


# Phytoplankton functional composition shows higher seasonal variability in a large shallow lake after a eutrophic past

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**Abstract.** Eutrophication is a well-known problem of global proportions with some easily recognizable and potentially harmful effects on aquatic habitats, but our knowledge on the underlying associated changes in ecosystem functioning is rather limited. Relevant studies suggest that seasonal variability in the functional composition of phytoplankton shows an increase as eutrophication progresses. The aim of the present study was to test this hypothesis through the assessment of long-term changes in the functional diversity and composition of phytoplankton in a large shallow lake situated in Central Europe with a history of dramatic changes in trophic state. Contrary to our expectations, results have shown that the maximum range of compositional variability had a significant negative correlation with the summer biomass maxima. On the other hand, average seasonal variability measured as annual beta diversity exhibited an increasing trend throughout the years from the period of early eutrophication to the recent period of reoligotrophication, seemingly following a decline in functional richness and a long-term rise in annual mean water temperature. The enhanced variability in phytoplankton succession implies that all the ecosystem processes connected to the phytoplankton follow more complex seasonal dynamics. Besides changing community structure, trophic state also seems to be an important factor in setting the limits to compositional changes during the annual cycle, whereas long-term warming is likely to enhance instability in the phytoplankton. The trajectories of these two factors and the changes in seasonal succession indicate a lake in transition, urging more in-depth research efforts to understand the impact of climate change on this specific ecosystem, and raise the question of whether the observed changes can also occur in other similar aquatic systems.

**Key words:** eutrophication; functional diversity; functional traits; long-term changes; phytoplankton; seasonal variability; temperature; trophic state; water depth.

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## INTRODUCTION

Anthropogenic eutrophication has long been recognized as a global problem with deleterious effects on the ecological status of aquatic habitats (Smith et al. 1999, Chislock et al. 2013). The phenomenon is caused by the increase in external nutrient supply, which in turn induces higher phytoplankton biomass and a higher risk of harmful algal blooms (Smith 2003). A frequently

occurring symptom of eutrophic conditions is the prominent presence of potentially toxic cyanobacteria, and a considerable portion of research related to the issue has consequently focused on this particular group and on the practical aspects of mitigating their proliferation (Paerl et al. 2011). While these direct and undeniably important effects are widely known and well documented, some subtler yet fundamental aspects are more difficult to assess. In agreement

with the general notion that changes in diversity affect ecosystem processes through the functional traits of the species (Chapin et al. 2000), eutrophication-induced shifts in phytoplankton biomass and composition also imply modifications in community functioning. Thus, the resulting alterations in algal community structure are reflected on the ecosystem level, for example, by affecting upper trophic levels due to reduced nutritional value of the phytoplankton (Taipale et al. 2018), or through changes in the importance of food web components (Schiewer 1998), which is also evidenced by high variability in the strength of food web interactions among lakes of different trophic state (Carney and Elser 1990, Yuan and Pollard 2018).

In the field of phytoplankton ecology, research efforts to grab the functional aspects of diversity gave rise to various approaches such as the functional associations of Reynolds et al. (2002), the morphologically based functional classification of Kruk et al. (2010) and the trait-based systems of Weithoff (2003) and Litchman and Klausmeier (2008). A number of papers particularly deal with the relationship between trophic state, or its indicator, phytoplankton biomass and diversity with varying results depending on the approach used and the scale of the research (Duarte et al. 2006, Pálffy et al. 2013, Weyhenmeyer et al. 2013). A comprehensive study from Török et al. (2016) described a unimodal relationship with decreasing diversity toward both low and high biomass values. Such studies contribute to a deeper understanding of eutrophication-related impacts; however, all of them are based on snapshot samples taken from a temporal continuum with variable frequency. Consequently, considering the short generation times of algae and the occasionally rapid compositional changes, we still have limited knowledge on how increased nutrient inputs affect the seasonal variability of phytoplankton and what ecological consequences it might entail.

According to a few studies directly addressing this issue, increasing eutrophy also involves an increase in the number of successional stages, suggesting a greater number of ecological niches during the annual cycle of more eutrophic lakes (Sommer 1986, Anneville et al. 2004). This assumption gained further support, when long-term oligotrophication was found to be

accompanied by just the opposite process, manifested in reduced annual community turnover (Pomati et al. 2015). A key question in this regard is whether the abovementioned increase also means a higher temporal variability of functional composition. If this assumption proved true, it would accordingly suggest that all the ecosystem processes connected to the phytoplankton follow more complex seasonal dynamics as eutrophication takes place. This complexity is further enhanced in the long run due to interannual variability and long-term trends in the environment. Increasing trends of temperature in the last few decades have been found to influence phytoplankton succession (Wiltshire and Manly 2004), induce dramatic compositional shifts (Hsieh et al. 2010), and favor cyanobacterial blooms (Paerl et al. 2016); thus, nowadays the effects of changing trophic state are expected to mingle with those of global climate change. Indeed, experimental and field observations have shown that there is significant interaction between these two impacts (Huber et al. 2008, Wilhelm and Adrian 2008, De Senerpont Domis et al. 2014), which all the more proves that the long-term effects of eutrophication on ecosystem functioning are still more or less unexplored.

The aim of our study was to elucidate long-term changes in the seasonal variability of phytoplankton functional composition using data collected in a large shallow lake (Lake Balaton) with a decades-long history of changing trophic state, and to compare it with trends in phytoplankton biomass, temperature, and water depth. On the basis of the abovementioned assumption, we hypothesized that in years of intensive eutrophication temporal variability was higher compared to the subsequent period of reoligotrophication.

