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Are recent protection strategies sufficient for maintaining diverse freshwater benthic diatom assemblages?

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ABSTRACT

Even though microscopic algae play pivotal role in the healthy functioning of freshwater ecosystems, recent water protection strategies rarely consider them and primarily focus on macroscopic organisms. Here, we studied the effect of protection level and utilization type of lowland standing waters on the composition and diversity of benthic diatom assemblages and on the diatom-based ecological status of waters. We hypothesized that (i) protected waters will sustain more diverse diatom assemblages and better ecological quality than not protected ones. We also hypothesized that (ii) the increase in number of utilization will affect negatively on biodiversity and on ecological quality. Clear taxonomic differences were revealed only in protected and not utilized waters while trait composition in protected waters was independent from the utilization type. Neither biodiversity nor ecological status of waters were influenced by protection level. The increase in number of utilization types, however, significantly decreased functional richness. Although high biodiversity of algae can effectively buffer the negative effects of climate change and anthropogenic impact, recent protection strategies are insufficient to support it.

1. Introduction

In the everyday life of human communities, freshwater ecosystems have been playing a pivotal role from the beginning of the history of mankind. However, at present these ecosystems have become one of the most vulnerable ones on the Earth (Porter et al., 2012). Thus, their protection or at least their careful use is essential not only from ecological but also from economic point of view. Management strategies of these systems have to be tailor-made and usually have to allow for three main concepts: The first one is the environmental protection approach requesting the achievement of good ecological status or potential of the freshwater ecosystems (in Europe: Water Framework Directive concept - EC, 2000). The concept of ecosystem services, however, emphasizes that the sustainable use of ecosystems is more important (Daily et al., 1997) than requesting an exact valuation of its quality. The third concept is linked to nature conservation and it derives both from international and national laws. These rules clearly specify which organisms or even ecosystems and concrete areas need to protect thus, the concept of nature conservation reflects both global and local considerations (Act of Nature Conservation, 1996; Council Directive, 1992; Ministerial Order, 2001). These three concepts related strongly to each other, and all of them strive to achieve and protect an appropriate status of environment but their purposes are sometimes not the same. While the concept of ecosystem services represent not only environmental but also social and economic viewpoints and therefore support the sustainable use of aquatic areas (Daily et al., 1997), the WFD-based concept stresses to decrease the human impact on ecosystems (Moss, 2008). It focuses primarily on the ecological status of waters and protection of communities living in them (EC, 2000). In contrast, the nature conservation concept is not limited primarily to the aquatic organisms, but also to terrestrial ones directly linked to rivers, lakes or wetlands (e.g. waterfowls – Ramsar Convention, 1971). It has a special and often exclusive interest to protect macroscopic organisms and to maintain their habitats (Ministerial Order, 2001).

Since the entire catchment area of rivers or the different basins of lakes rarely serve for the same purposes (Adams et al., 2015), it is hard to meet every expectation mentioned above in practice i.e. good ecological status, protection of habitats of aquatic organisms, sustainable use of

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freshwaters, and their protection by law. While certain sections of watercourses or lakes are protected, their other parts are used for recreation, fishing or water transportation (Adams et al., 2015; Kókai et al., 2019). In addition, it is rather unclear, what roles protected areas play in maintaining biodiversity or good status in freshwater ecosystems (Adams et al., 2015; Chessman and Cadotte, 2013). Both positive (Baird and Flaherty, 2005) and negative (Mancini et al., 2005) impacts of the protection by law on ecosystems has already been shown.

Protected freshwater ecosystems in the Carpathian Basin belong to the network Natura 2000. These protected areas are to maintain habitats which are considered to be European interest and to preserve and to conserve the "biodiversity hotspots" within a region (Council Directive, 1992). However, the nature and biodiversity laws focus on protection of macroscopic life forms and their habitats (see above), microscopic organisms such as benthic or planktic algae are excluded from this system. Therefore in some cases, it can be challenging to achieve algal based good ecological status required by EC directive (EC, 2000), since laws providing protection for macroscopic organisms can be against it.

