

# Stream order-dependent diversity metrics of epilithic diatom assemblages

Csilla Stenger-Kovács · Lívia Tóth ·  
Franciska Tóth · Éva Hajnal · Judit Padisák

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**Abstract** Diatoms are considered as an appropriate indicator group for ecological status assessment of surface waters. These organisms can be indicative not only of the waterchemical but also of the hydro-morphological characteristics (e.g., stream size, physical habitat diversity) of running waters. In this study, diatom diversity metrics (species number, Shannon diversity, and evenness) from 506 sites in Pannon ecoregion (Hungary) were compared to the Strahler stream order system established with ArcView GIS 3.2. SOM analyses were performed to exclude the effect of nutrients on diversity metrics along the stream orders. Mixed-effects linear models and

Tukey's post hoc test revealed a linear relationships between species number, diversity and stream orders on ecoregion level from first- to eighth-order streams. The species number increases with an average of 8%, and the diversity by 10% per unit increase of the stream order. However, we could not find relationships with evenness. Autotrophic diversity metrics based on diatom species data appear to increase parallel with the stream order while those of heterotrophic metrics (published in the literature) maximize at medium stream orders. We argue that stream order is a relevant typological parameter which can basically determine the diatom diversity metrics, and it is well applicable in biomonitoring.

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C. Stenger-Kovács (✉) · L. Tóth · J. Padisák  
Department of Limnology, University of Pannonia,  
Egyetem u. 10, Veszprém 8200, Hungary  
e-mail: stenger.kovacs@almos.uni-pannon.hu

C. Stenger-Kovács · J. Padisák  
HAS-UP Limnoecology Research Group of the Hungarian  
Academy of Sciences, Egyetem u. 10, Veszprém 8200,  
Hungary

F. Tóth  
Geoinformatics Research Group, Department of Physical  
Geography, Eötvös Loránd University, Egyetem tér 1-3,  
Budapest 1053, Hungary

É. Hajnal  
Alba Regia University Center Székesfehérvár, Óbuda  
University, Budai út. 45, Székesfehérvár 8000, Hungary

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## Introduction

Water body size is one of the most important factors determining the structure and function of aquatic ecosystems. However, it is difficult to define in absolute terms either in the field or in a geographic information system (Hughes et al., 2011). The Strahler stream order (Strahler, 1957) has been a useful indicator of stream size in stream biology (Miyamoto et al., 2011) regionally, nationally, and globally. It is a useful descriptor of the catchment area, distance from

the source, and the mean annual discharge (Hughes et al., 2011). Stream order may separate streams into different segments with similar internal characteristics (Seyfer & Wilhm, 1977). Several studies were published where stream order was used to classify and explain diversity and composition of lotic- [like macroinvertebrates (e.g., Pringle, 1985), phytoplankton (e.g., Garnier et al., 1995), fish (e.g., McCormick et al., 2000)] and riparian organisms (e.g., Dunn et al., 2011), and even the water quality (Crunkilton & Duchrow, 1991). Stream order has been successfully applied in freshwater biodiversity planning (Higgins et al., 2005).

So far, there has been no systematic attempt at studying the response of diversity of diatom communities to changes in stream order in Europe, although the investigation of benthic diatoms received distinguished interest in implementation of the Water Framework Directive (WFD) (EC, 2000) since most of the member states chose diatoms as proxies for phytobenthos (Kelly et al., 2009a). Only some publications can be found in the USA (Cushing et al., 1983; Molloy, 1992) about stream size and stream order-dependent diatom species compositions and morphological growth forms (ecological guilds). Stream order as an important component of understanding relationship between diatom assemblages and eutrophication/organic pollution was considered by Rott et al. (1998) in the Grand River (Ontario) and by as Pan et al. (1996) in a water basin consisted of 49 streams from 1st to 3rd order. Potapova & Charles (2002) found that the stream size is a major element in determining diatom assemblages in rivers. According to Leira & Sabater (2005), variation of the diatom assemblages can be explained not only by chemical characteristics but also by physiographical factors: thus, stream order may serve as a general descriptor summarizing the physical habitat. At large spatial scales, variation in diatom assemblages along stream longitudinal gradients is attributable to three main reasons: changes in morphology, in geology, and in land use of streams and their basins (Molloy, 1992). According to Kutka & Richards (1996) diatoms can be suitable indicators of stream physical characteristics for monitoring purposes. Present knowledge about the variation of diatom composition along stream order gradients on a larger basin or landscape level has been limited. This applies also for ecoregions which serve as basis of ecological status assessment of the WFD.

