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PHYTOPLANKTON

Phytoplankton functional and morpho-functional approach in large floodplain rivers

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Abstract Influence of hydrological characteristics and nutrient concentrations on phytoplankton was investigated in four large rivers (Mura, Drava, Danube and Sava) in the Pannonian ecoregion in Croatia to understand how phytoplankton of rivers can be explained by the "different functional group approach". To gain a clearer understanding of the factors that affect river phytoplankton, the present study examined phytoplankton biomass and composition in relationship with physical and chemical parameters assessed in detail by preparing self-organising maps using functional groups and morpho-functional groups. Total nitrogen along with water residence time showed to be the best predictor to determine phytoplankton biomass and chlorophyll *a*. Phytoplankton diversity increased with higher water discharge, but it had the consequence of diluting algae and decreasing biomass. Bacillariophyceae and Chlorophyceae species dominated the phytoplankton assemblages in all rivers. Diatoms predominated in rivers with shorter residence time. Dominant diatom codons of functional groups were C, D and TB while morpho-functional groups were represented by only diatom group VI. As residence time increased, the proportion of chlorococcalean green algae, represented by functional group codon T and morpho-functional group IV grew in summer. Since potamoplankton is dominated by diatoms, functional groups with its fine partition of diatom codons proved to be excellent descriptor of the potamoplankton. Application of morpho-functional groups originally developed from the lake data, showed to be limiting because of the predominating presence of only one diatom group.

Keywords Potamoplankton dynamics · Nutrients · River discharge · Water residence time · Large rivers

Introduction

Lake eutrophication has become one of the most intensively investigated areas of hydrobiology in

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recent decades. Several papers have been published on the relationship between nutrients and chlorophyll *a* content in lakes (reviewed in Phillips et al., 2008), and different trophic scales have been proposed for characterising the levels of lake eutrophication (Vollenweider & Kerekes, 1982). Running waters also suffer the negative consequences of anthropogenic eutrophication, such as high phytoplankton biomass and undesirable changes in species composition, but the literature focusing on the characteristics of phytoplankton in riverine ecosystems is much less extensive.

An explanation for the relative scarcity of studies on riverine phytoplankton may be that ecological assessment of rivers is usually based on investigation of the benthic elements of biota. Without a doubt, this approach has been successful over the 100-year history of river quality assessment. Rivers of lower order are naturally heterotrophic systems (Vannote et al., 1980; Reynolds et al., 1994; Dodds & Cole, 2007), and their benthic invertebrates are the biological elements most relevant for ecological assessment. The autotrophic component of these systems is the phytobenthos, which has been used successfully for monitoring (Van Dam et al., 1994; Kelly et al., 1998; Stevenson & Pan, 1999). Large floodplain rivers during medium and low-discharge periods can also be considered naturally autotrophic systems (Thorp et al., 1998; Wehr & Descy, 1998) because potamoplankton dominates the biota. Like lake phytoplankton, potamoplankton can be organised into functional groups (FGs) (Reynolds et al., 2002; Borics et al., 2007; Padisák et al., 2009) and morpho-functional groups (MFGs) (Kruk et al., 2010) on the basis of their morphological and physiological adaptive strategies for surviving in different environments. Such a classification of potamoplankton with FG approach has been described before (Devercelli, 2006; Várbíró et al., 2007; Abonyi et al., 2012) while MFG approach has never been used.

Several studies suggest that the biomass of river phytoplankton is determined by nutrients (Van Nieuwenhuyse & Jones, 1996) and by physical factors like catchment area, mean depth and flushing rate (Baker & Baker, 1979).

In light of previous studies on different FG and MFG approach on lake and river phytoplankton we hypothesised that they can also be applied in large floodplain Pannonian rivers. We tested FGs versus MFGs. In order to gain a clearer understanding of the factors that affect river phytoplankton, the present study examined phytoplankton biomass and composition of different FGs and investigated how those two are affected by nutrient composition and hydrological characteristics (water residence time and discharge).

Materials and methods

Study area

Croatia is located on the border of Western Europe and the Balkan Peninsula and it has two ecoregions: Pannonian and Mediterranean. The Pannonian ecoregion is influenced by Continental climate with average summer temperature between 20 and 22°C and annual precipitation between 700 and 120 mm, while the Mediterranean ecoregion is influenced by Mediterranean climate with average summer temperature between 20 and 26°C and annual precipitation between 700 and 1,000 mm. The Mediterranean ecoregion has short, fast-flowing, clearwater karstic rivers, while the Pannonian ecoregion has large, slow-flowing, lowland rivers.

Phytoplankton was investigated at nine sampling sites on the four largest Croatian floodplain rivers in the Pannonian ecoregion (Mura, Drava, Danube and Sava; Fig. 1). These rivers belong to the Black Sea watershed. Mura River is 493 km long and it flows through Croatian territory for only 53 km. It is the left tributary of the Drava River. Drava River flows through Croatian territory for 305 km, much of that along the Croatian-Hungarian Border and it is the right tributary of the Danube. Only 188 km flows through Croatian territory, mainly along the border with Serbia. Sava River is the longest Croatian river, with 562 km flowing through Croatian territory, mainly as a border river between Croatia and Bosnia and Herzegovina. Between our two sampling sites, Sava River receives two large tributaries from Bosnia and Herzegovina, the Bosna and Vrbas rivers.

All sites are Croatian National Monitoring Sites for tracking the ecological status of rivers according to the EU Water Framework Directive (WFD, 2000). Geological and hydrological properties of sampling sites are given in Table 1.

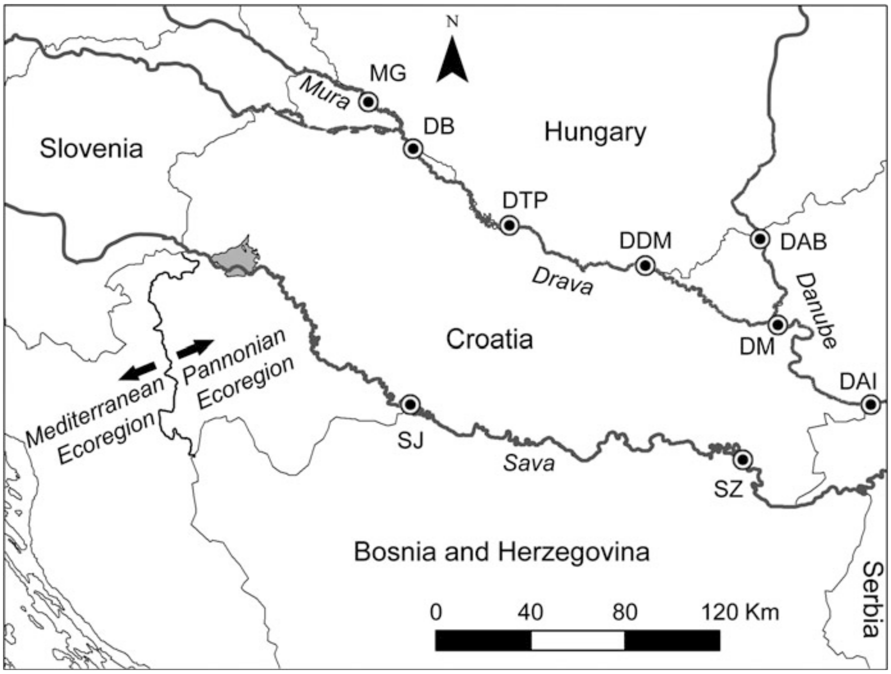

Fig. 1 Study area with the sampling sites indicated. Mura River, Goričan (MG); Drava River, Botovo (DB), Terezino Polje (DTP), Donji Miholjac (DDM) and river mouth (DM); Danube, Batina (DAB), Ilok (DAI); Sava River, Jasenovac (SJ) and Županja (SZ)