Not protected standing waters also could play a part in maintaining the diversity of habitats and communities both locally and at landscape level (Bolgovics et al., 2019), but rather they provide opportunities for social and economic well-being in many ways. Thus, their management is primarily under the pressure of expectations originated from social welfare demands (Lepšová-Skácelová et al., 2018). In general, fishing and other recreation activities are important ecosystem services provided by lowland lakes and ponds (Borics et al., 2016; Kókai et al., 2019; Lepšová-Skácelová et al., 2018). These social activities are prohibited in some standing waters protected by the law in the region, while in some others not only fishing but also water sports are allowed (Kókai et al., 2019). But it is still problematic, how these activities influence on structure and diversity of assemblages, or even the interactions between the populations living in these areas. It is also questionable how good ecological status based on algae could be achieved in waterbodies using primarily for recreation or fishing (Heino et al., 2018).

Here, we assessed the effects of the level of protection and the type of utilization focusing on human welfare (fishing, water sports) in lowland lakes and ponds in relation to composition and taxonomical and functional diversities of benthic diatom assemblages and also to the diatom-based ecological status of waters. Highlighting the similarities and dissimilarities between waters with different level of protection and/or different type of utilization, we tested the following hypotheses in natural benthic diatom communities: (i) Composition and biodiversity of benthic assemblages and diatom based ecological status of waters are strongly influenced by the level of protection of lakes and ponds: the protected freshwater ecosystems will maintain higher taxonomical and functional diversity and better ecological status than not protected ones. (ii) Composition and biodiversity as well as the ecological status of lowland lakes strongly relate to the utilization type of waters relating to human well-being. The increase in numbers of utilization will influence negatively on diversity and on diatom based ecological status of lakes and ponds.

2. Materials and methods

2.1. Sampling area, sample collection and preparation

Altogether 85 diatom samples were collected from 17 differently managed lowland lakes, oxbows, ponds, basins and reservoirs in the Carpathian Basin between 2005 and 2017 in the growing season (from May to September; Fig. 1). We defined the management of standing