## METHODS

### *Site description*

Lake Balaton is a 77 km long shallow lake situated in Central Europe, with a surface area of 594 km<sup>2</sup> and an average depth of 3.3 m (Fig. 1). Due to its shallowness and large, exposed surface area, Lake Balaton is thoroughly wind-mixed on a regular basis. The lake underwent intensive eutrophication from the 1970s onwards, resulting in eutrophic–hypereutrophic conditions and high summer biomasses of cyanobacteria (Vörös and

Nagy Göde 1993). After more than two decades, restoration measures aimed at reducing external nutrient loads eventually resulted in reoligotrophication from the mid-1990s (Istvánovics et al. 2007). These long-term changes in phytoplankton biomass were most prominent in the western basin of the lake (Fig. 2), since the major inflow carrying more than half of the nutrient input flows into the westernmost part of the lake. Regular sampling and microscopic analysis of phytoplankton began in the lake during the 1960s; however, the temporal distribution of the data series is uneven. Due to the nature of our study, we selected and included years in the analysis that matched the criteria that data coverage spans at least from February to October, and the distribution of the samplings is relatively even with a frequency of two to three weeks. Following these criteria, we analyzed 11 yr from our dataset, which meant a total of 189 samples. Two years were from the 1970s, the period when the lake was starting to turn into a eutrophic state (early eutrophic era), four years represented the period of intensive eutrophication, and five years came from the period of reoligotrophication (post-eutrophic era).

#### Sampling and laboratory analysis

Sampling and analysis were performed using the same methods during the entire period covered in the study. Water column samples were collected in the western basin of the lake (46°44'05.8" N, 17°16'32.0" E). Freshly collected

samples were transported into the laboratory to determine chlorophyll *a* concentration in order to estimate phytoplankton biomass. Chlorophyll *a* concentration was determined spectrophotometrically after hot methanol extraction using the absorption coefficients determined by Iwamura et al. (1970).

#### Taxonomic analysis and functional diversity indices

Fifty milliliter aliquots of the collected samples were fixed with 50  $\mu$ L Lugol's iodine and stored at 5°C for microscopic analysis. Species composition and abundance of nano- and microplankton

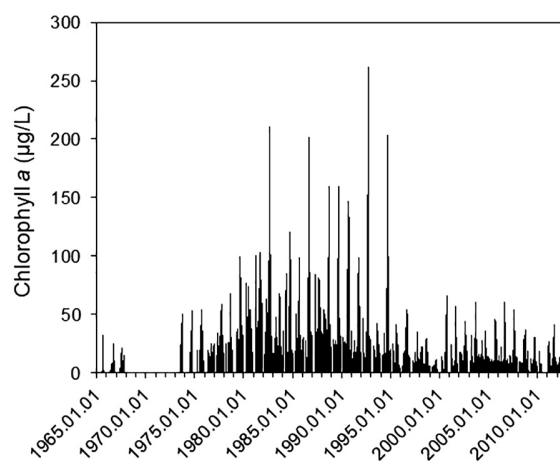


Fig. 2. Long-term changes of chlorophyll *a* concentration in the western basin of Lake Balaton.

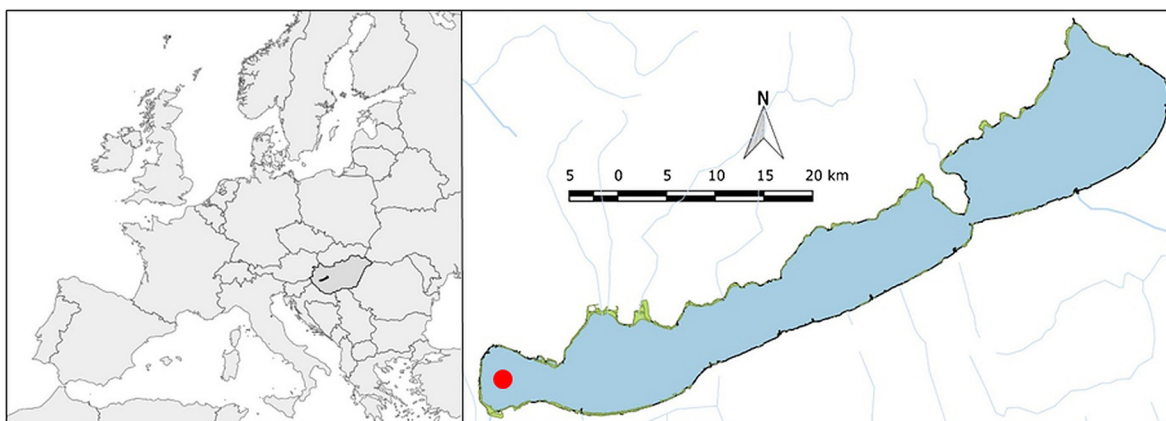


Fig. 1. Geographic position (left) and map of Lake Balaton (right) with position of the sampling site (marked by a red circle).

were determined with an inverted microscope (Utermöhl 1958) at 400× magnification. Biovolumes of the observed taxa were derived from average cell dimensions using the formulas of Hillebrand et al. (1999). The biomass of each species was calculated as the product of species abundance and cellular biovolume assuming an average cell density of 1 g/cm<sup>3</sup>.

