). Two hypothesises tested in Lake Vombsjön confirmed namely that a high total phosphorus (TP) level correlates with an abundance of cyanobacteria and that low N:P ratio (total nitrogen/total phosphorus < 20) favours the growth of cyanobacteria. To control the growth of cyanobacteria in Lake Vombsjön, the TP level should be kept below 20 µg/L and the N:P ratio be maintained at a level of over 20. The two species *Planktothrix agardhii*, and *Pseudanabaena spp.* should be carefully monitored especially in late autumn. Future work should consider any high degree of leakage from the sediment of the dissolved phosphorus available there.

Keywords: phosphorus; N:P ratio ; cyanobacteria; *Planktothrix agardhii*; Lake Vombsjön

1. Introduction

Harmful algae blooms (HABs), specifically those caused by cyanobacteria, have become one of the most critical concerns for drinking water supply, as well as for maintaining the ecological and economic sustainability of freshwater ecosystems worldwide [1](p.13) [2](p.57). Eutrophication is the major process stimulating the growth of algal and cyanobacterial biomasses, the key factors here being the maintaining of a high degree of availability of important nutrients, such as phosphorus (P) and nitrogen (N), and also a high P/N ratio[3] (pp.5-6), [4] (pp.11-14). Cyanobacterial blooms are predicted to become even more common as climate warming proceeds[5](p.1),[2](p.57), recent studies having also demonstrated that synergies between climate warming and increasing levels of runoff of humic substances are able to trigger an increase in cyanobacteria[6](p.3), as well as a reduction in the biodiversity of phytoplankton [7](pp.137-138), [8](1290-1301). Accordingly, both global and local scale adaptive management tools are important for being able to meet future challenges and secure water resources and adequate functioning of the ecosystem [7](pp.130-133).

Regarding drinking water supply, major reasons for the strong impact of cyanobacterial blooms are that too large amounts of algae tend to clog the treatment process, cause unpleasant smells, and also that many cyanobacteria are toxic [8](p.1291), [9], [10](pp.128–144) or are difficult to remove[11](pp.1705-1714), even there is a risk in the sludge management[12](pp.1192-1200). Nearly eutrophic freshwater bodies contain toxic cyanobacteria as a result of eutrophication, the size of cyanobacterial populations are also being dependent upon such environmental conditions, temperature, mixing regimes, transparency, and the availability of iron or carbon [13](pp.572-576),[14](pp.5-6),[15](pp.485–498),[16](pp. 297–309).

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Hence, to meet future challenges in regard to ecosystem services, such as the providing of clean drinking water, it is highly important to reduce the nutrient input to aquatic ecosystems [17](1051–1059),[18](1901–1916),[19](p.217). Phosphorus is commonly considered to be the limiting nutrient in freshwater ecosystems[20](pp.313-334), high concentrations of P often co-occurring with severe cyanobacterial blooms in many regions of the world, such as in the Great Lakes, in the U.S and Taihu Lake, in China [21],[22](pp. 760–773). To reduce the probability of HABs biomass concentrations reaching the WHO Alert Level 1 (1 mg/l), a TP concentration of below 20 µg/L for Swedish lakes, for example[23](pp.2345-2363).

Generally, phosphorus is the main limiting nutrient for cyanobacterial growth in freshwaters, although nitrogen is sometimes limiting too, since it is a quantitatively important bio-element [24](pp.411-425). However, contrary to most other planktonic algae, some cyanobacteria are able to fix atmospheric nitrogen in a nitrogen-limiting situation, which can lead to a lack of nitrates or of ammonia, and in turn to a dominance of N_2-fixing cyanobacteria such as *Anabaena spp.* and *Aphanizomenon spp.* [25](p.25). The drop of dissolved inorganic nitrogen (DIN) correlated to the increase of N_2 fixation rates and *Aphanizomenon* abundance in a eutrophic lake (Lake Mendota, Wisconsin, USA)[26]. Another example is that under nitrogen-limiting conditions the levels of various common toxic cyanobacteria, such as *Microcystis aeruginosa* which are incapable of fixing N_2, declines in the biomass which is present, whereas the level of these bacteria increases again as nitrogen becomes more available [27](pp.188-198).