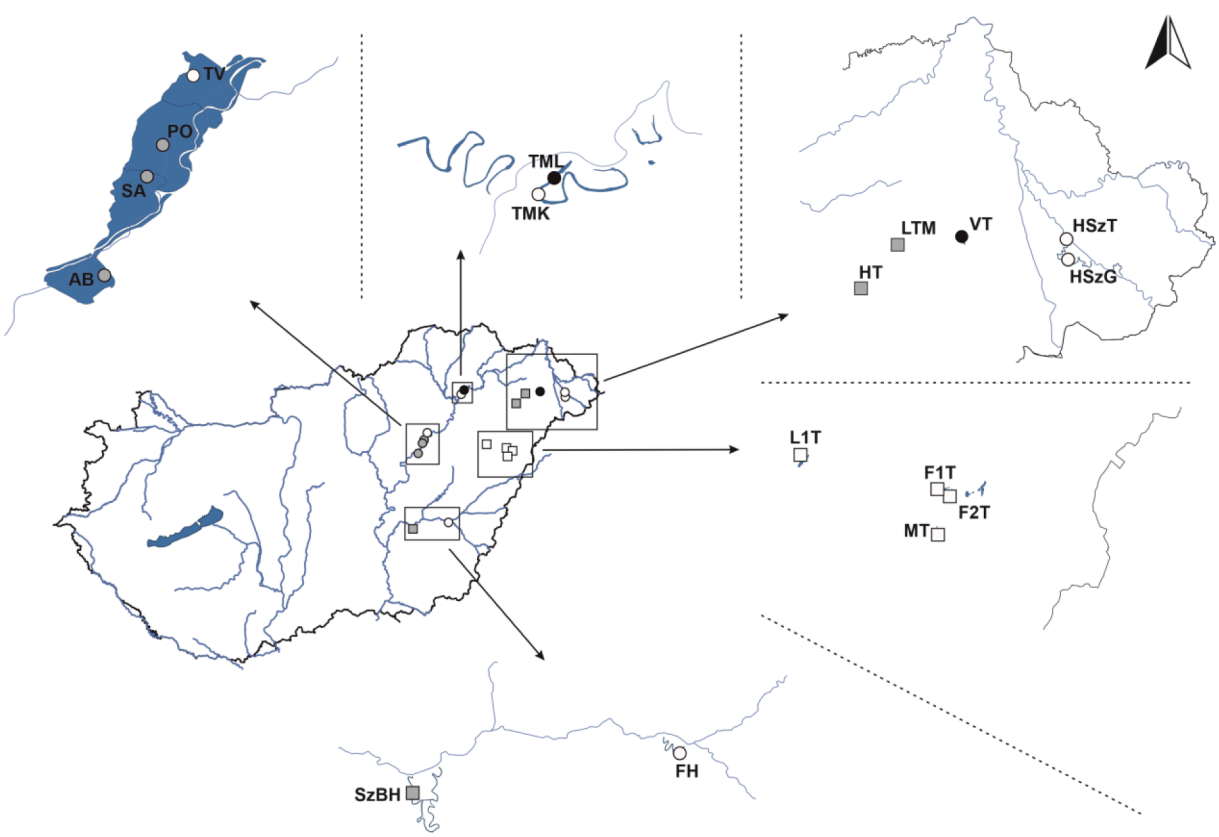

Fig. 1. The study area in the Carpathian basin, the Hungarian watercourses are marked with blue lines. The differently utilized standing waters were denoted with the following symbols: Empty square – not protected and utilized by fishing Grey square – not protected and utilized by both fishing and waters sports; Empty circle – protected and utilized by fishing; Grey circle – protected and utilized by both fishing and water sports; Black circle – protected and not utilized. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

waters as the combination of level of protection and utilization type. Based on the level of protection (either protected by the law – PF, or not protected – NP) and utilization type (not utilized – NU, utilized by fishing –F, utilized by both fishing and water sports – FW) we classified the studied standing waters into five combined categories (Table A.1; Fig. 1).

The sampling and preservation of benthic diatoms was done according to the European guideline (EN 13946, 2003). The cleaning of diatom valves from organic matter and the preparation of permanent slides were also performed according to the European guideline (EN 13946, 2003). At least 400 valves were identified and counted (EN 14407, 2004) using Leica DMRB microscope with 1000–1600-fold magnification. The taxa identification was carried out using reference and also up-to-date literature (Krammer and Lange-Bertalot, 1997a, 1997b, 2004a, 2004b; Potapova and Hamilton, 2007; Stenger-Kovács and Lengyel, 2015).

2.2. Data processing and analyses

A total of 273 taxa were identified in the samples. Classifying taxa into guilds (low profile, high profile, motile and planktic; Table A.2) was done according to Passy (2007) modified by Rimet and Bouchez (2012). Taxa were classified into five cell-size classes (Table A.2) by using a database containing the average biovolume of diatoms (Rimet and Bouchez, 2012). Length per width ratio of diatoms (LW ratio) was calculated by using the above mentioned database containing also the average length and width data of taxa (Rimet and Bouchez, 2012). Classifying taxa into six LW ratio groups was done according to Stenger-Kovács et al. (2018) (Table A.2).

The main components of functional diversity (functional divergence - FDiv, functional evenness - FEve and functional richness – FRich; Mason et al., 2005) were calculated by using 'FD' R package in R (Laliberté and Legendre, 2010; Laliberté et al., 2014). All calculations were performed using the f-Diversity software package (Casanoves et al., 2011).

We used indicator species analysis (Dufrêne and Legendre, 1997) to identify which species contributed to the distinctiveness between groups of sites with similar drying characteristics. Good indicators are found exclusively and consistently within a group at high abundance. Permutation tests (n = 9999) were used to assess indicator significance. Indicator species analysis was conducted with the package 'indicspecies' in R version 3.2.2 (R Development Core Team, 2010) using the 'multipatt' function (De Cáceres and Jansen, 2016).