Species-level dissimilarities were determined using six ecologically relevant traits: photosynthetic pigment composition, growth form/complexity, greatest axial linear dimension (GALD, as a measure of size), the ability to fix N<sub>2</sub>, phagotrophic potential and motility/buoyancy. According to their pigment composition, taxa were assigned to four different groups (as detailed in Graham et al. 2009): cyanobacteria (chlorophyll *a*, phycobilins); Chlorophyta and Euglenophyta (chlorophyll *a*, chlorophyll *b*); Heterokontophyta, dinophytes, and haptophytes (chlorophyll *a*, chlorophyll *c*); and Cryptophyta (chlorophyll *a*, chlorophyll *c*, phycobilins). All the other traits were included based on the works of Kruk et al. (2010), Longhi and Beisner (2010), and Pálffy et al. (2013). Growth form/complexity consisted of three categories: unicells, filaments, and colonies. The category thresholds for GALD were chosen based on the Kernel density plots of the respective values of the species found in the samples. This meant six different size classes: cells under 5 μm, between 5 and 15 μm, 15 and 25, 25 and 50, 50 and 100, and above 100 μm. In the case of multicellular organisms, the GALD of the whole filament or colony was taken into account. Motility/buoyancy consisted of three different types: non-motile taxa, those possessing gas vacuoles allowing vertical migration and flagellated cells with the ability of free movement. The abovementioned traits were considered as nominal variables, except GALD, which was regarded as an ordinal variable. The ability of N<sub>2</sub> fixation and phagotrophic potential were taken as asymmetric binary (presence/absence) variables. In order to investigate whether compositional variability shows similar patterns under different functional classifications, we also assigned each species to the appropriate functional association of Reynolds et al. (2002), later updated by Padisák et al. (2009), which is based on adaptive features linked to specific habitats.

Using the abovementioned trait categories, each species was considered a specific trait combination. Next, we determined four indices of functional diversity. We calculated the number of unique trait combinations (UTC, Erős et al. 2009) for each sample and the Shannon evenness of these combinations ( $J_{UTC}$ ) as a basic measure of functional diversity. Rao's quadratic entropy (FD<sub>Q</sub>) was used as a composite index of functional diversity, determined from the dissimilarity matrix of the species constituting the phytoplankton for each date separately. Species dissimilarities were calculated from the trait values with Gower's formula (Gower 1971, modified by Podani 1999), which is the appropriate measure of distance for mixed variables. FD<sub>Q</sub> is the sum of interspecific distances (dissimilarities) within a community in the multidimensional space, with each distance weighted by the product of the relative abundance or biomass of the species (Botta-Dukát 2005). FD<sub>Q</sub> without biomass weighting was also calculated in order to determine average undistorted functional distances between the species found in each sample. FD<sub>Q</sub> was determined using the R package FD. All data analysis was performed using the R software version 3.4.3 (R Core Team 2017).

#### *Determining temporal variability*

We used two different approaches to detect changes in seasonal variability. First, we visualized variability with correspondence analysis (CA). In order to include multiple traits in one ordination, we created six large, functionally distinct phytoplankton groups: cyanobacteria, chlorophytes, euglenophytes, diatoms, flagellates with phagotrophic potential (cryptophytes, chrysophytes, and haptophytes), and dinophytes (phagotrophic flagellates with a preference for eukaryotes). Using the CA performed on the group biomass values, we compared the distribution of the samples in terms of group composition and calculated the volume of the convex hull determined by the first three CA components in order to compare the (maximum) extent of annual variability between the years.

The second approach was temporal beta diversity ( $\beta_t$ ) using multivariate dispersion around site centroids in ordination space according to the method of Anderson et al. (2006), which proved

to be an effective tool to quantify the annual variability of community structure (Cook et al. 2018). This method determines pairwise dissimilarities among samples on the basis of community abundance/biomass data and calculates the mean distance of the samples to their centroid in the multivariate space of a principal coordinates ordination. Samples were grouped into years with respective group centroids; mean distances were determined for each year separately. These mean distances can be interpreted as the  $\beta_t$  of the phytoplankton. The Jaccard index was applied to calculate the dissimilarity between the samples; distances were determined with the betadisper function of the R package *vegan* (Oksanen et al. 2017). Standard error of the distances from the centroid was also calculated for each year. Significant differences in  $\beta_t$  between the years were determined by permutation tests with 9999 permutations (Anderson 2006). Beta diversity was determined for the divisions, for Reynolds' functional associations, for the UTC, and for each nominal trait separately.

In order to estimate long-term trends in water temperature, data for the western basin of the lake were acquired from the Hungarian Meteorological Service. Data were only available from 1981; thus, the data series did not cover all the years included in the analysis, and the dates did not fully coincide with those of the phytoplankton data set. Therefore, we used a generalized additive mixed model (GAMM) with an autocorrelation structure of order 1 (AR-1) to estimate a long-term trend in temperature as suggested in Chapter 18 in Zuur et al. (2009):

$$T_i = \alpha + f(\text{Year}_i) + f(\text{Day}_i) + \varepsilon_i$$

where  $T_i$  is water temperature on date  $i$ ,  $\alpha$  is the intercept,  $f(\text{Year}_i)$  and  $f(\text{Day}_i)$  are smoothing splines for the year (representing the long-term trend) and the day of the year (seasonal component) corresponding to date  $i$ , respectively, and  $\varepsilon_i$  is the residual.

## RESULTS

Among the diversity indices used, the number of UTC showed a conspicuous decreasing trend through time (Fig. 3). There was a high interannual variability in RaoQ values with regard to

both the level and the range of functional diversity, although generally higher variability could be observed in the post-eutrophic period, in 2011–2012 in particular. On the other hand, RaoQ without biomass weighting, which represents the diversity of the temporary trait pool, suggests a different trend. There is a gradual increase in the variability of the index in the eutrophic period, which is maintained in the years thereafter, and its values tend to be higher in the post-eutrophic years. No apparent trend could be observed in  $J_{\text{UTC}}$  with generally high within-year variability except the years 1974, 1987, and 1990.