In our research, diatom composition was analyzed in running waters of Pannon Ecoregion covering all of Hungary. Relationship between typological classification (Strahler rank) and diatom assemblages increases from class to order resolution and saturates at the finest levels (species and subspecies; Rimet & Bouchez, 2012). Therefore, diatom diversity metrics based on species data (species number, diversity, evenness) of Hungarian rivers and stream order data were collected to analyze this relationship. Our hypotheses were that (i) there is clear, positive, linear relationship between species number/Shannon diversity/evenness and stream order separately, and (ii) species number and diversity values are characteristic for a given stream order, indicate the hydro-morphological status of the running waters.

## Methodology

Benthic diatom data included in the PERIDAT Diatom Database (Hajnal et al., 2010) were used in this study. These data derive from different sources (i) ECO-SURV project (van Dam et al., 2005), (ii) thesis works (e.g., Kiss, 2004; Kovács, 2006), and (iii) publications of University of Pannonia, Department of Limnology (e.g., Pór et al., 2000; Kovács et al., 2004, 2005). Altogether 683 sampling sites were included in this study. Epilithic diatom sampling, identification, and quantification followed standard methods (Kelly et al., 1998; CEN, 2003). Diversity values were calculated by the widely used Shannon–Weaver function (Shannon & Weaver (1949)). The stream order was determined after the accurate identification and location of different sampling sites (Rostetter, 2009) using Arc-View GIS geographic information system, EOV coordinates and cartographs according to Horton–Strahler (Gordon et al., 1994). Of the 683 samples, it was possible to determine the stream order precisely in 506 cases (Fig. 1); these samples were taken at 357 different sampling sites in 189 streams (number of repeated samples per sampling site ranged 1–25, mean  $\pm$  SD =  $1.47 \pm 1.81$ ). To investigate the clustering and gradient features of the data, the self-organizing methods with SOM Toolbox of MATLAB software were applied (Vesanto & Alhoniemi, 2000). The first was supplemented with the weight matrix principal component projection. For the test, 117 randomly chosen samples were used from stream