Because of differences in the N_2 fixation capabilities involved, many studies have examined interactions between cyanobacterial blooms and the nutrients available to them. For example, in Taihu Lake in China, the availability of P has been found to control the pre-bloom conditions of Microcystis spp. during the spring months and the availability of N control the blooms of this species during the summers, the thresholds of the limitations of TN and TP that are targeted being below 0.80 mg/l and below 0.05 mg/l, respectively [17](1051-1059). In addition to the absolute concentrations of the nutrients, the ratio of nitrogen to phosphorus (the N:P ratio) has been considered to be one of the main parameters in determining cyanobacterial growth [28](pp.260-262),[4][5](pp.11-14). A high concentration of P and a low N:P ratio tend to favour the development of cyanobacterial blooms [29](pp. 669–671), since for some species nitrogen deficits may be compensated for by nitrogen fixation in the atmosphere. However, a low N:P ratio does not always lead to nitrogen limiting situations and to nitrogen fixation, since it is also affected by the phytoplankton biomass and by the nitrogen concentration[4](pp.11-14). Hence, a low N:P ratio alone does not suffice for appreciably limiting the development of cyanobacterial blooms. However, keeping the absolute concentrations of N and P at low levels and combined with this, maintaining a high TN:TP ratio (one higher than 30), reduces appreciably the risks of cyanobacterial blooms appearing[30](pp.379-390).

From both a research and a management perspective, it is highly important to identify and control environmental processes that have the potential of driving and sustaining cyanobacterial blooms [31](pp.1-17). One possibility if achieving this is through use of system analytic approaches of different kinds, such as CMT (cyanobacteria management tools), which define the monitoring levels both of the nutrient concentrations, in the biomass of cyanobacteria and of concentrations of the cyanotoxin [23](pp.2363-2365).

In order to obtain a better understanding of how nutrient concentrations and nutrient ratios affect the frequency and intensity of cyanobacterial blooms and the ecosystem services provided by lakes, long-term data for Lake Vombsjön, in southern Sweden, which serves as a raw water resource for more than 500 000 consumers, were collected and evaluated. We examined seasonal, as well as long-term trends in nutrient levels, in N:P ratios, in phytoplankton species compositions and in their interactions. The rationale for the study was to use a model lake, Lake Vombsjön, to be able to obtain a thorough going and general understanding of the formation of cyanobacterial harmful algal blooms, one that could serve as a basis for decision support and for the management of a eutrophic lake.

After it has been observed by Redfield in 1958 that phytoplankton generally have a molecular C:N:P ratio of 106:16:1 (the ratio by weight being 50:7:1), the use of elemental ratios became

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widespread in marine and freshwater phytoplankton studies. Although the Redfield ratio generally averages 16, local variations, within a range of 5 to 34 are possible [32](pp.1-17). Deviations from a stable molecular ratio have been assumed to suggest nutrient deficiency and N:P ratios of less than 22 have been suggested to favor cyanobacteria [30]. We employed system analysis and statistical analysis in attempt to identify the growth mechanisms behind algal blooms and discover seasonal patterns of phytoplankton and the interaction of these mechanisms with nutrient availability and with species variations. By using a long-term time series from a eutrophic lake in southern Sweden as model system, we tested specifically the hypothesis that periods involving large amounts of phosphorus (P) and low N:P ratios (<20) correlate with a high frequency of cyanobacterial blooms.

2. Materials and Methods

Lake Vombsjön is part of Kävlingeån River's catchment area (Fig.1). It is situated 20 km east of the city of Lund. The main type of land use within the catchment area are those of agriculture (72%) and forestry (23%), whereas urban living areas (3%) and lakes (2%) cover only a minor portion of the area. The lake has a surface area is approximately 12 km^2 and an average depth and a maximum depths of 9.4 and 16.0 m, respectively [33] as well as a turnover time of 1.04 years. The main inflow to the lake is that from the Björkaån River (76%). Some 20% of the water flow (of 31 Mm^3/yr) from Lake Vombsjön is used for drinking water, processed in the water treatment plant there (the Vombverket, belonging to the firm Sydvatten).

Due to Lake Vombsjön being located in what is both an agricultural and a highly populated region, the water quality of the lake suffers considerably from the leaching of nutrients and pesticides into it from the wastewater produced and from the agriculture carried on. It has been found that more than 85% of the external phosphorus and nitrogen that come into Lake Vombsjön are from agricultural activities (SMHI 2015)[34]. The accumulation of large amounts of nutrients in the lake sediments have also become a challenge for nutrient management of the lake.

Data on nutrients found to be present in water at the outlet of Lake Vombsjön from the year 1990 to 2016 was collected within the framework of regional and national environmental water recipient monitoring program termed the Vattenanknuten recipientkontroll program [35], considered by the Kävlingeåns Water Protection Agency [36].

Data from the year 1989-2010 on phytoplankton data was provided by the county board of Scania and was analysed by use of Utermöhl 1958 method for quantitative counting of phytoplankton [37](pp.143-170). Data at this sort for the year 2016 was provided by the firm Sydvatten, one of the largest water suppliers in southern Sweden. Samples of the incoming water that the drinking water treatment plant in the area (the Vombverket plant) received were taken.