The CA biplot on the larger functional groups demonstrates that the annual variability of phytoplankton composition has changed during the decades (Fig. 4). The most conspicuous differences between the periods occurred in the case of diatoms, cyanobacteria, chlorophytes, and flagellates with phagotrophic potential (bacterivory) (chrysophytes, cryptophytes, haptophytes; Appendix S1: Fig. S1–11). The overall share of diatoms showed a diminishing trend during the period of eutrophication, declining from an annual mean of 53% in 1974 to 11% in 1990, and varying between 11% and 26% in the post-eutrophic years. With the exception of 1974, the annual mean relative biomass of cyanobacteria showed a long-term decreasing trend, although it varied more between the years in the eutrophic period (25–47%) than in the post-eutrophic era (26–37%). However, this group still continued to be dominant during the summer biomass peaks despite considerable decreases in external nutrient load and chlorophyll *a* concentration (Fig. 2), with an annual maximum contribution rising from 74% in 1977 to 91% in 2012. The relative biomass of chlorophytes had a distinct long-term pattern with an increase in the annual mean from 5% in 1974 to 40% in 1990 and a subsequent decline during the post-eutrophic years. The maximum contribution of this group followed a similar pattern, and their spring dominance was generally characteristic for the eutrophic period with 59–81% relative biomass. Potentially phagotrophic (mixotrophic) flagellates were mostly characteristic for the post-eutrophic period (except 2011), with a winter/early spring dominance reaching annual maximum contributions of 77–97%.

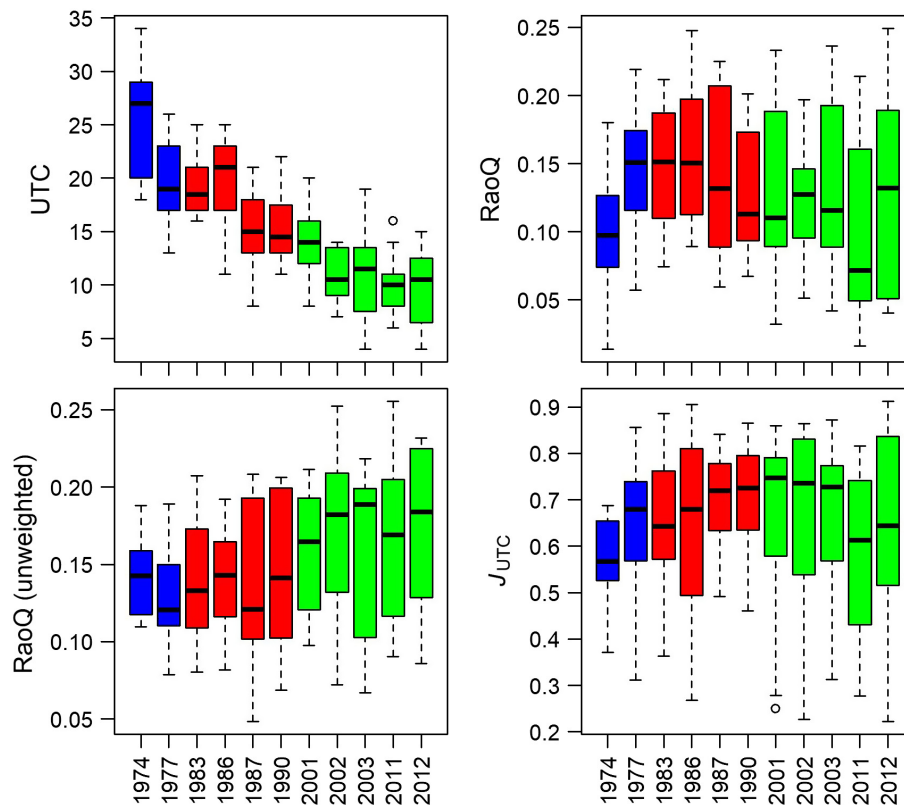


Fig. 3. Boxplots of the number of unique trait combinations (UTC), Rao's quadratic entropy (RaoQ), unweighted RaoQ, and the Shannon evenness of unique trait combinations ( $J_{UTC}$ ) in the western basin of Lake Balaton in years covering various periods of trophic state. Blue is years from the early period of eutrophication; red is eutrophic period; green is post-eutrophic period.

A comparison of each year in terms of the convex hull of the first three CA components (explaining 80.7% of total variation) shows that the volume occupied by the samples is generally higher in years after the period of eutrophication and negatively correlates with the annual chlorophyll *a* maxima (Pearson's  $r$ :  $-0.81$ ,  $P < 0.005$ , Fig. 5). A noteworthy difference between the eutrophic and post-eutrophic years is the change in water level (data were provided by the Central-Transdanubian Water Management Directorate). As seen in Fig. 5, annual mean water depth was higher and showed less interannual variance during eutrophication than during the years 2001–2003 and 2011–2012. In particular, years 2001–2003 saw a gradual decrease in mean depth accompanied by an increase in annual variability with no considerable changes in the summer biomass (chlorophyll *a*) maximum.

The trend in temporal beta diversity ( $\beta_t$ ) was somewhat different from what the convex hull values suggested. Regardless of what the calculation was based on (divisions, Reynolds' associations, UTC, or trait categories), there was an apparently increasing tendency in  $\beta_t$  through the decades, with the years of the post-eutrophic period characterized by generally higher values than those during the period of eutrophication (Figs. 6, 7). The difference was most apparent in the case of divisions, and the groups of GALD, motility, and pigment composition, despite the fact that phytoplankton biomass (chlorophyll *a* concentration) during the early years of the eutrophic era was comparable to that of the post-eutrophic years. This long-term trend consequently seems to be only loosely related to interannual changes in phytoplankton biomass, as evidenced by the lack of significant correlation