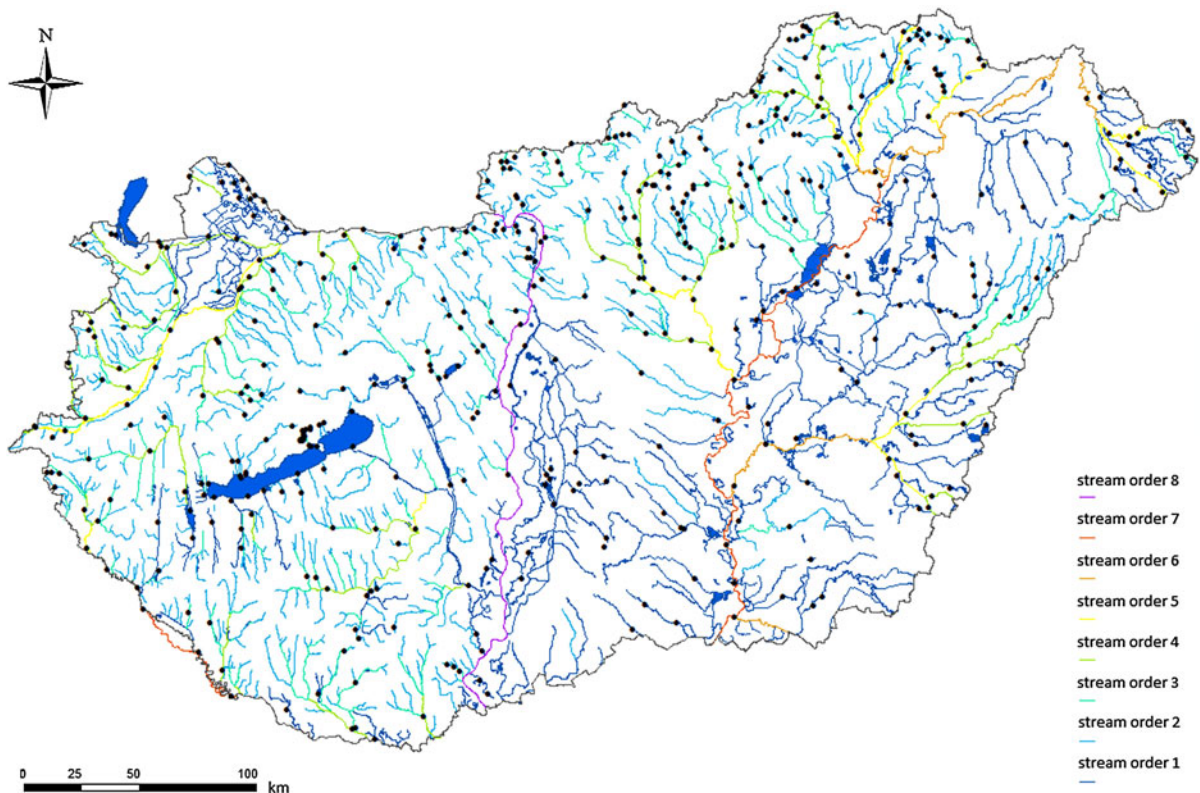
orders 1 to 8 (stream order 1—41 samples, stream order 2—22 samples, stream order 3—27 samples, stream order 4—8 samples, stream order 5—3 samples, stream order 6—2 samples, stream order 7—3 samples, stream order 8—11 samples). The Kohonen type self-organizing map analysis was executed both on ecological features (species number, diversity, evenness) and on water chemical data ( $\text{NH}_4^+$ ,  $\text{NO}_2^-$ ,  $\text{NO}_3^-$ , and total P) with hexagonal topology and equalized mask to show nutrients can influence the diversity metrics along the stream order. To analyze the relationship of stream order with number of species, diversity, and evenness, we used mixed-effects linear models in R statistical and computing environment (R 2.11.0, R Development Core Team, 2010). The effect of stream order was modeled by linear regression whereas the non-independence of samples taken at the same site and in the same stream, respectively, was taken into account by using hierarchical random factors, i.e., sampling site nested in stream. Then Tukey's post hoc tests were used to compare samples with different stream order pairwise.

## Results

The investigated Hungarian running waters represent first- to eighth-order rivers. Most of the sampling sites were first (317) and second (128) order. The number of the higher order streams was smaller. Eighth-order streams were represented (11 sites, 2.2% of the dataset) exclusively by the different sections of the Danube River; Table 1).

The average species number of the first-order streams was  $23 \pm 7$  (average  $\pm$  standard deviation) and that of the eighth-order rivers was  $44 \pm 16$ . The average diversity of the first-order streams ( $2.73 \pm 0.67$ ) increased gradually until it reached  $3.75 \pm 0.81$  in the eighth-order river. The evenness of streams varied from  $0.48 \pm 0.05$  (fifth-order) to  $0.73 \pm 0.27$  (second-order) (Table 1).

The self-organizing map of ecological data  $u_{\text{matrix}}$  does not show any clear clustering by the stream order, but a clear gradient in species number of the samples and the diversity values (map size  $11 * 5$ , final quantization error  $q_0 = 0.368$ , final topographic