3. Results and Discussion

3.1 Seasonal pattern of cyanobacteria

Temporal nutrient trends in the main source of water inflow to Lake Vombsjön, the Björkaån River, have shown there to be a slightly increasing trend in the inflow of both phosphorus and nitrogen concentrations during recent decades (Fig. 2a). In contrast, there has been a decreasing trend in both TN and TP concentrations at the main outlet of Lake Vombsjön, although the levels there have still remained high with average concentrations of around 1000 μg/l and 55 μg/l, respectively, being currently obtained (Fig. 2b). Thus, very large amounts of nutrients remain in the lake, as can be seen as in Fig. 2c, which also shows there to be for nutrients to accumulate in the lake. The average for the period reported on here shows that there can still be a phosphorus deposition in the sediment of the lake of just under 1 ton of phosphorus per year. For the internal load such produced, the stock of phosphorus created thus far was estimated to be one of around 590 tons altogether, some of which, 38% about 226 tons involve three P fractions, which are not stable but instead can readily release phosphorus into the water [38](p.21). The TN load has thus reached some 914 tons per year, 96% of it being from diffuse sources, specifically from agriculture, from forest and from pastures [39](pp.129-136). The large amounts of nutrients involved a considerable opportunity for algae to grow. Accordingly, heavy algal blooms have been observed in Lake Vombsjön almost every summer since the 1970s.

There is a clear seasonal pattern in the cyanobacterial development that takes place, which often start with a bloom sometime in the spring, followed by an increase most marked during July and August, its generally peaking in September and then declining (Fig. 3a). The red line in Figure 3a represents the WHO alert level 1 (a level of 1 mg/l), pertaining to the health risks that the presence of cyanobacteria create [1]. More than 50% of the samples obtained during the past 26 years have been of risk to health. Although the definition of what a bloom represents differs, we let the presence in a given biomass of more than 1 mg/l of cyanobacteria serve as a definition here as a bloom. Since in general 50-75% of algal blooms are toxic[40](p.1), this also means that raw water taken in from the lake for drinking water purposes is frequently toxic during bloom seasons. The later in the season a bloom occurs, the more likely to be dominated by cyanobacteria (Fig. 3b). During the period of September to November the percentage of cyanobacteria present in the phytoplankton community is often above 80% (Fig. 3b). This suggests that special attention should be paid to algal blooms in late autumn.

In accordance with what we had hypothesized, we found periods in which there are large amounts of phosphorus and the TN:TP ratio in low (<20) to correlate with the biomasses involved contains large amount of cyanobacteria (Figs. 4 and 5), which also suggests since our study took place in Lake Vombsjön, that phosphorus is a limiting nutrient there. It can thus be recommended that in order to ensure that there be a low probability of a cyanobacterial bloom, the concentration of phosphorus should not exceed 20 μg/L [23].

Since many cyanobacteria species are nitrogen fixing, they can retrieve nitrogen from the air during periods when the amount of nitrogen in the water are limited. Hence, for nutrient management purpose, one should not implement nitrogen reduction measures without a simultaneous decrease in concentration of phosphorus [41](p.77-86). In a Swedish national research project entitled WATERS aimed at obtaining a dataset for the ecological assessment of Swedish water bodies, it was found that levels of TP > 20 μg/l or of TN> 500 μg/l indicate there to be a risk of health-related problems connected with the cyanobacteria found in Swedish lakes[42](p.40). In line with this, one can conclude that Lake Vombsjön is not in a healthy lake since the TP level there about 2 times as high and the TN 4 times as high as level that is recommended, suggesting the conditions present to promote the development of cyanobacterial blooms, thus implying there to be a health risk in connection with the ecosystem services provided, specifically that of supplying drinking water.

The long-term time series of samples from Lake Vombsjön also demonstrates there to be a clear seasonal pattern in terms of the TN:TP ratio being lower during the summer and autumn than during the rest of the year (Fig. 6), and accordingly, the summer and autumn period periods being dominated by cyanobacteria (Fig. 3b). The highest biomass level for the cyanobacteria generally occur in September and correspond to the lowest TN:TP ratios being found during that month. In line with this, high cyanobacterial biomass levels are strongly correlated in temporal terms with the occurrence of low TN and low TN:TP ratio, as well as with high TP levels (Figs. 4 and 5; and Table 1) The very clear increase in phosphorus concentrations at the outflow of the lake (Fig. 7), suggests that a likely reason for the seasonal pattern in the TN:TP ratios that are found is that of phosphorus leakage (resulting in internal phosphorus loading) stemming the phosphorus sediments in the lake, leading to a decline in the TN:TP ratio during the summer and autumn (Fig. 7). The likelihood of this is further strengthened by the elevated phosphate fraction found in the lake during the late summer and the fall (Fig. S2). The internal loading of phosphorus from the sediments can thus provide appropriate growth conditions for N_2-fixing cyanobacterial species.