Table 1 Geological and hydrological properties of study sites

Sampling station	Code	Latitude	Longitude	Altitude (m)	Watershed (km^2)	Distance from mouth (km)	Width (m)	Depth (m)
Mura River								
Goričan	MG	46°24′43.40″	16°42′3.80″	140	13,148	33.5	94	4.7
Drava River								
Botovo	DB	46°14′29.76″	16°56′18.51″	123	31,038	226.8	127	3.1
Terezino Polje	DTP	45°56′44.15″	17°27′42.63″	98	33,916	152.3	210	5.5
Donji Miholjac	DDM	45°47′0.41″	18°12′3.79″	84	37,142	80.5	175	2.7
River mouth	DM	45°32′43.11″	18°54′45.49″	79	39,982	1.0	231	6.0
Danube								
Batina	DAB	45°53′21.90″	18°49′38.63″	82	210,250	1424.8	560	8.3
Ilok	DAI	45°13′57.16″	19°24′6.12″	73	253,737	1301.5	400	8.5
Sava River								
Jasenovac	SJ	45°16′7.85″	16°54′55.19″	89	38,953	500.5	210	8.1
Županja	SZ	45° 2′17.14″	18°42′10.63″	76	62,891	262.0	330	13.3

Sampling and sample analysis

Surface samples were collected monthly from the main-flow (thalweg) of the rivers between April and September 2010 (Kiss et al., 1996).

Phytoplankton samples were fixed with acidic Lugol's solution and stored in the dark at 4°C. Cells were counted with an inverted microscope following Utermöhl's (1958) method. A minimum of 400 settling units were counted in transect at 400× magnification, providing a counting error of <10% (Lund et al., 1958). Biovolumes were calculated by determining an average individual size from 30 randomly chosen cells of each species, and then multiplying by the observed species abundance (Rott, 1981). Biomass (freshweight) was derived from biovolumes and used for further analyses, where $1\ \mathrm{mm}^3\ \mathrm{l}^{-1} = 1\ \mathrm{mg}\ \mathrm{l}^{-1}$ (Wetzel & Likens, 2000). Taxa were assigned to FGs based on Reynolds et al. (2002), Borics et al. (2007) and Padisák et al. (2009) and MFGs based on Kruk et al. (2010).

Samples for water chemistry were taken simultaneously with phytoplankton samples. They were stored in special bottles and analysed in the laboratory. Oxygen and total suspended particles were analysed following the APHA (2005). Conductivity and pH were measured with electrodes of the SevenMulti Modular Meter System (Mettler Toledo). Total phosphorus (TP) and dissolved silicates were detected using a UV–VIS spectrometer (Perkin Elmer Lambda 25). Total nitrogen (TN) and total organic carbon (TOC) were analysed using a Shimadzu TOC-VCPH equipped with an analyser for TN and TOC.

Chlorophyll *a* was filtered with Whatman GF/F glass filters, extracted in 96% ethanol and measured using a UV–VIS spectrophotometer (Perkin Elmer Lambda 25) according to standard guidelines (APHA, 2005).

Average monthly water discharge data were obtained from the Croatian National Hydrometeorological Institute. Theoretical water residence time (WRT) was calculated as a function of drainage area (A_d, km^2) and discharge (Q, $\mathrm{m}^3\ \mathrm{s}^{-1}$) using the equation $\mathrm{WRT} = 0.08 \times A_\mathrm{d}^{0.6} \times Q^{-0.1}$ (Soballe & Kimmel, 1987; Leopold et al., 1995).

Data analysis

Principal component analysis (PCA) was carried out using the program PRIMER 6 (Clarke & Gorley, 2006) in order to group sampling sites according to their environmental parameters. Data were normalised prior to PCA. Results of PCA were plotted together with similarity clusters after calculating Euclidean distances.

The Shannon–Wiener diversity index (Shannon & Weaver, 1963) was calculated for each sample using phytoplankton abundance values.

Self-organising map (SOM) was used to analyse and visualise the phytoplankton groups parallel to the environmental variables. SOM is a neural network analysis tool for visualisation of high-dimensional data by reducing its dimensionality, but still preserving the most important features of the data. In this case it is the topological and metric relationships of the original data that are preserved (Kohonen, 2001). SOMs have frequently been used in diatom ecology and in studies describing benthic algal assemblages in France, Luxembourg or to define (Rimet et al., 2004; Gosselain et al., 2005) riverine phytoplankton assemblages Hungary (Várbíró et al., 2007). The multidimensional data of environmental variables can be visualised using a SOM, initially training it with only biological variables by applying a mask function that assigns a weight of 1 to the biological variables and a weight of 0 to environmental variables (Céréghino & Park, 2009).

The SOM Toolbox (http://www.cis.hut.fi/projects/somtoolbox) was used to implement the SOM in a MATLAB™ environment. The data matrix consisted of 52 samples and 36 variables (24 codons, 5 physical–chemical–hydrological variables, 7 functional traits). In the selection phase of creating the SOM, the weights of the output layer were initially assigned randomly. Then a sample was chosen randomly and the best matching unit (BMU) was selected by calculating the Euclidean distance between the weights of the input layer and the output layer. Selection of the BMUs was based exclusively on the FGs coded biomass.

Physical–chemical–hydrological variables and functional traits were masked out during this selection phase of the neural network. When the learning phase was finished, a map with hexagons was obtained in which each hexagon contained a virtual unit containing the calculated weight/codon composition. The resulting hexagon map with its weights was visualised using the SOM Toolbox as component planes (CPs). Each CP represents the supplied variables that the SOM algorithm has learned.

Results

Physical, chemical and hydrological characteristics

Median values and ranges for all physical, chemical and hydrological data, as well as for the level of chlorophyll *a*, are presented for each sampling site in Table 2. Mura River had lower values for temperature, conductivity, alkalinity and oxygen than Drava River, but a higher concentration of nutrients. Most physical and chemical parameters tended to increase downstream along the Drava River. The Danube had the highest parameter values among the four rivers investigated, but the values tended to decrease downstream. The same tendency was observed downstream the Sava River.

Many of the variables were correlated with each other, thus leaving only 11 distinct uncorrelated parameters for the consideration of multivariate statistical (Table 2). PCA grouped sites according to the rivers to which they belonged. The Mura River sampling site was statistically close to the Drava River sampling sites, while both Sava River sampling sites differed from those of the other rivers, and from each other. Danube sites were grouped separately from other investigated sites. The two axes in PCA analyses accounted for 56.3% of the cumulative variance in physicochemical data set with eigenvalues of 4.12 and 2.07, respectively. Four potamal rivers highly correlated with conductivity, TN, TP, oxygen and temperature. Axis 1 was presented by conductivity ($r = -0.41$), TN ($r = -0.40$) and TP ($r = -0.37$), explaining 37.4% of the variance. PCA axis 2 was positively influenced by temperature ($r = 0.56$) and oxygen ($r = 0.53$) and negatively by saturation ($r = -0.38$), explaining 18.8% of the variance.