Assessing the diatom-based ecological quality of waters, Multimetric Index for Lakes (MIL) was calculated (Kelly et al., 2014) according to the JRC Technical Report (2014).

$$\mathrm{MIL} = 1/3 \times (\mathrm{IBD} + \mathrm{EPI} - \mathrm{D} + \mathrm{TDIL}_{1-20})$$

where IBD is the Indice Biologique Diatomées (Prygiel and Coste, 1999), EPI-D is the Eutrophication Pollution Index Diatoms (Dell'Uomo, 1996) and $\mathrm{TDIL}_{1\text{-}20}$ is the Trophic Diatom Index for Lakes (Stenger-Kovács et al., 2007). The indices (IBD, EPI-D and $\mathrm{TDIL}_{1\text{-}20}$) were calculated by using the weighted average equation of Zelinka and Marvan (1961) modified by Coste (1982). Data required for these calculations are contained in the software OMNIDIA 5.5 (Lecointe et al., 2003) and the software DILSTORE (Hajnal et al., 2009).

For the comparison of the taxonomic and trait composition of the six combined protection and utilization type categories, we performed Principal Components Analyses (PCA) applying CANOCO 5.0 software package (ter Braak and Šmilauer, 2002). For the trait-based analyses, we used the community-weighted mean (CWM) matrix, in which the mean trait values in the community were weighted by the relative abundances of the species.

Effects of nature protection and utilization type were analyzed using two-way Generalized Linear Mixed Models (GLMM; Zuur et al., 2009), we assumed normal distribution of dependent variables and used the identity link in SPSS 20.0. In the analyses, 'nature protection' and 'utilization type' (both ordinal scale) were included as fixed factors with 'site identity' as a random factor. We analysed the following as dependent variables: richness, Shannon diversity, species evenness, Rao quadratic entropy, Functional richness (FRich), Functional evenness (FEve), Functional divergence (FDiv), and Multimetric Index for Lakes (MIL). Species evenness was calculated following Pileou (1975): $J_{\mathrm{Evenness}} = \mathrm{H}/\log(\mathrm{S})$, where 'H' was Shannon diversity and 'S' was the species richness. We used Fisher's Least Significant Difference (LSD) method for paired comparisons.

3. Results

3.1. Composition of diatom assemblages

With the exception of PF_NU waters, taxonomy-based PCA analyses did not indicate clear separation among the assemblages living in differently managed waters (Fig. 2). The different management of waters explained 28.86% of the variance in the taxonomical composition of benthic diatoms. The eigenvalues of the first and second axes were 0.12 and 0.06. Large sized mostly motile taxa such as *Epithemia turgida* and *Rhopalodia gibba* were indicator species ($p < 0.001$) in PF_NU standing waters (Fig. 2, Table A.3). However, some indicator species were also identified in NP_F and PF_FW waters. While *Fragilaria vaucheriae* and *Sellaphora bacillum* were identified as characteristic indicator species to NP_F standing waters, mainly motile *Navicula* and *Nitzschia* spp. and planktic taxa (*Aulacoseira distans*) were characteristic to PF_FW waters (Table A.3).