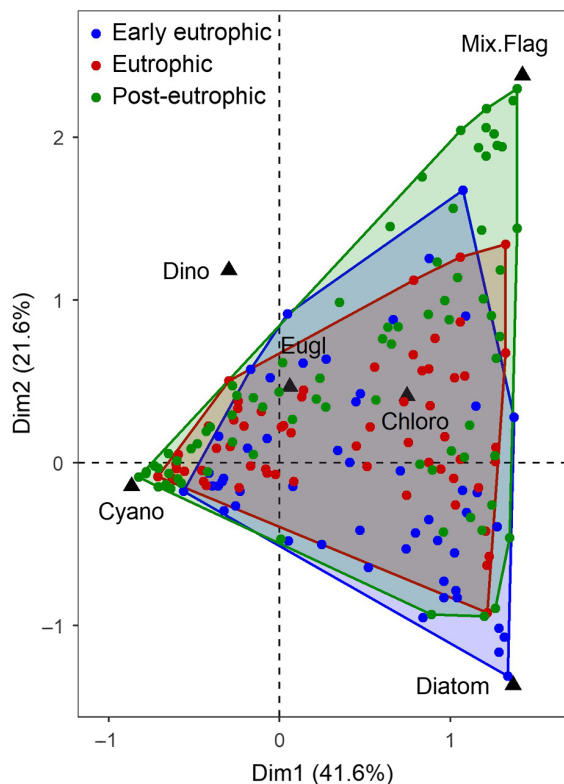


Fig. 4. Asymmetric (row principal) biplot of correspondence analysis on the biomass of larger functional groups in years covering various periods of trophic state in Lake Balaton. Abbreviations are Cyano, cyanobacteria; Chloro, chlorophytes; Eugl, euglenophytes; Dino, dinoflagellates (eukaryvorous mixotrophs); Mix.Flag, flagellates with phagotrophic potential (cryptophytes, chrysophytes, haptophytes). Years in early eutrophic period: 1974, 1977; in eutrophic period: 1983, 1986, 1987, 1990; in post-eutrophic period: 2001, 2002, 2003, 2011, 2012.

with the annual chlorophyll *a* maximum, with only the  $\beta_t$  of GALD and motility showing significant negative correlation (Pearson's  $r$ :  $-0.63$  for both,  $P < 0.05$ ). Permutation tests have shown that  $\beta_t$  of divisions in 1974, 1977, and 1990 was significantly lower than any year of the post-eutrophic period, while in 2011, it significantly exceeded all the years from the eutrophic period (Appendix S1: Table S1f). In terms of GALD, motility, and pigment composition,  $\beta_t$  significantly different from the post-eutrophic period mostly occurred also in 1974, 1977, and 1990 (Appendix S1: Table S1b–d). Differences in the  $\beta_t$

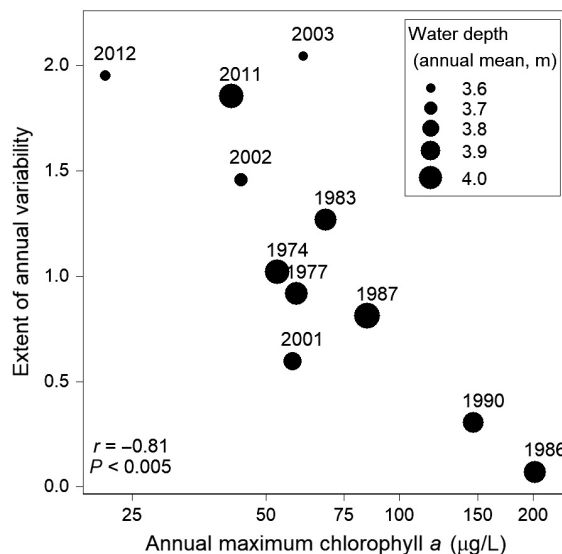


Fig. 5. Maximum extent of annual variability\* in functional composition versus the annual (summer) maximum of chlorophyll *a* concentration in the western basin of Lake Balaton (\*: convex hull volume determined from the first three components of the correspondence analysis shown in Fig. 4, explaining 80.7% of total variation). Larger symbols correspond to higher annual mean water level; numbers above symbols represent years.

of Reynolds' groups were less significant (Appendix S1: Table S1g). Concurrently with this enhancement of  $\beta_t$ , the outcome of GAMM on water temperature also implied a significantly increasing long-term trend ( $P < 0.05$ ; Fig. 8; Appendix S1: Fig. S12, Table S2).

## DISCUSSION

Contrary to our expectations, our assessment of long-term changes in the investigated large shallow lake has demonstrated that the seasonal variability in phytoplankton functional composition showed an increasing trend after a long period of eutrophication. This trend can be associated with multiple long-term shifts in the environment, as the two fundamental components of functional variability exhibited different temporal patterns. While eutrophic conditions tended to reduce the probability space within which phytoplankton functional composition varies during the seasonal cycle (the maximum