3.2 Cyanobacteria's species

Which cyanobacteria species are dominant can vary from year to year, although the most frequent species occurring in the blooms found in Lake Vombsjön are *Planktotrix agardhii*, *Cyclotella spp.* and *Aulacoseira spp.*, followed by several species of *Microcystis* (Table 2). The dominant cyanobacterial group during booms (biomass >1 mg/l) was found to vary during the period of 1989-2016, as shown in Fig.8. *Planktothrix agardhii*, which is dominant in many shallow eutrophic lakes, including Lake Vombsjön, can produce hepatotoxic microcystins. During some years cyanobacterial blooms were found to have included considerable amounts of diatom species, such as in 1993, 1998, 1999, 2001 and 2002. The dominance of *Pseudanabaena* that could be noted in 2016 should receive continuing attention in the future, since some species of *Pseudanabaena*, such as *P. tenuis* can produce substances that generate acute toxicity in neonates of *Daphnia magna* and *Ceriodaphnia dubia* [43].

Table 1. Correlations between concentration of cyanobacteria and TN, TP and TN:TP in Lake Vombsjön, and resulting P-values.

Item	TN	TP	TN:TP
Correlation Coefficient	-0.56	0.58	-0.63
P-Values	0.001	0.001	0.001

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Table 2. Types of cyanobacteria found to be most frequent in Lake Vombsjön during the period of 1989 to 2002.

Types	Number of times between 1989 to 2002 that their concentration was found to exceed 1mg/l
Planktothrix agardhii	29
Cyclotella	21
Aulacoseira	20
Ceratium hirundinella	18
Microcystis viridis	16
Microcystis wesenbergii	12
Microcystis aeruginosa	11
Stephanodiscus	9
Microcystis flos-aquae	7

4. Conclusions

To conclude, once can say that reducing the amount of phosphorus that is present either as TP or as phosphate represents a sound prevention practice for reducing the risk of cyanobacterial blooms developing. A recommendation in this respect is that TP be kept at a level below that of 20 μg/l. A lesson to be learned from Lake Constance is of the TP level there being reduced over a 15 year period from 80 μg/l to a level of less than 20 μg/l, through international cooperation and investments totaling over six billion Swiss francs for construction and modernization of sewage canals and 220 water treatment plants. This is an inspiring story that can be used to motivate Lake Vomsbjön management teams to take effective measures in this respect in the coming decades. There are many options for obtaining control over the P levels such as increasing the efficiency of the phosphorus uptake by crops in the farmland, recycling and reusing large amounts of phosphorus in water bodies, and promoting efficient wastewater treatment, especially at individual wastewater treatment plants around Lake Vombsjön, which of course requires the involvement and investment of multi stakeholders. We show that control not only of the phosphorus level but also of the N:P ratio is important for the prevention of cyanobacterial bloom and that a low N:P ratio tends to trigger cyanobacterial blooms in Lake Vombsjön. We found that an N:P ratio of below 20 should be regarded as an indicator of a potentially high risk of cyanobacterial growth. Analysis of the long-term dataset obtained for Lake Vombsjön indicated to us the importance of monitoring cyanobacterial bloom during the period of July to November, in particular, above all September. Careful attention is also called for as regards the potential impact of certain bioactive compounds, such as those present in *Planktothrix agardhii* and *Pseudanabaena spp.*. Future work should also consider the effects of the hydrodynamic conditions present in the lake and the effects of the internal loading of phosphorus on the development of cyanobacterial blooms.

Acknowledgments: This work was mainly executed during Young Scientist Summer Program 2017 at IIASA with support by FORMAS, Sweden. Many thanks to Professor Peter Burek for his kind supervision and the whole WATER group at IIASA for their support. This project is also financially supported by Sydvatten and Sweden Water Research.

Author Contributions: Jing Li was contributing to the project design, data sourcing, data analyzing, results formulating and article writing. Professor Kenneth M Persson was involved in the project idea discussion and project supervision. Professor Lars-Anders Hansson was involved in results discussion and manuscript polishing.