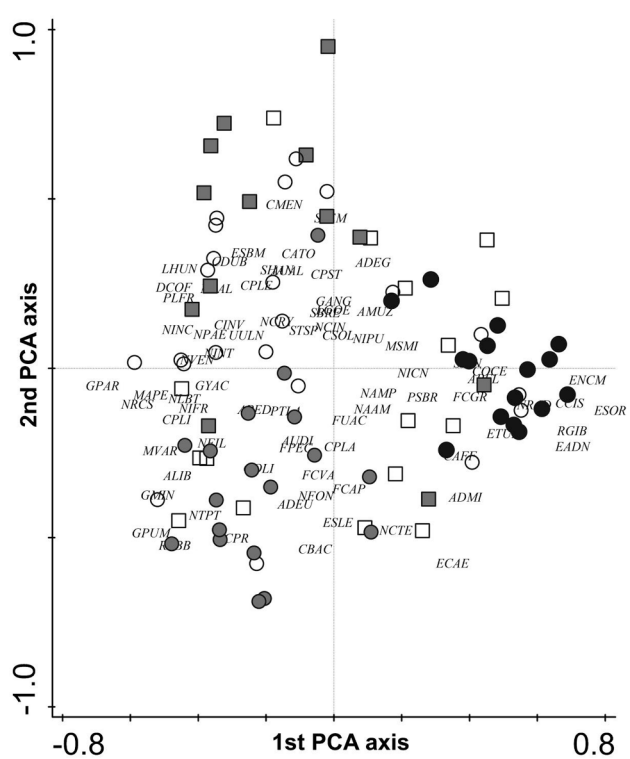

Fig. 2. Composition of diatom assemblages in protected or not protected and differently utilized standing waters. The explained variations were 12.47% and 6.15% for the first and second axes, respectively. Four letter OMNIDIA codes indicate the name of dominant diatom taxa (relative abundance >5%). The differently utilized standing waters were denoted with the following symbols: Empty square – not protected and utilized by fishing Grey square – not protected and utilized by both fishing and waters sports; Empty circle – protected and utilized by fishing; Grey circle – protected and utilized by both fishing and water sports; Black circle – protected and not utilized.

Based on the trait composition, the PCA analyses indicated only weak differences among the differently managed waters (Fig. 3). Differences in utilization and protection of waters explained 60.94% variance in functional composition of benthic diatom assemblages (Fig. 3). The eigenvalues of the first and second axes were 0.21 and 0.16, respectively. While low profile guild, the smallest size category (S1) and the intermediate LW category (LW3) were characteristic in protected standing waters, no trait category exclusively characterized the not protected ones were found (Fig. 3).

3.2. Diversity and the diatom-based ecological quality

The level of protection and type of utilization had not significant effect on the classical diversity metrics. There were no interactions between the level of protection and utilization (Table 1, Fig. A.1).

The level of protection had no significant effect on the functional diversity metrics, while differences of utilization type strongly predicted the FRich ($p = 0.023$). The functional richness was significantly lower in standing waters utilized by both fishing and water sports than in waters utilized only by fishing (Table 1). Analysis of interaction between level of protection and utilization type did not reveal significant relations to functional diversity metrics (Table 1, Fig. A.1). The level of protection and the utilization type neither separately nor combined with each other did not predicted the diatom based ecological status of the studied standing waters (Table 1).

4. Discussion

4.1. Level of protection

Although protected freshwaters often have to provide social and economic services (e.g. Kókai et al., 2019), which do not differ from services characterized the not protected areas, the main purpose of these ecosystems is to maintain the biodiversity "hot spot" habitats. We