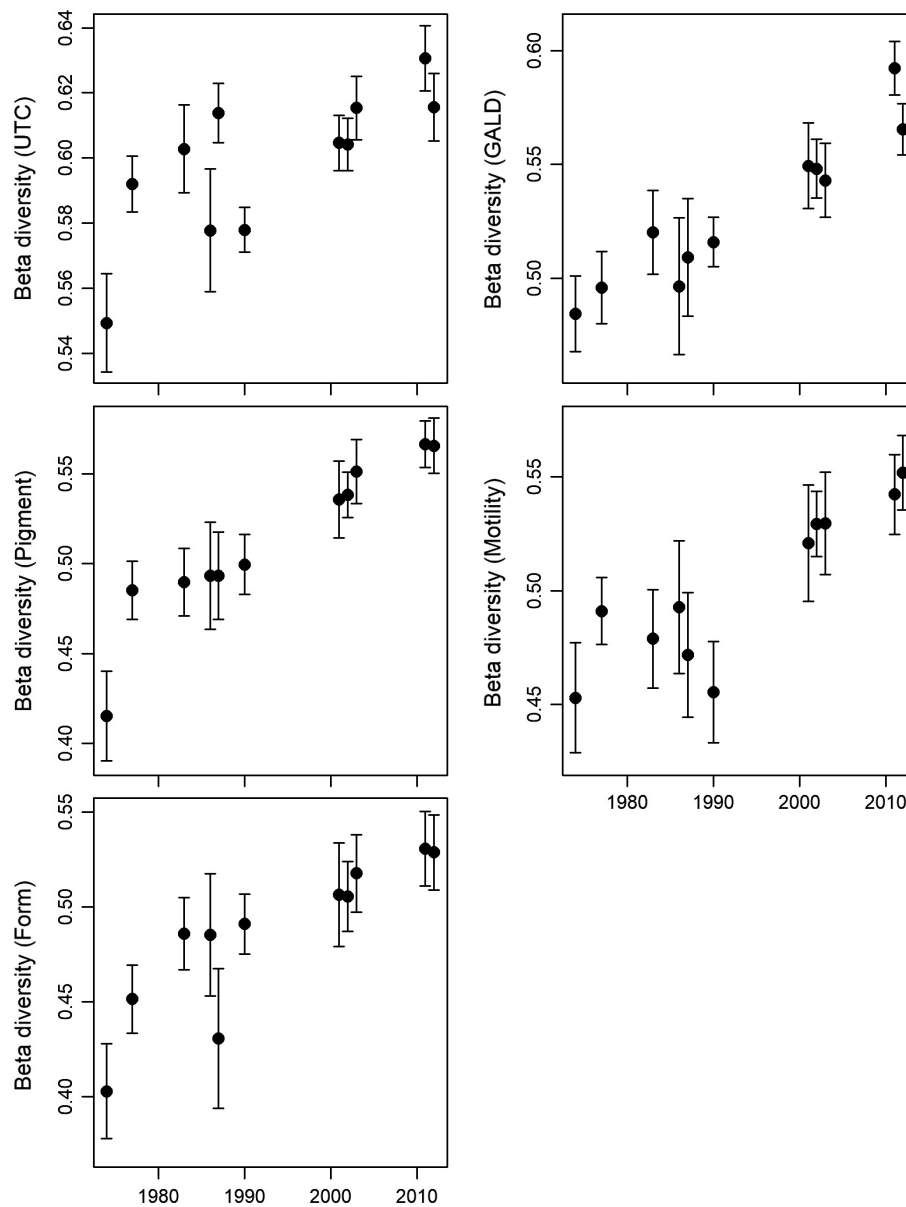


Fig. 6. Changes in the temporal beta diversity of unique trait combinations (UTC) and various functional trait categories through the years in the western basin of Lake Balaton. Error bars represent standard errors. Significant differences are shown in Appendix S1: Table S1a–e.

extent of annual variability), annual mean compositional dissimilarity (temporal beta diversity,  $\beta_t$ ) showed an increasing tendency regardless of the shifts in trophic state, seemingly following the long-term increase in water temperature.

The shrinking of the probability space in eutrophic years and its expansion in mesotrophic years meant a change in the diversity of instances

with uneven community structure. In this respect, mixotrophic flagellates undeniably formed an important group, although, being a successful strategy when resources are limiting (Rothhaupt 1996), the emerging winter/early spring dominance of mixotrophic potential in the post-eutrophic period of the lake is not surprising. Similar compositional shifts were observed



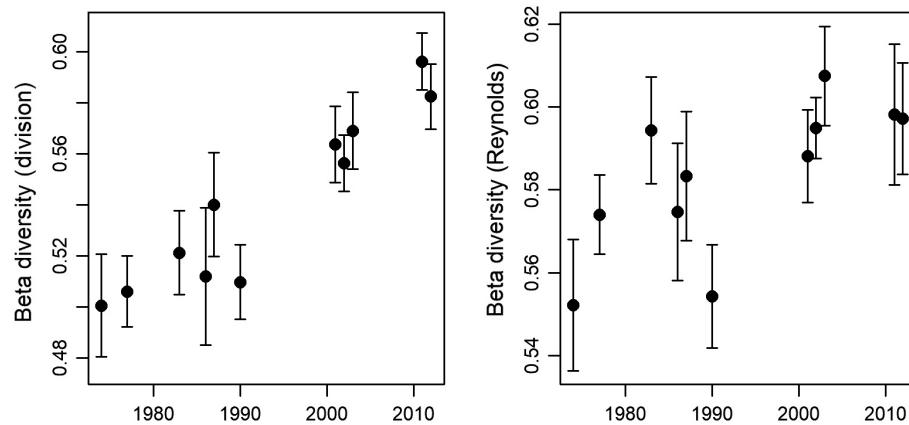


Fig. 7. Changes in the temporal beta diversity of divisions and Reynolds' functional associations through the years in the western basin of Lake Balaton. Error bars represent standard errors. Significant differences are shown in Appendix S1: Table S1f–g.

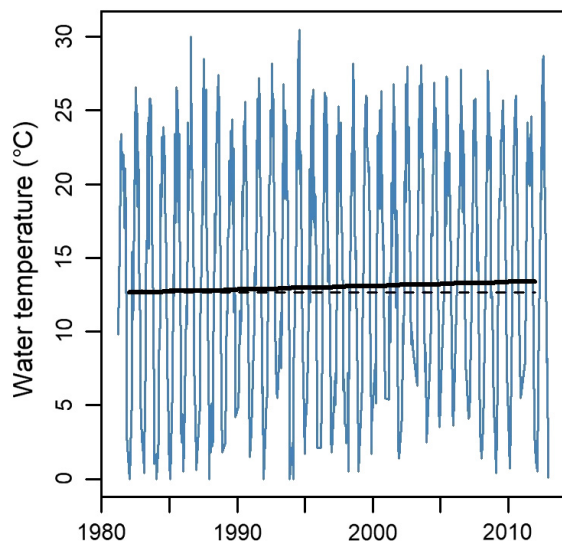


Fig. 8. Seasonal changes in water temperature in the western basin of Lake Balaton. The thick black line denotes generalized additive mixed model-estimated long-term trend of annual means ( $R^2 = 0.897$ ;  $P < 0.05$ ). Model results and estimated smoothers are included in Appendix S1: Table S2, Fig. S12.