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A: Figures

Figure 1. Location of Lake Vombsjön in Scania

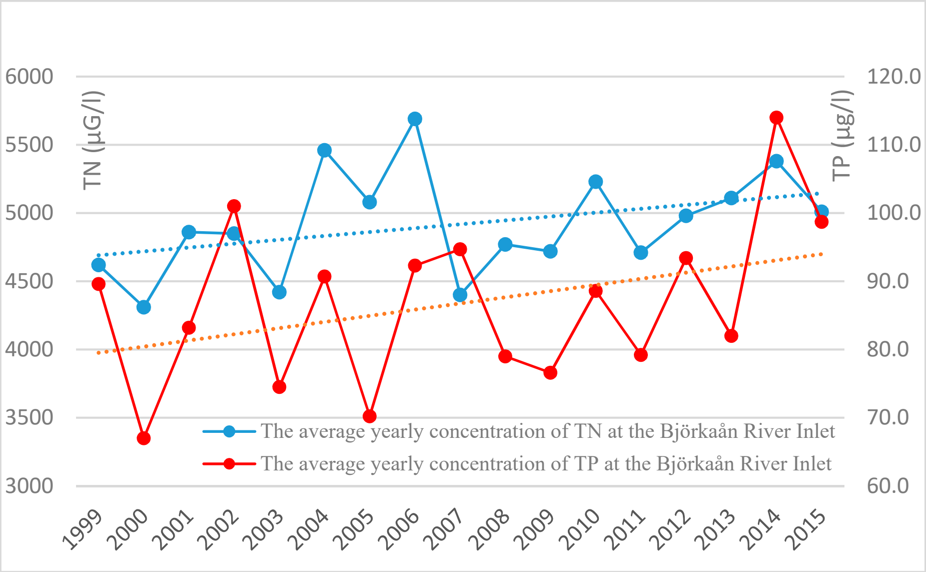

Figure 2a. The yearly total phosphorus and total nitrogen trends at the inlet of the Björkaån River.

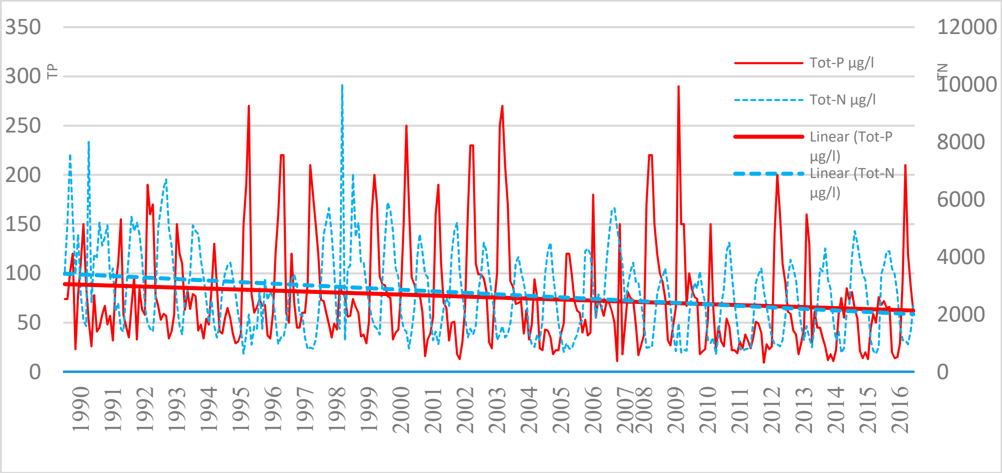

Figure 2b General trends of changes (in terms of µg/l) in the concentration of Tot-P and Tot-N at the outlet of Lake Vombsjön

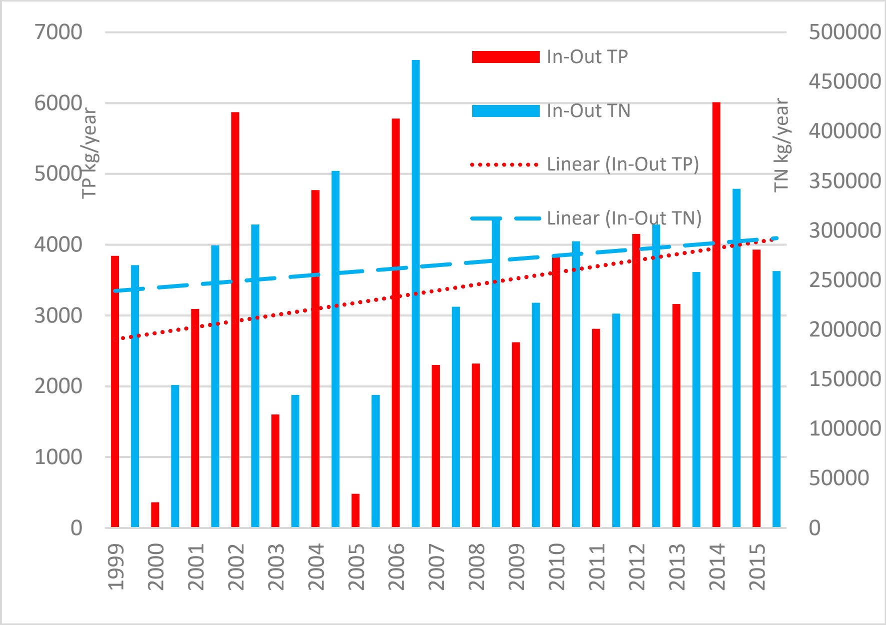

Figure 2c. Yearly differences between the main inlet and the outlet of Lake Vombsjön during the period of 1999-2015 as regards yearly TP and TN transport (measured in terms of kg/year)