**Fig. 1** Map of the sampling sites

**Table 1** Characteristics of the different stream orders in the Pannon Ecoregions, Hungary

Stream order	1	2	3	4	5	6	7	8	Total
Number of stream sites	317	128	33	8	3	2	4	11	506
Ratio from the total stream sites (%)	62.6	25.3	6.5	1.6	0.6	0.4	0.8	2.2	100
Species number $\pm$ SD	23 $\pm$ 7	28 $\pm$ 7	35 $\pm$ 8	33 $\pm$ 5	34 $\pm$ 14	34 $\pm$ 10	43 $\pm$ 6	44 $\pm$ 16	
Diversity $\pm$ SD	2.73 $\pm$ 0.67	2.95 $\pm$ 0.69	3.21 $\pm$ 0.76	3.14 $\pm$ 0.71	3.27 $\pm$ 0.93	3.11 $\pm$ 0.12	3.61 $\pm$ 0.9	3.75 $\pm$ 0.81	
Evenness $\pm$ SD	0.63 $\pm$ 0.14	0.73 $\pm$ 0.27	0.56 $\pm$ 0.15	0.61 $\pm$ 0.14	0.48 $\pm$ 0.05	0.62 $\pm$ 0.03	0.66 $\pm$ 0.14	0.69 $\pm$ 0.11	

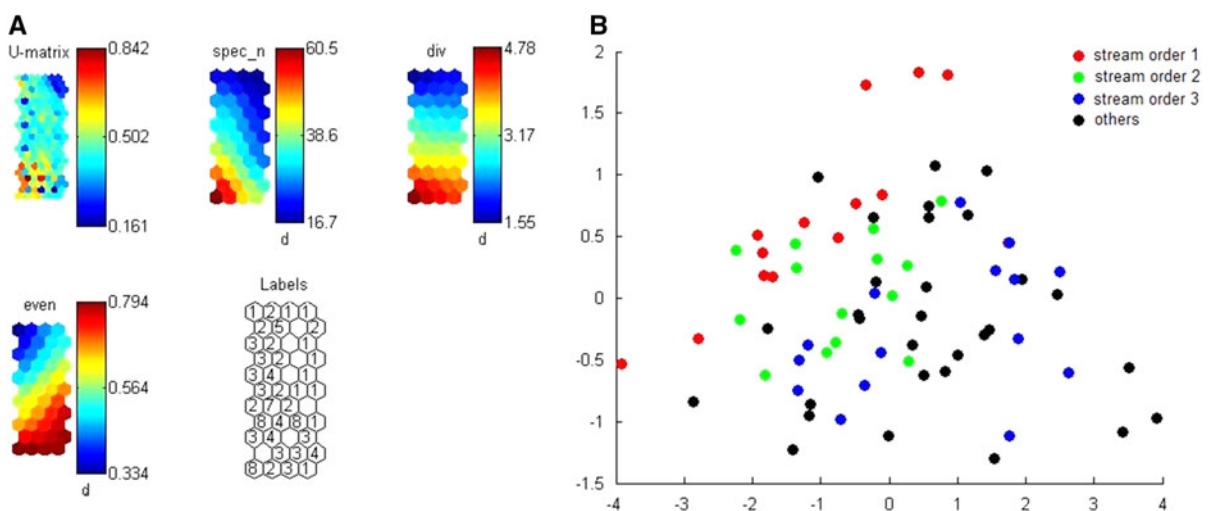
error  $t_0 = 0.013$ ) (Fig. 2A). The evenness values are not seems to be related to the stream order (Fig. 2A). The gradient feature is supported by the principal component projection of the eigenvector of the weight matrix (Fig. 2B). The self-organizing map of water chemistry data showed neither clustering nor gradient tendency in relation to stream order (map size 11 \* 5, final quantization error  $q_0 = 0.465$ , final topographic error  $t_0 = 0.009$ ) (Fig. 3).

The mixed-effects linear models showed that the number of species increased significantly with the increase of stream order (Table 2). At average, 8% increase in species number was observed per 1 U increase in stream order (Fig. 4A). Post hoc tests indicated that first-order streams had significantly less species than streams with order 2, 3, 7, and 8, and 2nd streams had less

species than eighth-order streams (Fig. 4A). Additionally, a marginally non-significant difference was found between stream orders 2 and 3 ( $P = 0.052$ ).