Average monthly discharge (Q) and WRT significantly differed among the four tested rivers (ANOSIM analysis, $P = 0.001$). Both parameters tended to increase downstream in all cases (Table 2; Fig. 2). Mura River had the lowest average monthly discharge, with small fluctuations over time between 133 and 242 $m^3\ s^{-1}$. Drava River had the next-highest discharge, which ranged from 395 $m^3\ s^{-1}$ at the most upstream site (Botovo) to 878 $m^3\ s^{-1}$ at the mouth, with small fluctuations over time. The Danube had the highest average monthly discharge, between 2,286 $m^3\ s^{-1}$ at the upstream Batina site and 6,496 $m^3\ s^{-1}$ at the downstream Ilok site. The discharge peaked in June, but otherwise fluctuated little during the other months of the year. The Sava showed the greatest oscillations at both sampling sites and throughout the study period. The average monthly discharge ranged from 306 $m^3\ s^{-1}$ at the upstream Jasenovac site to 2,131 $m^3\ s^{-1}$ at the downstream Županja site.

Phytoplankton

A total of 222 algal taxa were identified in the samples. These belonged to nine major groups: Cyanobacteria (30), Euglenophyceae (13), Cryptophyceae (4), Dinophyceae (4), Chrysophyceae (9), Bacillariophyceae (65), Xanthophyceae (2), Chlorophyceae (91) and Zygnematophyceae (4).

Bacillariophyceae was the dominant taxonomic group in the majority of samples and contributed 6.4–93.7% to the total phytoplankton biomass. This group was the most dominant in all samples from Mura River, accounting for 73.1–93.7% of phytoplankton biomass. Chlorophyceae contributed 1.0–89.4% to the total biomass in Drava River, with its contribution to biomass peaking in August and September. This group contributed 4.1–49.1% to the total biomass of the Danube throughout the study period. On the contrary, it contributed only 0.4–11.1% to the total biomass in Sava River. Chryptophyceae were sometimes co-dominant with Bacillariophyceae and Chlorophyceae, contributing 0.1–46.8% to the total biomass, but the contribution varied widely and erratically over the study period. Euglenophyceae contributed significantly to the phytoplankton of the Sava River at the Jasenovac site, 16.9% in July and 26.8% in September. Other taxonomic groups made little or no contribution to the total phytoplankton biomass.

Taxa contributing more than 5% of the total biomass in individual samples were defined as descriptive species. A total of 41 descriptive species were identified (Table 3). The number of descriptive species was similar for the Mura (15), Danube (17) and Sava (18) rivers. Drava River had the greatest number (27). Three of these taxa were present in all four rivers: *Stephanodiscus hantzschii*, *Stephanodiscus* cf. *minutulus* and *Navicula lanceolata*. Nevertheless there were species that, despite accounting for a very small proportion of the total biomass, occurred in almost every sample: *Plagioselmis nannoplanctica*, *Cryptomonas* sp., small-celled *Stephanodiscus* sp., *Nitzschia acicularis* and *Scenedesmus* sp.

Total biomass was lowest in Sava River throughout the study period (0.03–0.71 mg l^{-1}), followed by the Mura (0.17–0.85 mg l^{-1}) and Drava River (0.14–3.92 mg l^{-1}). The Danube had the highest total biomass (0.21–20.94 mg l^{-1}) (Fig. 2).

Total phytoplankton biomass in relation to Shannon–Wiener diversity index and average monthly discharge is presented in Fig. 2. Two patterns of the total phytoplankton biomass were observed. At the Drava River sampling sites at Terezino Polje, Donji

Table 2 Median values and ranges of physical, chemical, and hydrological parameters and chlorophyll *a* concentration at all sampling sites from April to September 2010

	Mura		Drava							
	Goričan		Botovo		Terezino Polje		Donji Miholjac		Mouth	
	Median	Range	Median	Range	Median	Range	Median	Range	Median	Range
Water temperature (°C)	16.4	9.4–21.4	17.0	9.9–21.3	18.1	10.7–23.4	19.3	10.8–23.6	19.1	11.7–24.1
pH	8.01	7.85–8.15	8.03	7.95–8.18	8.11	7.95–8.21	8.14	7.93–8.17	8.16	7.92–8.38
Conductivity (μS cm^{-1})	318	262–509	299	279–379	302	283–377	318	301–386	319.5	288–393
Total suspended particles (mg l^{-1})	23	13.5–88.0	9.6	6.8–42.0	12.8	6.4–29.2	16.0	5.2–43.2	17.2	6.4–45.6
Dissolved oxygen (mg l^{-1})	8.9	7.8–10.2	8.8	8.0–10.6	8.7	8.3–10.5	8.85	8.4–10.6	8.5	6.9–10.8
Dissolved oxygen (%)	90	85.1–93.9	93.6	87.0–95.9	93.4	92.1–97.5	98.0	90.1–105.0	95.0	72.3–105.5
TN (μg N l^{-1})	1500	1360–2060	1170	960–1540	1235	920–1500	1280	930–1730	1310	920–1370
TP (μg P l^{-1})	81	58–215	51	28–109	67	30–90	69	40–114	79	42–130
Dissolved silicates (mg SiO_2 l^{-1})	6.3	5.1–7.6	5.1	4.2–6.2	5.06	4.7–6.5	4.8	3.9–6.7	5.4	2.8–6.2
TOC (mg l^{-1})	2.5	1.97–2.65	1.86	1.51–2.31	1.97	1.5–2.9	2.17	1.64–3.16	2.12	1.86–2.99
Q (m^3 s^{-1})	178	133–242	542	395–690	559	417–730	588.5	439–814	595.5	444–878
WRT (day)	14.1	13.9–14.5	21.2	20.6–21.8	22.2	21.6–22.9	23.4	22.6–24.0	24.4	23.4–25.1
Chlorophyll *a* (μg l^{-1})	2.7	0.5–7.9	3.8	1.7–5.1	4.9	1.4–8.8	8.51	2.2–20.3	8.4	2.7–28.4

	Danube				Sava			
	Batina		Ilok		Jasenovac		Županja	
	Median	Range	Median	Range	Median	Range	Median	Range
Water temperature (°C)	18.7	10.9–22.6	17.9	13.8–27.2	20.7	10.8–25.8	22.6	11.8–26.2
pH	8.32	7.98–8.63	8.01	7.91–8.23	7.81	7.63–8.09	7.95	7.87–8.06
Conductivity (μS cm^{-1})	464	387–565	392.5	367–450	394	365–434	413	381–468
Total suspended particles (mg l^{-1})	44.2	22.8–51.6	21.6	14.0–48.8	12.4	1.0–39.2	8.6	3.0–18.0
Dissolved oxygen (mg l^{-1})	9.2	7.7–12.0	8.1	6.5–10.1	6.8	5.1–9.0	6.8	6.1–9.3
Dissolved oxygen (%)	95.3	74.5–130.6	88.2	70.4–101.0	75.3	60.1–84.1	79.3	73.3–86.4
TN (μg N l^{-1})	1985	1760–2730	1755	1460–2440	1420	1270–2000	1380	1210–1910
TP (μg P l^{-1})	115	75–190	108	57–147	129	72–396	113	62–190
Dissolved silicates (mg SiO_2 l^{-1})	5.2	0.3–6.7	5.9	3.1–7.8	4.1	2.5–5.4	5.1	3.9–6.1
TOC (mg l^{-1})	3.11	2.52–3.51	3.30	2.74–4.16	3.82	1.82–4.91	3.10	1.63–4.79
Q (m^3 s^{-1})	3101.5	2286–5258	3740	2988–6246	905.5	306–1163	1171	441–2131
WRT (day)	9.1	8.7–9.4	24.2	23–24.8	23.0	22.4–25.6	29.9	28.1–32.9
Chlorophyll *a* (μg l^{-1})	6.2	3.6–80.5	14.2	3.3–22.6	3.8	0.2–9.2	0.9	0.2–5.6