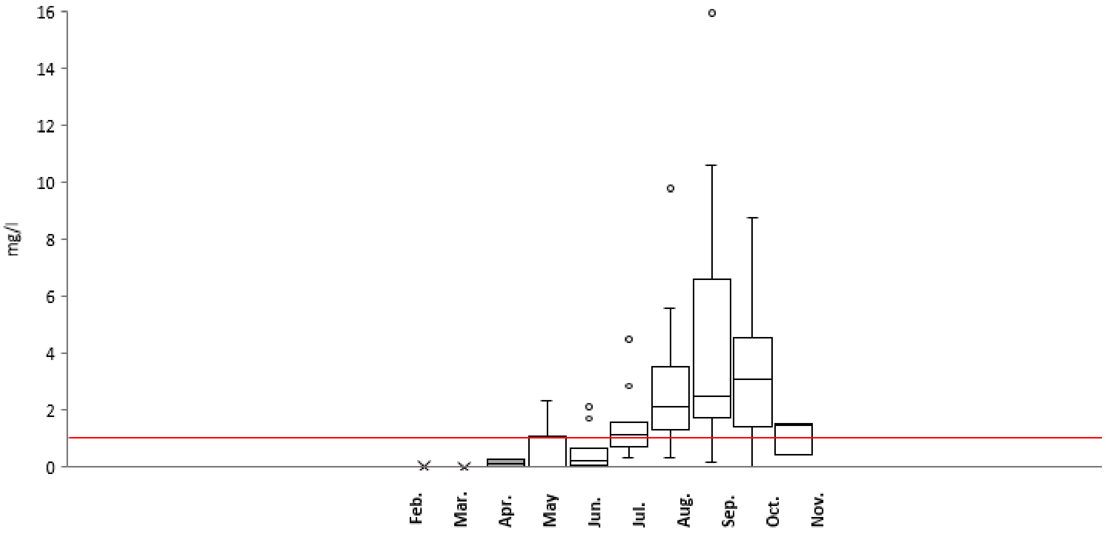

Fig. 3a. Seasonal patterns of the presence of cyanobacteria in Lake Vombsjön during1989 to 2002

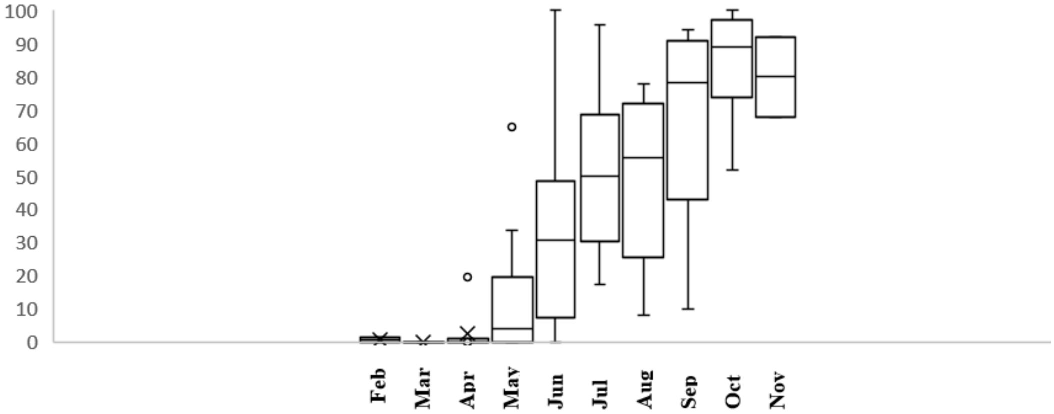

Figure 3b. Seasonal patterns of percentages of cyanobacteria belongs to the Phytoplankton group found in Lake Vombsjön 1989-2002

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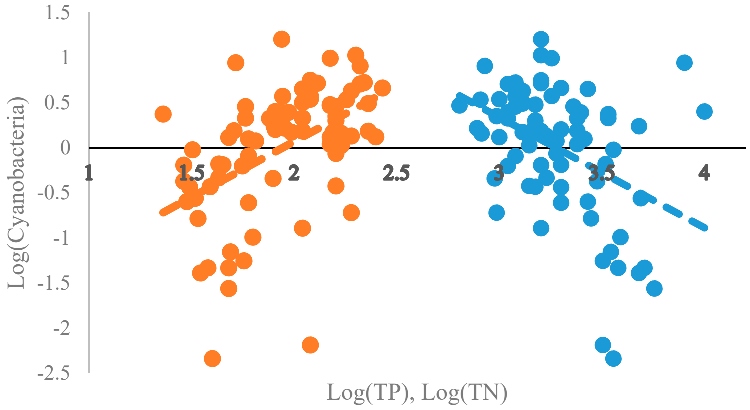

Figure 4. The relationship between log (Cyanobacteria) and log (TN) and log (TP)