in other lakes, where chrysophytes were mostly found at low total phosphorus concentrations (Olrik 1998), and the same group proved to be able to cover most of its phosphorus demand through bacterivory during reoligotrophication (Kamjunke et al. 2007). Under such conditions,

mixotrophs can play an important role in material fluxes within the plankton (Jones 2000), broadening the range of effects elicited by the phytoplankton on ecosystem processes during its annual cycle of succession. When the lake was eutrophic, the probability space was reduced because of the general lack of flagellate dominance and the longer duration of green algal and/or diatom dominance. Such a shift in the temporal pattern of community structure also meant a change in the seasonal variability of specific functional traits: pigment dominance, motility and phagotrophic potential (facultative bacterivory) in particular. It is important to note that the maximum extent of annual variability would not necessarily have increased during the post-eutrophic era without the late summer dominance of cyanobacteria still remaining characteristic. The persistence of cyanobacterial dominance during reoligotrophication is not unusual (Jeppesen et al. 2005, Ostermaier et al. 2012), but their share is expected to eventually diminish in the future in case the oligotrophication process continues (Fastner et al. 2016), which is a probable scenario for the lake due to the significant reduction in nutrient loads (Istvánovics et al. 2007). From this, we can assume that the inflated probability space may depict a transitional period, and how the borders of annual variability will shift in the future is hard to tell.

The trend in temporal beta diversity suggests a different story. Its long-term increase cannot be

simply explained by the processes of eutrophication and reoligotrophication, and thus may be rather linked to a unidirectional change in the lake, such as the moderate rise in annual mean water temperature detected using a GAMM modeling approach (Fig. 8; Appendix S1: Fig. S12). Warming can have a destabilizing effect on plankton dynamics (Strecker et al. 2004), which could be an explanation for higher seasonal variability. However, the effect of warming on phytoplankton is obviously species-specific (Huertas et al. 2011), and besides, changes in composition can also be an indirect consequence of the impact of warming on the grazer community. Whether this complexity in the impact of elevated temperatures leads to higher temporal variability in phytoplankton composition remains a question open for further discussion. What also seems to be a plausible answer comes from the fact that a long-term rise in temperature can simultaneously mean a longer vegetation period. Using a modeling approach, Klausmeier and Litchman (2012) demonstrated that changes in the duration of the growing season can have a considerable impact on food web structure and dynamics. This has not yet been tested in such a frequently mixed environment as Lake Balaton, but the earlier the annual phytoplankton succession begins and the longer the favorable temperatures allow its further development, the longer time is available for dynamic interaction between the planktonic components and their environment, which increases the possibility for more varied temporal patterns. An indication of this extended successional path could be the observed higher seasonal variability in functional composition.

A similarly increasing tendency occurred in the seasonal variability of Reynolds' groups, although with higher interannual variability (Fig. 7). This more or less uniform long-term shift, regardless of the type of functional approach used, also implies that there is an apparent change in phytoplankton dynamics beneath the alterations in community structure, following higher compositional fluctuations in the post-eutrophic era. Less significant differences between the periods when using Reynolds' system most probably originate from the theoretical basis of its classification. Reynolds' groups contain species with similar adaptive features (tolerances and sensitivities) linked to specific

types of habitats, but species of the same group can differ in certain functional traits while sharing other traits with species from other groups.

An intriguing question, which comes from our finding, is whether and how functional diversity and variability are interconnected. We detected a remarkable long-term decline in the number of UTC, coupled with a moderate increase in the annual mean and variance of unweighted functional diversity (RaoQ without biomass weighting; Fig. 3). Drawing conclusions from these trends needs special care, since both measures are highly dependent upon the number and trait composition of rare species. The contrasting direction of change in these two indices can be interpreted as a long-term decrease in functional redundancy, that is, increasing within-sample functional dissimilarity, but this might partially be a natural consequence of the decrease in UTC. Lower UTC can also result in reduced compositional overlap and higher dissimilarity among the samples, which thus might have also contributed to the increase in temporal beta diversity over the years. An interesting point here is a relationship found in experimental planktonic communities, according to which increasing functional redundancy can enhance reliability/predictability; that is, it can diminish variance in community responses due to the phenomenon of compensatory growth (Naeem and Li 1997). It might be a far-fetched assumption, but our finding thus raises the question of whether the observed rise in temporal beta diversity can actually be a direct manifestation of increasing instability as a result of decreased functional redundancy. In contrast with UTC and unweighted RaoQ, biomass-weighted RaoQ and UTC evenness did not show any clear tendency (Fig. 3), which demonstrates that certain composite indices used to quantify functional diversity may not be the most appropriate tools for detecting long-term tendencies in such a highly dynamic environment. The main problem in our case might be that besides being strongly influenced by the distribution of biomass among the species, these indices blur or completely mask the importance of functional identity, which yields little explanatory power when compositional changes are the focus of research.