TN total nitrogen, *TP* total phosphorus, *TOC* total organic carbon, *Q* mean monthly discharge, *WRT* water residence time

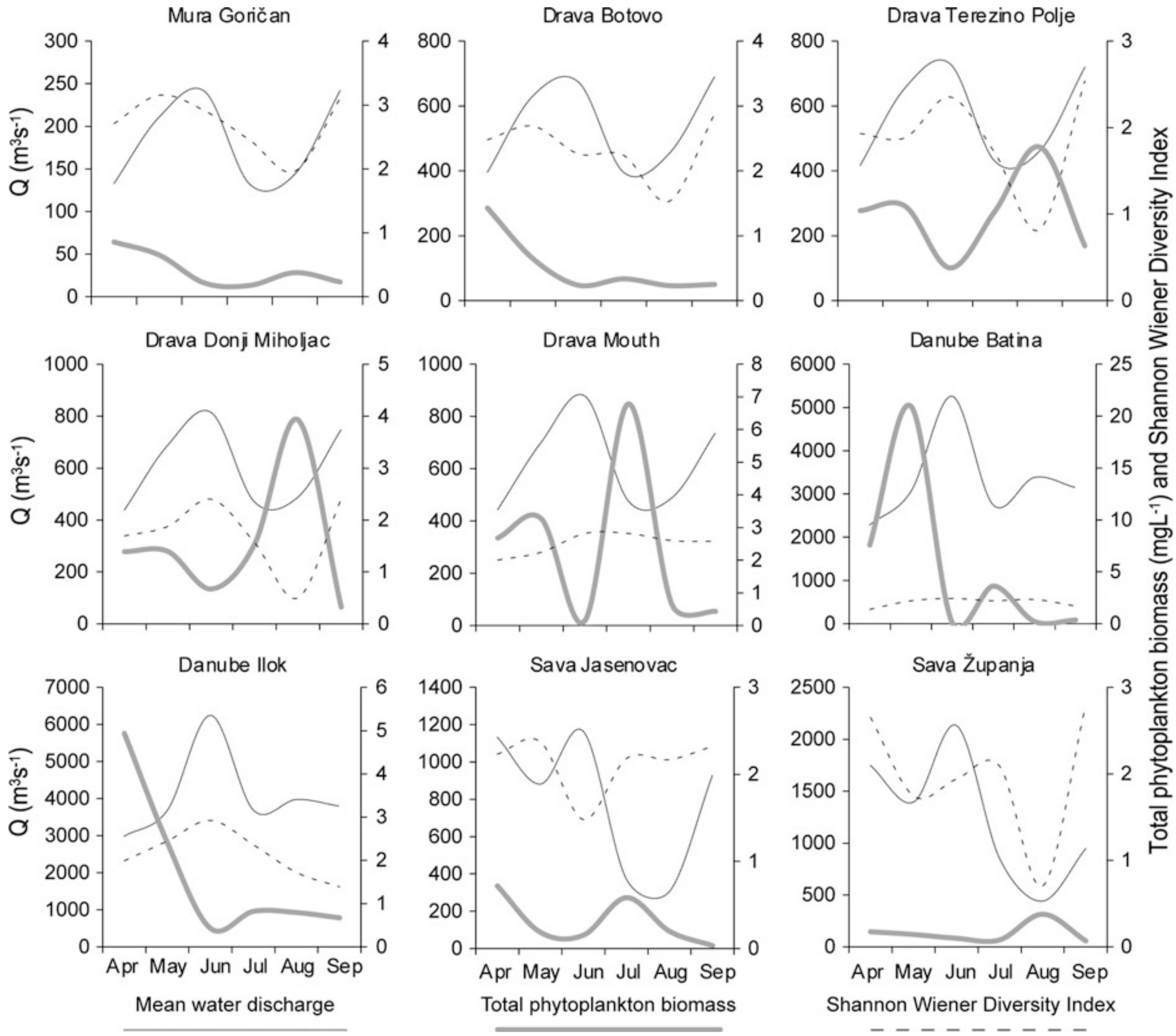

Fig. 2 Variations in average monthly discharge (*Q*), total biomass and Shannon–Wiener Diversity Index at the investigated sample sites

Miholjac and the river mouth, a maximum in biomass appeared in August. At all other sampling sites, the biomass reached a maximum in April or May.

Values of the Shannon–Wiener diversity index changed throughout the study period. It behaved similarly at all sampling sites at all four rivers (Fig. 2): it varied directly with average monthly discharge and inversely with total phytoplankton biomass, but without significance (linear regression, $P = 0.928$, $P = 0.250$).

A total of 24 FGs were detected. Those that contributed with low biomass (<10%) were grouped together as Others (Fig. 3). Throughout the study period, the dominant FGs were diatom groups with codons C, D and TB. Occasional dominance of codon T was also found in mid-summer, while other codons J, P, TD, W1, Y and X2 showed occasional co-dominance. Figure 3 also shows increasing tendency of codon T relative biomass in summer months with increase of WRT in Mura and Drava rivers. Downstream stations have higher relative biomass contribution with its maximum in Drava Donji Miholjac station, while in Drava Mouth station it decreases again, similar to Danube Batina. Sava River, as isolated hydrological system from other three investigated rivers had its specific FGs composition.

Table 3 List of taxa of dominant and subdominant species that contributed >5% to the total phytoplankton biomass recorded in the Mura, Drava, Danube and Sava Rivers during the study period