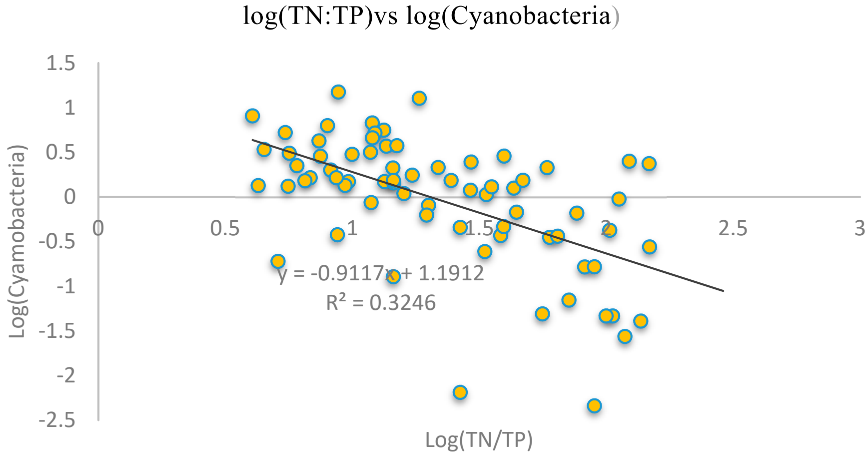

Figure 5. Plot of log (N:P) in the relation to log (cyanobacteria) for the period of 1990 to 2002

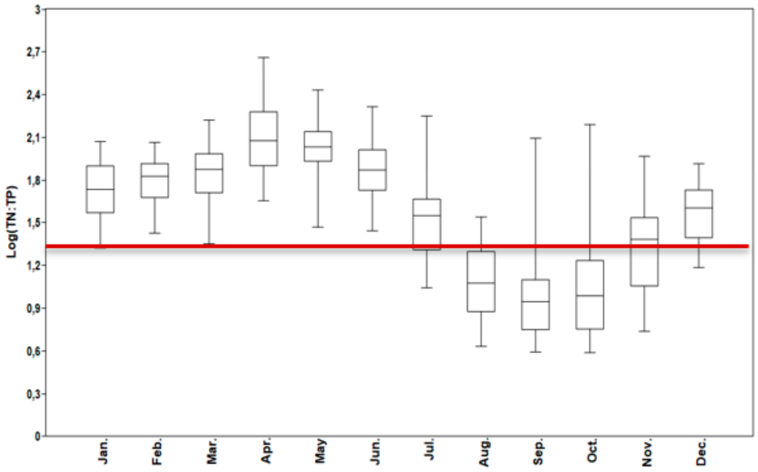

Figure 6. Seasonal patterns of TN:TP ratios at the outlet of Lake Vombsjön; monthly Biplot results for the period of 1999 to 2002

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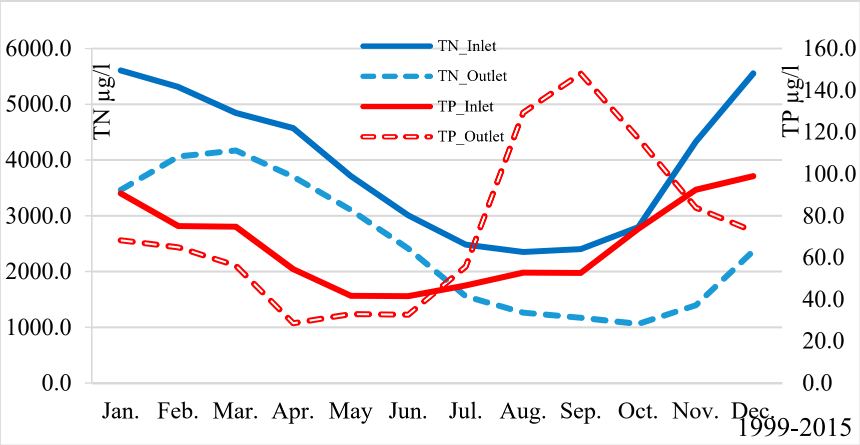


Figure 7. Average monthly values for the amount of TN and TP found in the main inlet and the main outlet of Lake Vombsjön during the period of 1999 to 2015.