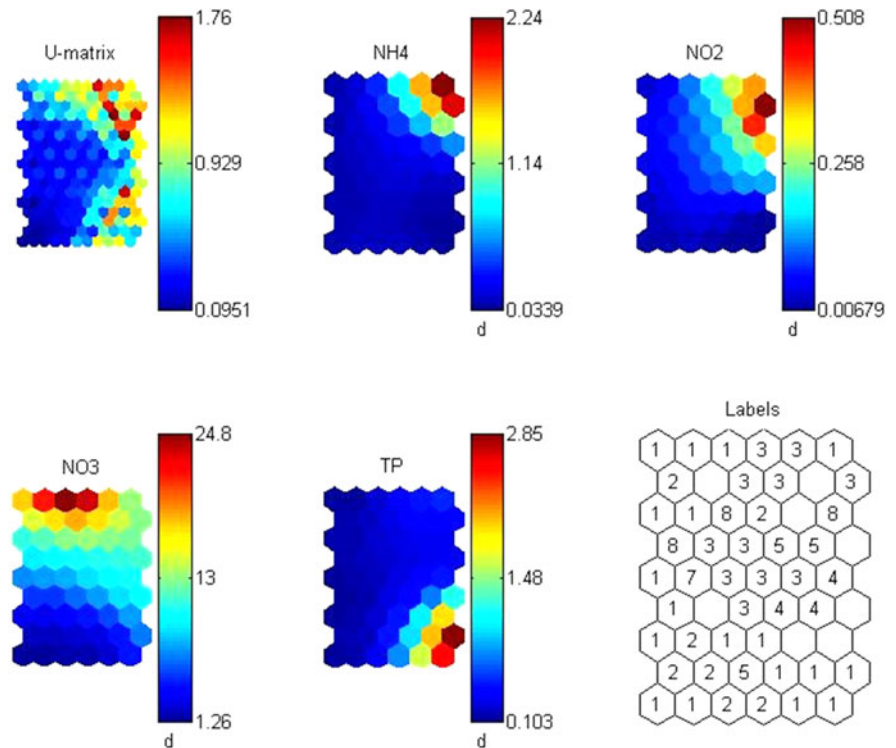
A unit increase in stream order is associated with an average of 10.3% increase in Shannon diversity (Table 2; Fig. 4B). Post hoc tests indicated that the low order streams had significantly lower diversity than the high order streams (Fig. 4B). Additionally, a marginally non-significant difference was found between stream orders 1 and 3 ( $P = 0.053$ ) and between stream orders 2 and 8 ( $P = 0.061$ ).

Evenness was not significantly related to stream order (Table 2; Fig. 4C). This was also the case when we compared the two highest stream orders with the rest (difference between these two categories:  $b \pm SE = 0.061 \pm 0.049$ ,  $P = 0.219$ ).



**Fig. 2** **A** Self-organizing map based on species number, diversity, and evenness data. **B** Principal component projection of the previously described data set

**Fig. 3** A self-organizing map based on  $\text{NH}_4^+$ ,  $\text{NO}_2^-$ ,  $\text{NO}_3^-$ , and total P data related to stream order



## Discussion

Metrics such as species number and diversity are frequently used in ecological status assessment for explaining spatial and temporal patterns of biotic communities (Borics et al., 2012) to better understand their functioning. Parallel to the release of the WFD, Gray (2000) discussed the need to re-evaluate whether diversity indices alone are useful to detect environmental changes. Recent studies recalled these ideas: diversity would provide a fast and complex overview of the ecological status, since the Shannon index and species richness, as the most widely used diversity metrics, appear to be able to collect information from the bulk of the diatom assemblages (Blanco et al., 2012).