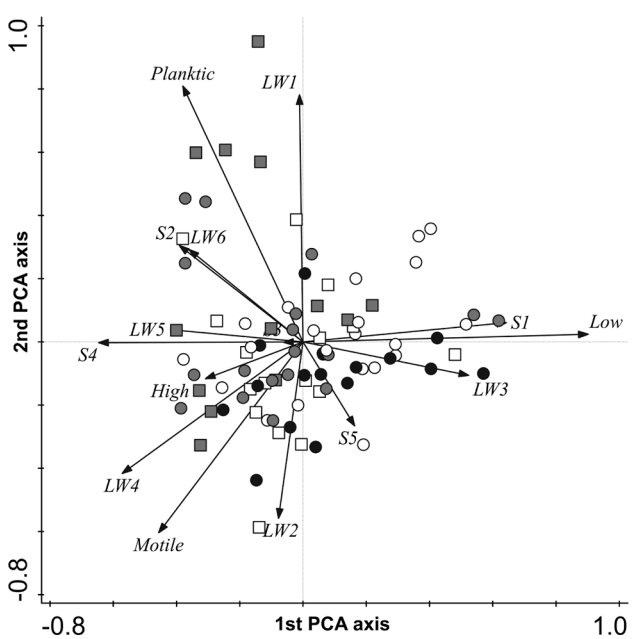

Fig. 3. Trait composition of diatom assemblages in protected or not protected and differently utilized standing waters. The explained variations were 21.26% and 15.98% for the first and second axes respectively. The differently utilized standing waters were denoted with the following symbols: Empty square – not protected and utilized by fishing Grey square – not protected and utilized by both fishing and waters sports; Empty circle – protected and utilized by fishing; Grey circle – protected and utilized by both fishing and water sports; Black circle – protected and not utilized.

Table 1
Effect of level of protection and utilization type on classical and functional diversity metrics. Significant effects (in bold) were detected using Generalized Linear Mixed Models, where 'level of protection' and 'utilization type' (both ordinal scale) were included as fixed factors, 'site identity' (=plots nested into sites) as random factor.

	Level of protection		Utilization type		Interaction	
	$F_{1,80}$	p	$F_{2,80}$	p	$F_{1,80}$	p
Characteristic						
Species richness	0.741	0.390	1.289	0.281	1.546	0.217
Shannon diversity	0.302	0.584	0.512	0.601	0.574	0.451
Species evenness	0.145	0.705	0.185	0.831	0.053	0.818
Rao quadratic entropy	0.278	0.600	0.079	0.924	0.517	0.474
Functional richness (FRich)	0.725	0.397	**3.897**	**0.023**	2.668	0.106
Functional evenness (FEve)	1.519	0.221	0.312	0.733	0.006	0.941
Functional divergence (FDiv)	1.050	0.309	0.120	0.887	1.956	0.166
Multimetric Index for Lakes (MIL)	0.868	0.354	0.331	0.719	1.701	0.196

hypothesized that there is a clear compositional difference between protected and not protected standing waters. This hypothesis was only partially supported by the results. Taxonomic composition based PCA analysis stressed clear separation only in the case of protected and not utilized lakes and ponds (Fig. 2). The trophic states of these standing waters are eu- and hypertrophic, their TP content is > 30 μg L^{-1}, additionally the TN:TP ratios here are <14 (Török et al., 2016; web1), which suggest a nitrogen limitation (Downing and McCauley, 1992). Most of the motile large-sized indicator species here (*Epithemia turgida* and *Rhopalodia gibba*) live in endosymbiosis with nitrogen-fixing cyanobacteria (DeYoe et al., 1992; Satncheva et al., 2013). This endosymbiotic relationship ensures these diatoms' advantages in nitrogen-limited environment (DeYoe et al., 1992; Satncheva et al., 2013). Protected standing waters utilized by both fishing and water sports are the basins of the largest artificial reservoir in Hungary (Kisköre reservoir) that water level is regulated with water from the Tisza River in the spring. One of the indicator species here was *Aulacoseira distans*, which is a planktic species. Hatvani et al. (2019) highlighted that planktic diatoms are characteristic members of Tisza River, especially in spring. The high number of these algae in the basins of Kisköre reservoir was already stressed and explained by the spring water level modification (Kókai et al., 2019). These reasons may be in the background of the significant role of *A. distans* in this water type. Further indicator species here were such *Navicula* and *Nitzschia* species, which usually indicate meso-, and/or eutrophic conditions (van Dam et al., 1994). According to the reference scale of trophic classes introduced by Besse-Lototskaya et al. (2011), Kisköre reservoir is in meso-eutrophic conditions with its average P value (web 1). The studied not protected standing waters managed by fishing are threatened by drying up within the last few decade (see more below). Indicator species here can tolerate low water level or even aerophilic conditions (van Dam et al., 1994).