In a certain respect, our results are comparable to those found in the deep Lake Zurich; however,

while the relationship between functional richness and seasonal variability was similar, the trend was quite the opposite. The lake went through long-term reoligotrophication and a gradual increase in temperature resembling the observed shifts on Lake Balaton; however, these changes were accompanied by an increase in functional richness (Pomati et al. 2012) and an overall decline in annual compositional turnover, an index fundamentally equivalent to temporal beta diversity (Matthews and Pomati 2012). The contrast in trends most likely stems from differences in hydromorphological conditions. Pomati et al. (2012) have found that spatial (depth) heterogeneity in Lake Zurich significantly contributed to the rise in functional richness by promoting niche partitioning. In the shallow Lake Balaton, the water column becomes frequently unstable due to wind-induced mixing (Padisák et al. 1988), which influences algal community structure (Jacobsen and Simonsen 1993), and due to coupled sediment resuspension, it can also play a major role in upward nutrient flux formation (Nöges et al. 1998). This feature and its consequences simultaneously mean that in shallow, wind-exposed lakes disturbance-driven changes and autogenic succession can be equally important in shaping phytoplankton composition (Padisák 1993, Honti et al. 2007). For this reason, niche partitioning is not likely to have a fundamental role in shaping phytoplankton functional richness and thus cannot support a higher level of functional redundancy in Lake Balaton, which may be one explanation for the contradiction in long-term trends between the two lakes. Another source of difference lies in the approaches used to describe compositional variability. We focused on functional composition, the changes of which suggest modifications in community functioning, whereas Matthews and Pomati (2012) determined compositional turnover in terms of species. Although there is a certain degree of correlation between taxonomic and functional diversity, the two approaches cannot be interpreted the same way, not just because of the obvious differences in meaning but also due to the fact that the two measures can exhibit both synchronous and diverging seasonal patterns (Weithoff et al. 2015).

An additional implication of our study also related to the abovementioned water column

instability is the degree of influence a decrease in annual mean water depth seems to have on the extent of annual variability, weakening the relationship between trophic state and compositional variability (Fig. 5). Certain years after 2000 have seen serious drought eventually leading to a considerable drop in the water level of Lake Balaton, which obviously increased the susceptibility of the water column to wind-induced turbulence. Thus, in addition to the long-term decrease of functional redundancy, higher interannual variation in water depth during the post-eutrophic years might have also contributed to a higher unpredictability reflected in the fluctuations of the probability space. The year 2001 differed from the other years in one particular respect, with consistent diatom dominance from early to late spring (Appendix S1: Fig. S7), which was presumably caused by the lack of a continuous ice cover over the lake (data provided by the Hungarian Meteorological Service). This prevented the formation of a more stable water column, which could have favored the proliferation of flagellates, as it was observed in the years thereafter as well as in other lakes with seasonal ice cover (Phillips and Fawley 2002, Vehmaa and Salonen 2009). The lack of a flagellate-dominated period consequently contributed to a reduced functional probability space, just the same way as during the eutrophic period. Considering current predictions on global warming, the duration of ice cover in temperate shallow lakes is expected to decrease in the future (Niemistö and Horppila 2007); thus, the limited compositional changes of 2001 might support our previous assumption that the observed increase in seasonal variability may represent a period of transition.

When dealing with phytoplankton dynamics in shallow, frequently mixed environments, one cannot forget about the biomass contribution of meroplanktonic diatoms, which can from time to time be resuspended from the sediment surface (Padisák and Dokulil 1994). In order to determine whether meroplankton presence had a significant contribution to seasonal variability in phytoplankton composition, we repeated the analyses with the exclusion of meroplanktonic species. This resulted in somewhat modified values, but the general tendencies in functional diversity, in the maximum extent of annual

variability, and in temporal beta diversity remained highly similar to those determined from the whole dataset.

In view of the results, our study also carries a more theoretical message. While eutrophication alters phytoplankton composition and variability, the reversal of the process (reoligotrophication) does not necessarily return the community to its original, pre-eutrophication seasonal pattern, and dynamics. This observation also suggests that any relationship between eutrophication, phytoplankton diversity, and ecosystem functioning inferred from short-term data using multiple sites along a trophic gradient should be interpreted with caution, as it also seems important what changes a water body went through prior to its state at the time of observation. Such a historical aspect, which consequently seems to involve a hysteretic effect, is often neglected from such studies. A similar message is carried by the notion of ecological memory, coined by Padisák (1992), intending to imply that the species pool of the community (and the inherent set of trait combinations) is, on the one hand, a fingerprint of the past, and on the other, it represents the capacity of the community to adapt to and influence future conditions. Nevertheless, the idea of hysteresis remains highly hypothetical until further, more convincing evidence is available, and the interactive effects of climate change and altering nutrient loads make it difficult to reach a definite conclusion about the causal relationships surrounding long-term shifts in phytoplankton dynamics. The emphasis here is on the alterations in seasonal succession, which is not always accounted for when addressing the combined effects of climate change and eutrophication. Using summer data, a long-term study on planktonic communities in the Baltic Sea found that warming and eutrophication induced shifts in the size structure of the planktonic food web (Suikkanen et al. 2013); however, our analysis (although dealing with a different type of water body) has demonstrated that the seasonal variability of size in the phytoplankton can go through a significant change over the decades. This might reflect a shift in the phenology of zooplankton, different groups of which suppress different size classes of phytoplankton (Sommer et al. 2001). This is a good example supporting our opinion that to achieve a more accurate understanding of

community functioning, we must work on data sets that match both the scale and the temporal resolution of compositional changes.

In summary, our findings suggest that the observed long-term changes in the seasonal variability of phytoplankton in the investigated shallow lake are the consequence of multiple environmental shifts. Our hypothesis is that trophic state is an important factor in setting the limits to compositional changes, whereas increasing temperature and its consequences (e.g., the extension of the growing season) are likely to enhance seasonal variability in the phytoplankton, which, coupled with a decline in functional redundancy, means a higher level of functional instability within the community. The relationship between the trajectories of these two factors and the observed changes in seasonal succession indicates that the lake is in a phase of transition. In order to verify our assumptions and to establish whether the observed transition is a unique or a frequently occurring phenomenon during warming, further testing is needed both in this lake and in other similar water bodies of the temperate zone.

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