- Cyanophyceae
  - *Anabaena* sp.—M, Dr, Da, S
  - *Aphanizomenon flos-aquae*—Dr, Da
  - *Oscillatoria limosa*—M, Dr, Da
  - *Planktothrix agardhii*—Dr, Da, S
- Euglenophyceae
  - *Euglena* sp.—M, Dr, Da, S
- Cryptophyceae
  - *Cryptomonas* sp.—M, Dr, Da, S
  - *Plagioselmis nannoplanctica*—M, Dr, Da, S
- Bacillariophyceae
  - *Acanthoceras zachariasii*—M, S
  - *Asterionella formosa*—M, Dr, Da, S
  - *Asterionella ralfsii*—M, Dr, Da
  - *Aulacoseira granulata*—M, Dr, Da, S
  - *Aulacoseira* sp.—Dr
  - *Cocconeis placentula*—M, Dr, S
  - *Cyclotella meneghiniana*—M, Dr, Da, S
  - *Diatoma ehrenbergii*—M, Dr, S
  - *Diatoma vulgaris*—M, Dr, Da, S
  - *Encyonema silesacum*—M, Dr, S
  - *Fragilaria crotonensis*—M, Dr, Da, S
  - *Gyrosigma acuminatum*—Dr, Da, S
  - *Gyrosigma scalproides*—Dr, Da
  - *Melosira varians*—M, Dr, Da
  - *Navicula lanceolata*—M, Dr, Da, S
  - *Navicula* sp.—M, Dr, Da, S
  - *Nitzschia acicularis*—M, Dr, Da, S
  - *Nitzschia sigmoidea*—M, Dr, Da
  - *Nitzschia* sp.—M, Dr, Da, S
  - *Pinnularia* sp.—Dr
  - *Stauroneis* sp.—S
  - *Stephanodiscus* cf. *minutulus*—M, Dr, Da, S
  - *Stephanodiscus hantzschii*—M, Dr, Da, S
  - *Stephanodiscus* sp.—M, Dr, Da, S
  - *Surirella brebissonii*—M, Dr, Da, S
  - *Ulnaria acus*—M, Dr, Da, S
  - *Ulnaria ulna*—M, Dr, Da, S
  - *Vibrio tripunctatus*—M, Dr, S
- Chlorophyceae
  - *Actinastrum hantzschii*—M, Dr, Da, S
  - *Actinochloris* sp.—Da
  - *Chlamydomonas* sp.—M, Dr, Da, S
  - *Gleotilla* sp.—M, Dr, Da, S
  - *Pandorina morum*—M, Dr, Da, S
  - *Spermatozopsis exsultans*—S

M Mura River, *Dr* Drava River, *Da* Danube, *S* Sava River

All seven MFGs were found (Fig. 4). The dominant MFG was group VI, corresponding to non-flagellated organisms with a siliceous skeleton. Group IV, corresponding to medium-sized organisms lacking special traits, showed mid-summer dominance. Group V, corresponding to unicellular flagellates of medium to large size, often showed subdominance.

The following MFGs did not contribute significantly to the total biomass of river phytoplankton: II, small flagellated organisms with siliceous exoskeletal structures; III, large filaments with aerotopes; and VII, large mucilaginous colonies. MFG I, corresponding to small organisms with high S/V, was present in only six samples and made an extremely low contribution to total biomass. Figure 4 shows that group IV has a tendency to increase in summer months following the increase of WRT in Mura and Drava rivers, where it is also observed that downstream stations have higher relative biomass contributions, with its maximum in Drava Donji Miholjac station. However, it was observed that Group IV decreases in Drava Mouth station similar to Danube Batina, while Sava river had different MFGs composition from other investigated rivers.

Different FGs and MFGs showed different relationships with nutrients and WRT, as described below.

SOM analysis

SOM analysis was performed based on relative codon biomass (Fig. 5). The rivers were clearly separated on the SOM map, which can be attributed to the different codon distribution in the four rivers. The darker colours of the CPs in Fig. 6 indicate higher biomass of the given codon.

Relationships were also investigated among the background variables TP, TN, WRT and biomass, expressed both in terms of chlorophyll *a* (μg l^{-1}) and biomass (mg l^{-1}) (Fig. 7). The SOM showed that the variables WRT and TN explain the variation in phytoplankton biomass. In contrast, TP showed an inverse relationship with algal biomass.

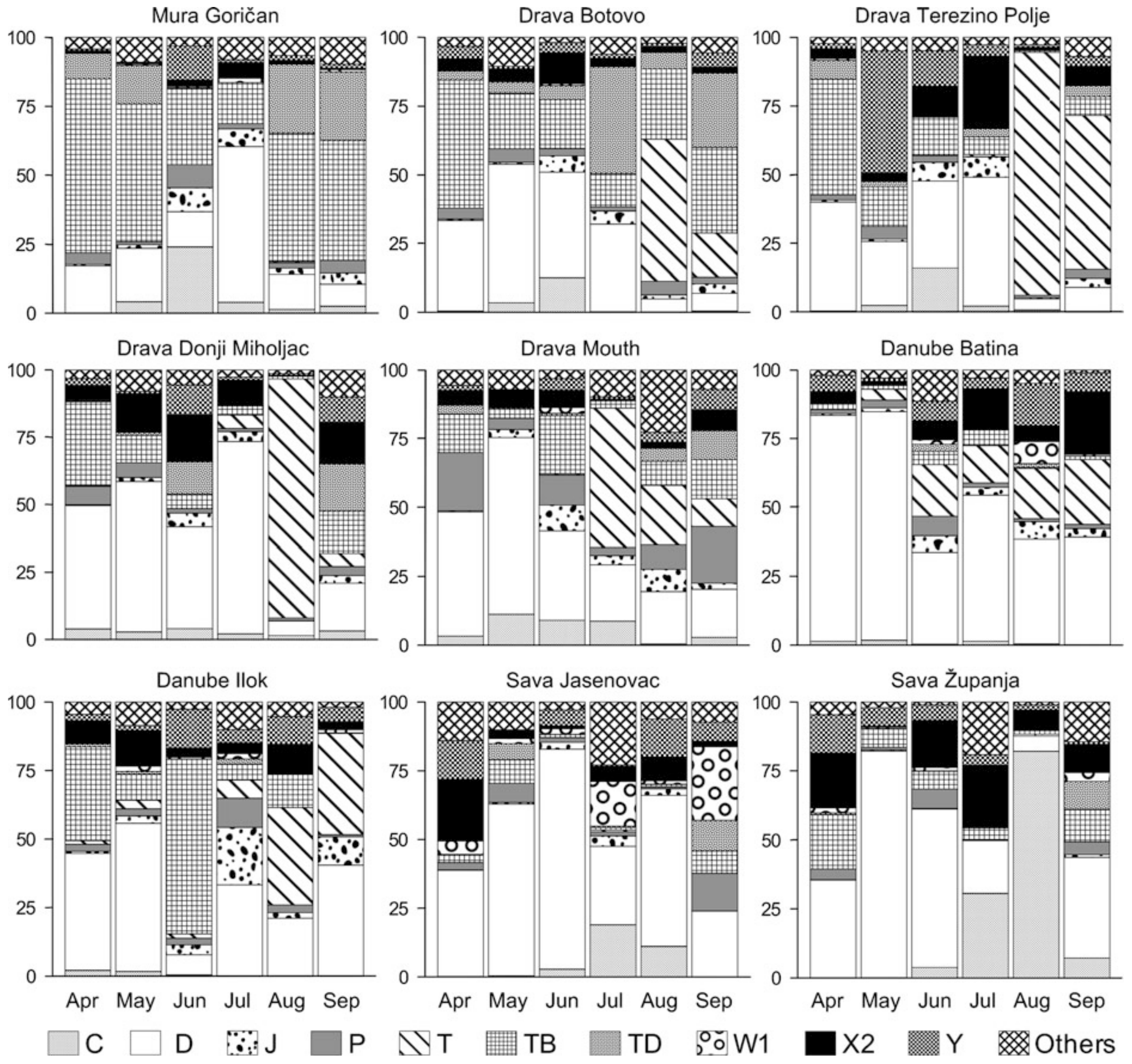

Fig. 3 Relative biomass of phytoplankton functional groups (Reynolds et al., 2002; Borics et al., 2007; Padisák et al., 2009) at all sampling sites. Functional groups that contributed with low biomass (<10%) presented as Others are: A, B, E, F, G, H1, K, L_M, L_O, N, TC, W2, X1 and X3

Comparison of the CPs of the physical and chemical variables with that of codons reveals that FGs have different preferences. The euplanktic C, D, J, L_O, Y, X1 and P codons prefer longer WRT. Conversely, the TB codon, corresponding to benthic diatoms that are the most characteristic of the upper river segments, preferred shorter WRT.