Trait composition-based analysis did not revealed clear separation among differently managed standing waters. Only some values of traits such as low profile guild, cell size S1 and length per width ratio LW3 were more characteristic to protected standing waters than in not protected ones (Fig. 3). Those standing waters, which were dominated by these trait categories are the parts of Tiszadob and Holt-Szamos oxbow systems and they are not utilized or only utilized by fishing. In addition, the ratio of these trait categories was also high in a protected basin utilized by both fishing and water sports (Abádszalóki basin). Probably, the reason why these trait categories were dominant in the above mentioned oxbows and basin, is different and it is strongly related to their hydrological characteristics. The oxbows are flushed by riverine water from the Tisza River during high-water conditions especially in

spring or early summer. The basin, however, is a part of the Kisköre reservoir whose water level as we mentioned above is regulated. In both cases (flushed by riverine water and water level regulation), small-sized, disturbance-tolerant taxa, such as *Achnanthidium minutissimum*, with high spreading ability can reach the oxbows and also the basin and colonize them rapidly. This taxon is characteristic member of the Tisza River (Várbíró et al., 2012). It is also well-known as pioneer species which small size inhibits the fast settling of cells supporting its spreading (e.g. Rimet and Bouchez, 2012). But not just flooding can support the dominance of low profile guild taxa in these oxbows. In late summer, the increase in biomass of planktic algae decreases the light-availability in biofilm, while the grazing pressure caused by macroinvertebrates is enhanced. These circumstances also benefit the low profile guild (Kókai et al., 2019; Liess et al., 2009).

We hypothesized lower diversity in not protected waters than in protected ones, but the results did not confirm this (Table 1). As we mentioned above, protected waters often serve for multiple purposes which are the same than in not protected standing waters. However no interactive effects of utilization type and level of protection on diversity were found. These results clearly highlighted that current protection system is not suitable for maintaining the diversity of benthic diatom communities. Since the management of waters protected by law are developed for protecting macroscopic organisms such as macrophytes, fishes or water birds and maintaining their habitats (Act of Nature Conservation, 1996; Council Directive, 1992; Ministerial Order, 2001; Ramsar Convention, 1971). This situation can create undesirable circumstances for diatoms preferring low/moderate trophic state and indicating good water quality. For example, increased abundance of water birds in an area can lead to an increased natural nutrient load in waters. Furthermore floating macrophytes - several of them protected by law in Hungary (Ministerial Order, 2001) - are not appropriate substrate for diatoms and an increase in their abundance can lead to a decrease in local diversity of benthic algae (Hinojosa-Garro et al., 2010). The most not protected standing waters studied here are non-flushed standing waters. These ecosystems are located far from rivers and they are not flushed by riverine water therefore they are not exposed to community forming/shaping effects of floods. In contrast, protected standing waters are located along the Tisza River and the Szamos River and they are usually flushing by riverine water during floods or in high-water level periods. These events result a change in assemblages composition and lead to periodic dominance of disturbance-tolerant taxa with high spreading ability. Because these taxa have very similar traits values, their dominance can diminish the functional diversity within the community even during the whole vegetation period. Here, we do not suggest that not protected standing waters are more valuable habitats than oxbows. In long term, flushing has key role in establishment in healthy ecosystem functioning (see more in Lóczy et al., 2019). Moreover, ongoing climatic changes threat exactly those freshwaters which water regime depend extremely on precipitation and on groundwater (Stubbington et al., 2017) and have no inflow such as most of the studied non-protected waters. Recently, these ponds have been drying up due to the serious droughts in the last very few years in the region (not published observations of the authors).