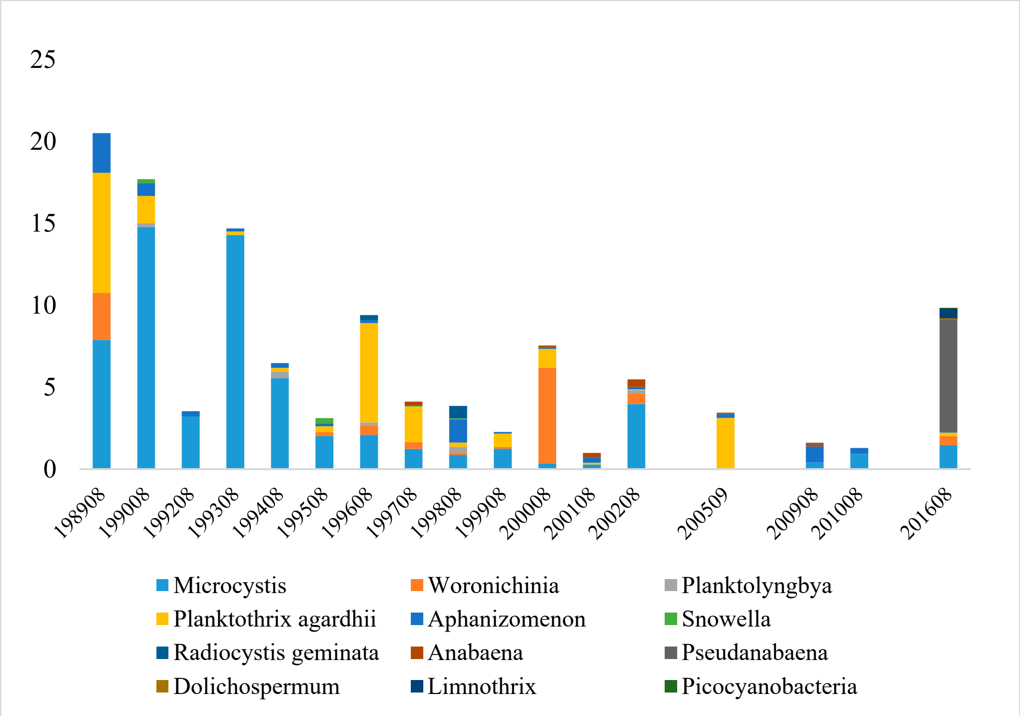
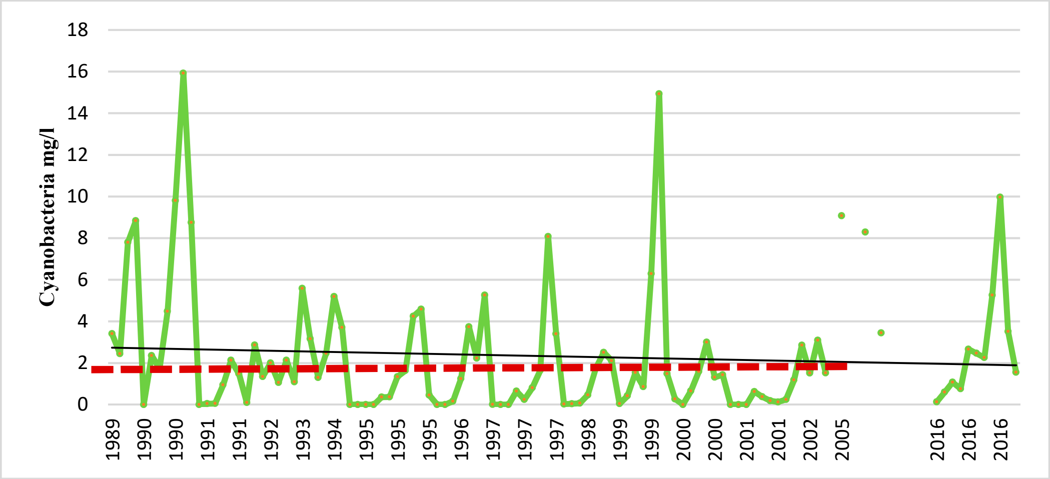
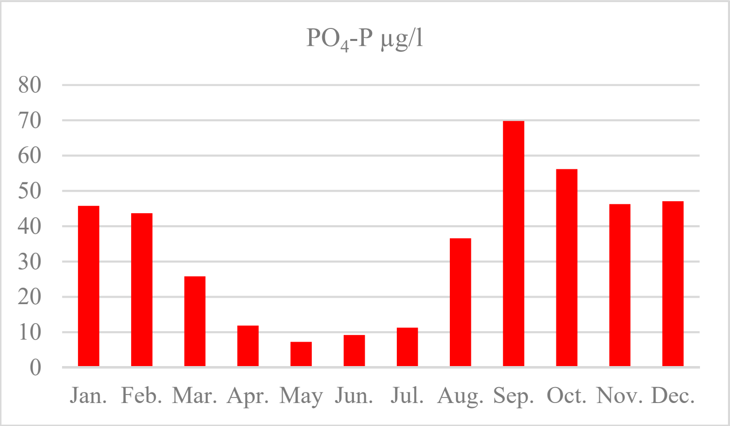


Figure 8. Variations from one year to another during the period of 1989-2016 in the frequency of different cyanobacterial groups found to be the dominate ones during blooms (periods in which amount of biomass >1 mg/l)

Appendix B: Supplementary figures



Supplementary Figure 1. Distribution of the cyanobacteria biomass distribution during the period of 1989-2016



Supplementary Figure 2. The average monthly dissolved phosphorus concentrations during the period of 1999-2015.

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