Analysis of the MFGs showed that groups V, VI and VII are not separated (Fig. 8). All of these MGFs were associated with higher WRT, while no MFG was associated with the shortest WRT values.

Discussion

Lotic systems have extremely dynamic hydrological regimes, leading to large fluctuations in abiotic and biotic factors along the river continuum (Vannote

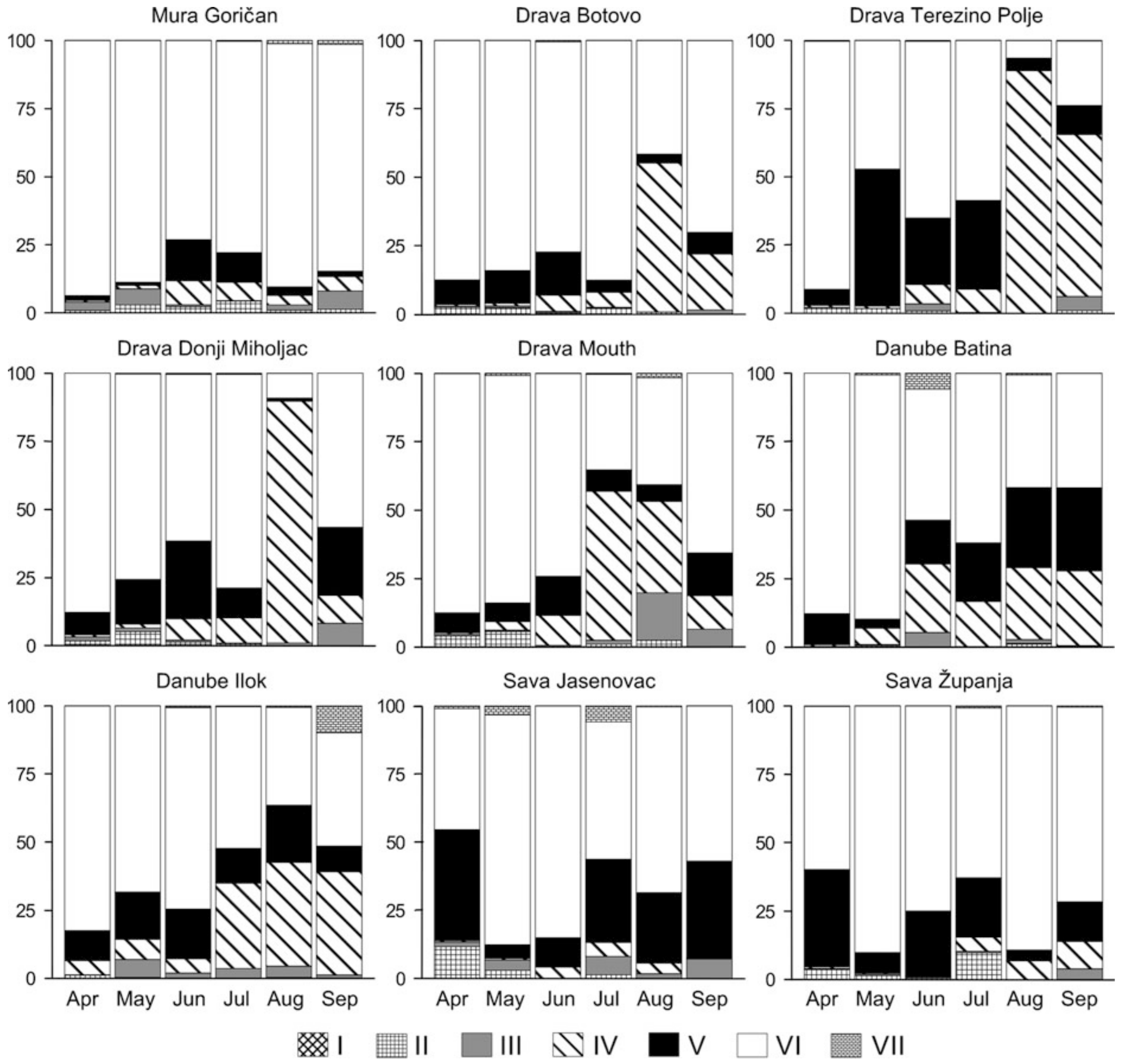

Fig. 4 Relative biomass of phytoplankton morpho-functional groups (Kruk et al., 2010) at all sampling sites

et al., 1980). Geology and geography, together with hydrology, are key factors of lotic environments that can make them more or less suitable biotopes for living organisms despite the turbulence in their characteristics (Wetzel, 2001). The rivers in the present study are large and flow in Pannonia lowlands; therefore they are predicted to be suitable habitats for the phytoplankton community known as potamoplankton (Borics et al., 2007).

The composition of the microflora of the four rivers in the present study is similar to that of other potamal rivers in the region (Várbíró et al., 2007). Similarly to the present study, a comprehensive, long-term evaluation of Danube microflora found that Chlorococcalean green algae and centric diatoms are the most relevant elements of the potamoplankton (Kiss, 1997). Indeed, the species identified in the present study as constant elements of the microflora are nearly identical to those identified in that dataset collected over more than 20 years. The tendency of the proportion of chlorophytes in the phytoplankton to increase with the size of the river, showed also in this study downstream in Mura and Drava rivers, can be considered a general feature of potamal river phytoplankton (Kiss & Genkal, 1996). In

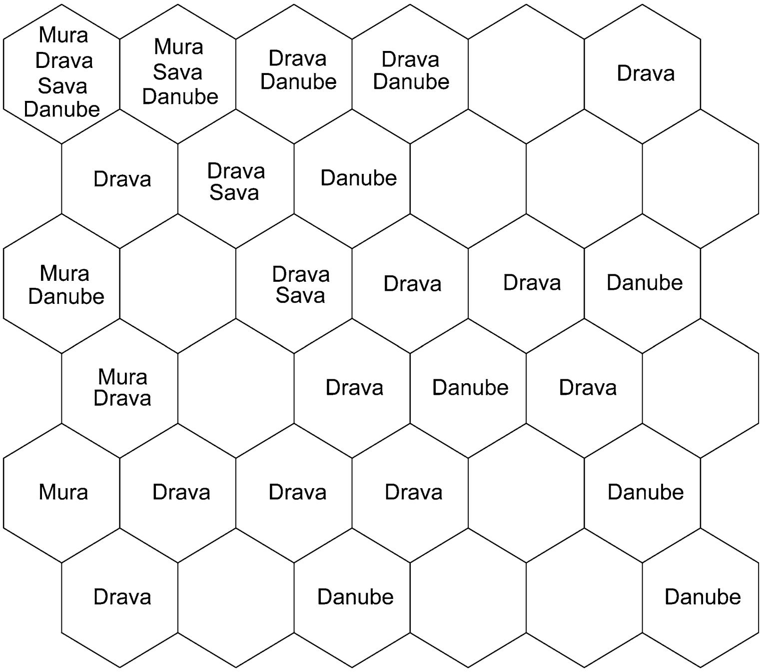

Fig. 5 The location of river sampling sites on the self-organising map (SOM). The map was produced as a result of the training and learning process based on functional group biomass