To assess properly the influence of protection on ecological status of aquatic ecosystems some basic facts should be taken into account such as the purpose or the time lags of protection, and obviously the extension of protected area (Adams et al., 2015). Here, protected standing waters are located in the area of National Parks and the waterbodies are protected in whole. We hypothesized better diatom-based ecological status in protected freshwaters than in non-protected ones. The results, however, did not support this hypothesis; there were no significant differences in MIL values between protected and not protected standing waters. These results strongly highlight that protection status per se is not enough to form diverse assemblages of diatoms and to maintain good diatom-based ecological status. Since protection activities do not focus directly on microscopic organisms but recent protection directives concerning freshwater ecosystems target macroscopic life forms (e.g. Council Directive, 1992). Freshwater benthic algae including diatoms, however, contribute to ecosystem services in multifaceted ways and they are key elements of aquatic ecosystems (Kireta et al., 2012; Stevenson, 2014). Excluding them from protection strategies finally can negatively affect the whole aquatic ecosystem.

4.2. Utilization type

In general, utilization of waters can have negative impact on community structure by reducing biodiversity in aquatic ecosystems (e.g. resulting nutrient load and/or physically disturbed environments; Kókai et al., 2019; Lotter, 2001; Tilman, 1982; Wang et al., 2019). Thus, we hypothesized compositional differences between differently utilized standing waters. Significant decrease in diatom diversity was also supposed with increasing numbers of water-use. Our presumptions were supported only very partially. No differences were found in taxonomic-based and in trait-based composition of differently managed standing waters (Figs. 2 and 3). Furthermore, utilization type had no significant effect on diatom diversity. The only exception was functional richness its value was lower in standing waters utilized by both fishing and water sports than in waters which are used only for fishing (Table 1). Lower functional richness can indicate lower resilience against extremities, thus these communities can be more vulnerable in fluctuating environments (Mason et al., 2005). Since most of the studied waters utilized by both fishing and water sports are reservoirs, their water regime is regulated depending on the actual weather conditions. In the Carpathian Basin, however, the number of extreme climatic events is expected to increase resulting flash floods or extremely low water level even frequently (Bartholy and Pongrácz, 2005). Therefore, the above mentioned reservoirs have to face strong climatic pressure in the near future which can finally lead to diversity loss similarly to small watercourses in the region (B-Béres et al., 2019; Várbíró et al., 2020).

Benthic diatoms respond quickly to environmental changes thus they considered to important indicators in WFD-based monitoring (e.g. Birk et al., 2012; EC, 2000; Kelly et al., 2009; Poikane et al., 2016). Since human activities which mainly target welfare services usually influence negatively the ecological status (Aristi et al., 2012; Vörösmarty et al., 2010), we hypothesized decrease in diatom-based ecological status parallel with increase in numbers of utilization. Our results did not confirm this hypothesis. As it was mentioned above, the trophic state is high in all of the studied standing waters and most probably, the very similar MIL values were due to the high nutrient supply of waters.

5. Conclusions

Our findings highlighted the shortcomings of recent water protection strategies in Hungary since these strategies focus only on macroscopic organism and do not on microscopic ones. Furthermore, protected freshwaters are usually utilized by fishing and water sports that can interfere the effective protection managements too. We revealed clear taxonomic differences only in protected and not utilized standing waters. Trait categories which characterized mainly protected waters independently their service numbers support high spreading of taxa. Their presence here related strongly to hydrology of the studied waters as flushing by riverine water or water level regulation. In contrast, no trait categories were found dominated mainly not protected standing waters. Although protected waters can serve as diversity “hot spot” which suppose good ecological status too, different taxonomical and functional diversity metrics and diatom-based index did not shown significant differences between protected and not protected waters stressing the insufficiency of recent protection strategy on microscopic organisms like benthic diatoms. Utilization type influenced only on functional richness significantly, using standing waters both for fishing and water sports had not negative effect on diversity of diatom assemblages or on diatom-based ecological status comparing to waters utilized

by only fishing. Since microscopic primary producers as benthic diatoms are not only important members but also the base of the food chain in freshwater ecosystems, supporting their high taxonomic and functional diversity is essential for effective protection against ongoing climatic changes and anthropogenic impacts. Therefore, microscopic life forms should be urgently involved in direct protection acts (e.g., habitat protection, habitat restoration).

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

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecolind.2021.107782.

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