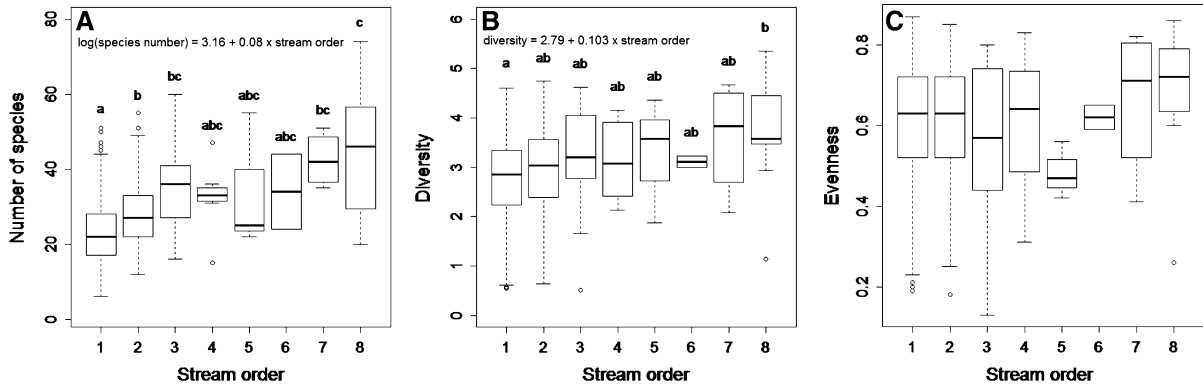
The river continuum concept (RCC) (Vannote & Sweeney, 1980) separates stream segments by size and location along the river course and therefore serves as measure of position in the river continuum. The catchment area and discharge increase with Strahler order (Hughes & Omernik, 1983; Hughes et al., 2011) and stream order is also applicable to describe distribution of human activities in the river basin and it was found to be a useful tool in river basin

classification in Japan (Miyamoto et al., 2011). Additionally, it is relatively easy to obtain and visualize. Along the river continuum from the first (tiny springbrook) to the highest stream orders (mighty river), a number of biological changes can be observed that are related to the pattern of the energy inputs. The carbon turnover lengths increase in large rivers (Naiman et al., 1988). Functional group composition of the consumers (e.g., macroinvertebrates) basically depends on the resource inputs. The heterotrophic inputs dominate in lower order streams and in large rivers. In mid-order streams, the variation of energy inputs might be the greatest (minimal shading and CPOM, profuse sunlight, and substantial amount of FPOM) consequently it predicts a peak in biological diversity (Allan, 2001). In mayfly and stonefly taxa, diversity values were the highest in 4th to 5th order streams and declined both at lower and higher stream orders due to the physical changes from the source to the mouth and to the geomorphology of the catchment basins (Crunkilton & Duchrow, 1991). The hump-shaped productivity–diversity relationship was supposed to be universal by Huston (1999) since at higher nutrient levels anoxia may delimit diversity. Mean annual respiration rates associated with FPOM,



**Table 2** Results of the mixed-effects linear models

	Value	SE	DF	<i>t</i> value	<i>p</i> value
Log (species number)	0.080	0.016	148	5.081	0
Diversity	0.103	0.035	148	2.970	0.0035
Evenness	−0.001	0.006	148	−0.0995	0.921



**Fig. 4** Relationship between species number, diversity, evenness, and stream orders. Letters above the errorbars mark significant differences in Tukey's post hoc tests (groups with the

percent organic matter, and chlorophyll showed a clear, significant increase with the stream order until the sixth-order streams and then slightly decreased (Naiman, 1983). Diatom species and genus number showed hump-shaped pattern along the pH (Schneider et al., 2013).

Neither the above pattern nor the humped-shaped relationship appeared in case of benthic diatoms along stream order in the study of Minshall et al. (1983). Rott et al. (1998) found that diatom species composition was arranged well along the stream order in the Grand River, following water quality where the upper part (orders 2–4) of the stream included the clear water sites, the middle part was characterized by highly variable turbidity (orders 4–5), and the lower part of the stream by high silt load (orders 5–6). The tributaries enrich ecosystems in (i) oxidized nitrogen compounds originating from runoff of cultivated areas; (ii) phosphorous; and (iii) ammonium from wastewater treatment in populated areas (Rott et al., 1998). Parallel with the increasing order of the stream the trophic level is also increasing (Vilbaste & Truu, 2003; Morgan & Kline, 2011). An increase of the species number and diversity along the course was also found by Szabó et al. (2004). Seyfer & Wilhm

same letters are not different whereas groups with different letters differ significantly). Cursive equations represent regression fits

(1977) supported this relationship from fourth- to sixth-order streams as did Vilbaste & Truu (2003) from first- to fifth-order streams, but in the latter study they could show only a tendency without statistical support of the differences. However, their multivariate analysis justified that the order of the stream is the master variable for the benthic diatom composition (Vilbaste & Truu, 2003). Molloy (1992) obtained similar results in three rivers of the Kentucky River system (first- to fifth-order). In two of these rivers Shannon diversity increased from the source to the mouth, and in the third river diversity was the highest at the mouth. Our results are in line with these observations and our original assumption as we found a clear, significant relationship between two of the diversity metrics (species number and Shannon diversity) and the stream order on ecoregion level in first- to eighth-order streams. The species number and the diversity change unequivocally on the stream order gradient.