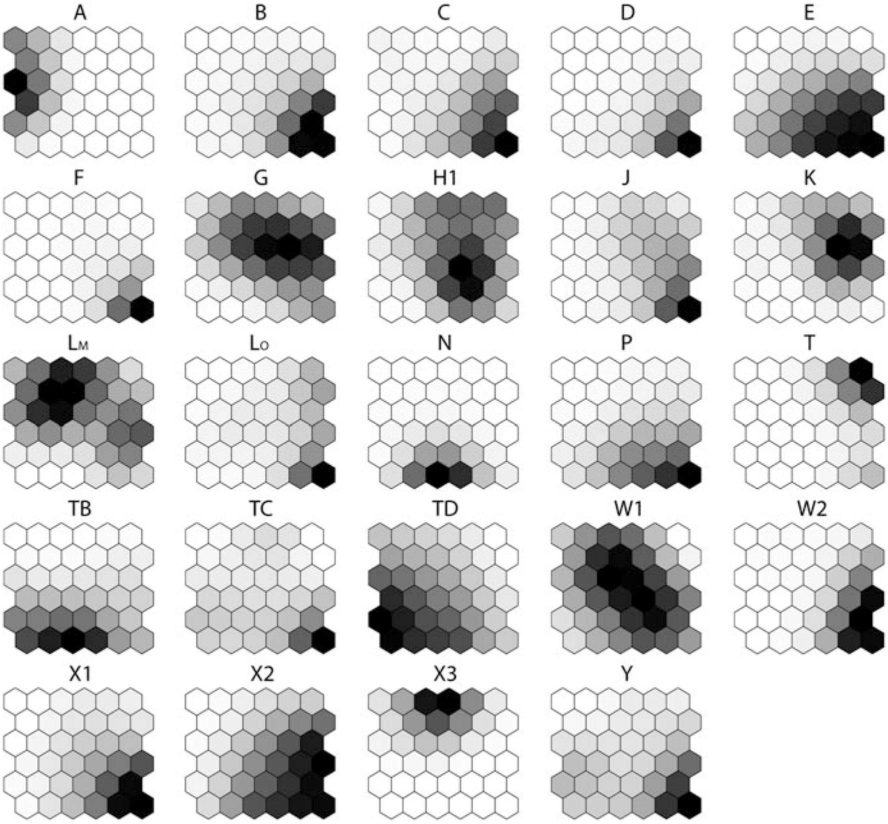

Fig. 6 Component planes of the self-organising map (SOM) showing gradient distribution of important functional codons. *Darker colours* indicate greater biomass

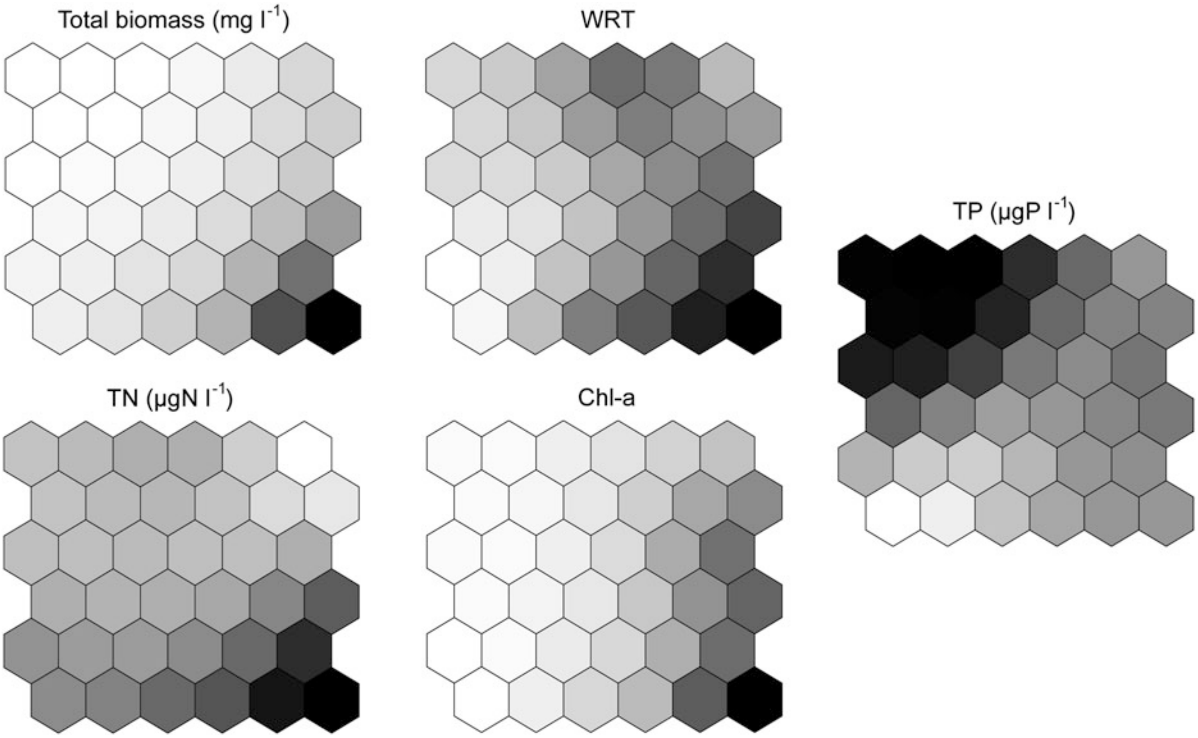

Fig. 7 Component planes of the SOM showing gradient distribution of the chemical and hydrological variables measured. *Darker colours* indicate greater values for the variable measured. *WRT* water residence time, *TN* total nitrogen, *TP* total phosphorus, *Chl-a* chlorophyll *a*

fact, chlorophytes can account for as much as 60% of the species diversity (Kiss & Schmidt, 1998). Euglenophytes occasionally appear as abundant members of microflora. For example, these taxa were observed to be more abundant in the Danube (Kiss, 1997) and in the Tisza River (Uherkovich, 1971). In both of those studies, the authors suggested that the organisms had arrived via the slow-flowing side arms of the rivers and/or via the oxbows of the floodplain. We found these taxa in the Sava River, which features a near-natural floodplain in the central, unregulated section.

Changes in phytoplankton diversity, not only in lakes but also in rivers, can be explained by the intermediate disturbance hypothesis (IDH) (Carvajal-Chitty, 1993; Padisák et al., 1993). The most frequent disturbances are river floods (Descy, 1993), which alter the entire river ecosystem. All discharge-driven variables like WRT, amount of suspended solids and concentration of nutrients are fundamental determinants of phytoplankton composition and biomass (Pieterse & van Zyl, 1988). During low-discharge periods, the phytoplankton of large rivers is similar to that of shallow lakes (Reynolds et al., 1994), dominated by euplanktic elements belonging to FGs J, X1, C and D (Várbíró et al., 2007). Floods restructure the phytoplankton, and the relative biomass of the tichoplanktic taxa increases. In the present study, the flood periods were associated with phytoplankton assemblages of high diversity and low biomass. Consistent with the predictions of the IDH, we found that the lengthy low-discharge periods in late summer allowed the development of high-biomass, low-diversity phytoplankton assemblages.

For both rivers and lakes, a clear non-linear relationship exists between TP and concentration of chlorophyll *a* (Van Nieuwenhuyse & Jones, 1996; Phillips et al., 2008). In rivers, this relationship is very strong when an extremely broad TP range is considered, but it is much less clear over narrower TP ranges. In the present study, the investigated rivers were neither phosphorus- nor nitrogen-limited because TP and TN values exceeded both mesotrophic (29 $\mu g\ l^{-1}$ for TP and 285 $\mu g\ l^{-1}$ for TN) and eutrophic boundary (71 $\mu g\ l^{-1}$ for TP and 714 $\mu g\ l^{-1}$ for TN) as suggested

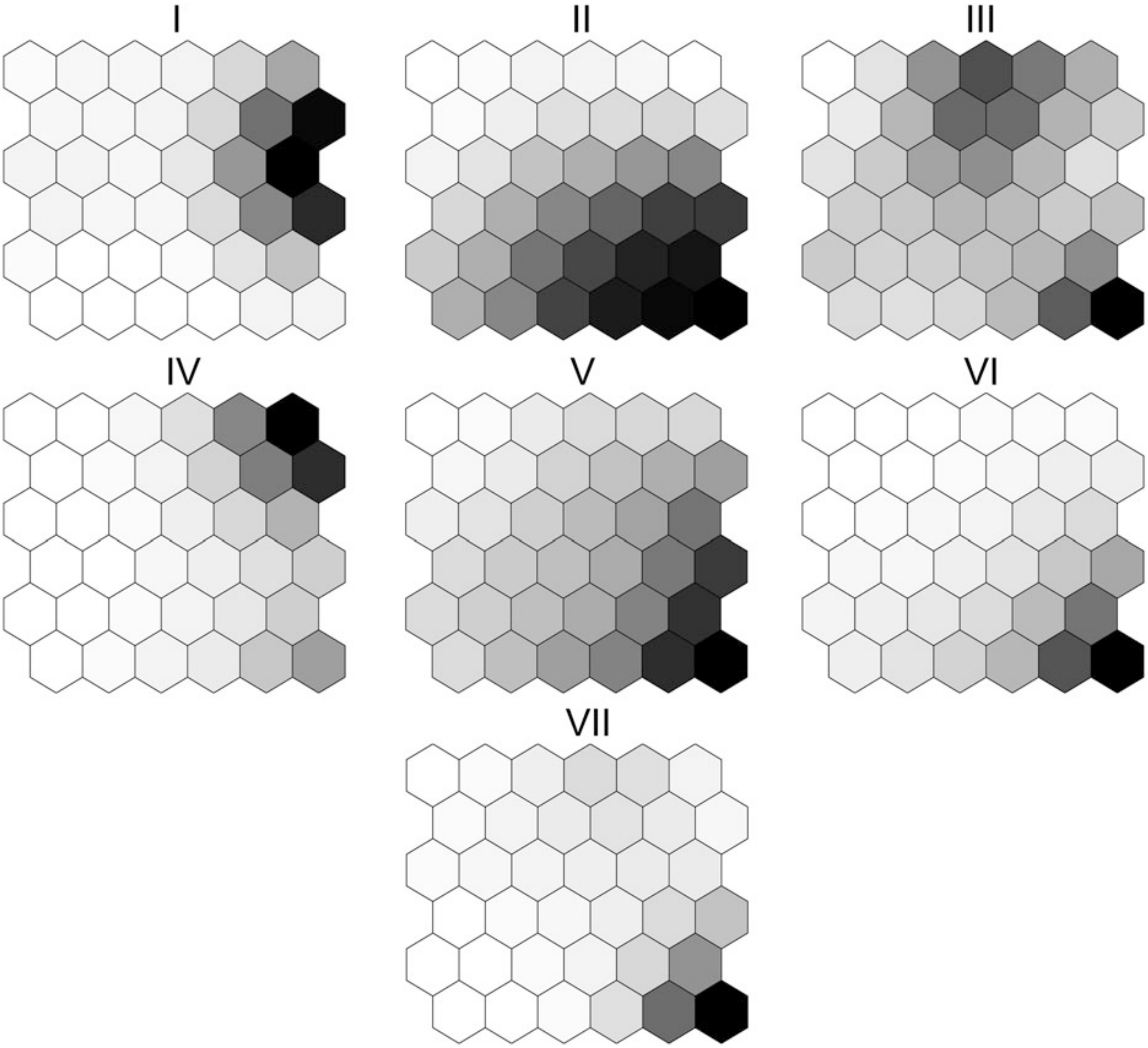

Fig. 8 Component planes of the SOM showing gradient distribution of the morpho-functional traits. *Darker colours* indicate greater biomass of the given functional trait

by Dodds (2006). The concentration of chlorophyll *a* highly varied from oligotrophic to hypertrophic situation with highest values (Dodds, 2006). In such nutrient-rich systems biotic interactions can be important, although phytoplankton biomass is controlled primarily by physical factors (Reynolds, 1988) which was confirmed by our SOM analysis that clearly indicated that in the four potamal rivers TP was not predictor of autotrophic biomass In this study, WRT and TN appeared as the best predictors of chlorophyll *a* and total biomass, in coincidence with Salmaso & Braioni (2008) and Salmaso & Zignin (2010) who asserted that discharge and variables directly linked to water fluxes significantly impacted phytoplankton biomass while nutrients showed occasional influence.

The analysis of the relationship between WRT and algal FGs revealed different behaviours. Not surprisingly, FGs D, J and X1 were strongly associated with high WRT. These groups frequently dominate the phytoplankton of shallow lakes and large, slow-flowing rivers (Reynolds et al., 1994; Schmidt, 1994). The tichoplanktic FGs TB, TD and TC (tichoplanktic Bacillariophyceae, desmids and cyanobacteria) show the opposite tendency (Borics et al., 2007): these algae dominate rivers of lower order, but during high-discharge periods they can also dominate potamal river phytoplankton, since the habitat zones can shift downstream significantly during such periods. Indeed, this shift can be more than 100 km in the large lowland river Tisza (Uherkovich, 1971). Our data suggest that WRT did not influence the biomass of other FGs.

Abonyi et al. (2012) successfully used FGs for understanding seasonality and longitudinal changes of river phytoplankton and also like Borics et al. (2007) used FGs for water quality assessment. The results of our SOM analysis of MFGs in these four rivers that we obtained and discussed previously in detail were not as clear as those we found for FGs. In our rivers, MFG analysis did not show clear separation of euplanktic and tichoplanktic organisms because as already mentioned, MFGs that we tested are originally developed from lake data. The dominance of diatoms in potamoplankton also does not favour MFGs because they are represented by only one MFGs group and that is group VI, while FGs have fine partition of diatom codons. Thus, we found that the ability of MFGs analysis to reveal characteristics of river phytoplankton tested here for the first time is limited, while FGs were once more confirmed to be good predictor (Devercelli, 2006; Borics et al., 2007; Abonyi et al. 2012).

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