Though increase of species number and diversity of diatom assemblages along the river continuum seems to be a general phenomenon, disturbances may confuse this regular pattern. The frequency of disturbance can also be a strong driver of diversity patterns

in streams (Cardinale et al., 2005). Robinson et al. (1994) investigated species number and diversity indices after a wildfire for 4 years. The watershed burnt most seriously showed the biggest changes in the diatom assemblages and no correlation was found between the stream order and diversity metrics.

The relationship between diatom diversity metrics and productivity has remained unclear either because of complex environmental influences or because other variables than nutrients determine diatom diversity (Soininen, 2009). Archibald (1972) found linear negative and Lavoie et al. (2008) found positive relationships between diversity and nutrients, Schneider et al. (2013) between species richness and nutrient (TP), while Bellinger et al. (2006) did not find any significant patterns between them, similar to our results. This is the reason why some authors did not suggest these indices for evaluating ecological conditions (Blanco et al., 2012). In large geographical areas where productivity (assessed by resource supply) can change significantly on a large scale, there is relationship between diversity and productivity (Soininen, 2009). Proceeding to global scales, historical factors explain significantly more of the observed geographic patterns in generic richness than do contemporary environmental conditions (Vyverman et al., 2007).

The second assumption, that species number and diversity values are characteristic for a given stream order, was not supported doubtlessly by our results. However, they show that the diversity metrics of low and high order streams is different. Small, undisturbed streams are characterized by most rapid uptake and transformation of inorganic nitrogen (Peterson et al., 2001), and by high sensitivity to nutrient loading and human alteration (USEPA, 2000), and their species number and diversity is lower. The diatom assemblages of medium fourth- and fifth-order streams are more stable compared with lower order sites based on their variations (Vilbaste & Truu, 2003), but in our study their values were not significantly different from lower or higher order streams. Diatom assemblages of large rivers differed substantially from those in smaller streams as shown by Winter & Duthie (2000) because in high order rivers water chemistry indicates not only the geological features of the basin but also the more intensive and extensive land-use characteristics. The effects of these factors on diatom composition are mixed and difficult to separate (Rimet et al., 2004). Furthermore, it has been shown that

higher order streams with a greater abundance and variety of food (Lotrich, 1973), bigger habitat size and diffuse pollution (Harrel et al., 1967) support a more diverse assemblage of biota such as fish and macro-invertebrates (Seyfer & Wilhm, 1977).

The WFD regulates the protection of the surface waters on uniform ecological bases. The member states can involve hydrological and geological factors which have effect on the biota (Kelly et al., 2009a). Considering the structure and function of ecosystems, communities naturally vary on spatial and temporal gradients, which should be taken account also in the status assessment (Kelly et al., 2009b). The recent typology in Hungary does not take the stream order (as hydrological factor and proxy of spatial gradient) into account directly, however, it does consider the size of the watershed. Running waters with a larger watershed are likely to be of higher stream order; therefore, these data are comparable and can be considered in the biological characterization. In a biomonitoring system, stream order is a relevant typological parameter which can basically influence the diatom species number and diversity—which can be supplementary parameters in the ecological status assessment. This thus is an applicable tool for planning biomonitoring programs in such a way as where to avoid over sampling of low- and under sampling of high order streams (Hughes et al., 2011). Equal and high sample number in the different stream orders would enable us to determine characteristic diversity metrics for a given a stream order if we exclude or minimize the gradients of eutrophication, organic and toxic pollution, and even of the hydro-morphological alterations. In this case, the results will be comparable (Kelly et al., 2009a) not only on ecoregion but on European level in the intercalibration exercises following similar sampling practices (Kelly et al., 2012).

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