Effect of Land Use on the Benthic Diatom Community of the Danube River in the Region of Budapest

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Abstract: (1) Urbanization significantly influences the ecosystems of rivers in various ways, including the so-called loading effect of wastewater production. Benthic diatoms are used in ecological status assessments of waters. Beside species composition, traits can be used as indicators. We aimed to evaluate how the loading of the large city of Budapest manifests in the physico-chemical variables of the River Danube and what species composition and trait response this loading results in for the benthic diatom communities. (2) Weekly samplings were performed at points upstream and downstream of Budapest on both riverbanks. Samples were compared, based on general physical-chemical variables and the concentration of thirty-four elements, as well as species composition and seven traits of species of diatom communities. Ecological status was assessed using the Specific Pollution Sensitivity Index (IPS). (3) Only a few measured environmental variables showed differences between the sampling points, suggesting that the nutrient loading has significantly decreased due to the installation of several efficiently working wastewater treatment plants since the introduction of the European Union Water Framework Directive. In contrast, the species composition and traits of species showed the effect of land use. Benthic diatoms indicate the environmental changes caused by land use in the longer-term, while chemical measurements reflect instantaneous status.

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1. Introduction

Cities have been a part of human history for millennia and urbanization is a pervasive and rapidly growing form of land use [1]. Urban areas drive environmental change at multiple scales, altering the land use and cover, biodiversity and hydrosystems locally to regionally and affecting local to global biochemical cycles and climate [2].

Many urban centers developed around rivers, which were the lifeblood of commerce [1]. Urbanization affects riverine ecosystems in various ways, including their hydrology (e.g., more frequent, larger and more rapid flow events), geomorphology (e.g., channel erosion), temperature (e.g., higher summer and lower winter values than in forested rivers) and chemical characteristics (e.g., nutrient load, introduction of contaminants such as metals, pesticides) due to increased catchment imperviousness, engineering interventions, riparian deforestation and introduction of effluents of wastewater treatment plants [1,3]. Via these changes, urbanization considerably influences all levels of aquatic biota and the ecosystem function. The consistent effects of urbanization on stream ecosystems are termed the urban stream syndrome [4].

One of the most important parts of this syndrome is the so-called loading effect due to wastewater production which strongly depends on the daily amount of treated and untreated communal and industrial wastewater introduced into the river, the efficiency of wastewater treatment technology for removal of different organic and inorganic pollutants, as well as the water yield of the river resulting in a given dilution of effluents. The wastewater treatment plants (WWTPs) should remove the main nutrient compounds (P and N), the toxic heavy metals (e.g., Cu, Cr, Ni, Hg) and organic micropollutants (e.g., pharmaceutical residues, microplastics); however, the biodegradation of synthetic organic molecules is often hampered by xenophore groups (e.g., $-F$, $-CF_3$, etc.) or their chemical structures [5]. To characterize the removal efficiency of WWTPs, the pH, electric conductivity, alkalinity, total phosphorous, nitrogen and carbon content, the chemical oxygen demand, concentrations of toxic heavy metals and metalloids, anions (Cl^-, SO_4^{2-}, PO_4^{3-}, NO_2^-, NO_3^-), cations (NH_4^+, Na^+, K^+, Mg^{2+}, Ca^{2+}) and suspended solids are usually determined [6,7]. Beside measuring chemical parameters, biological elements are used for the investigation of the loading effect of large cities on the water quality (e.g., References [8,9]). In general, the community composition changes and tolerant species become more abundant while the proportion of sensitive species decreases and therefore diversity also declines [1,3].

The diatoms are eukaryotic algae with a silica cell wall called a frustule [10]. They constitute a frequently dominant group in aquatic ecosystems, playing significant roles in primary production, as well as silica cycling [11]. Their large species diversity [12] and their ability to respond directly and sensitively to specific physical, chemical and biological changes make them efficient indicators of environmental conditions [13]. Diatoms are often dominant in benthic habitats and they are a good proxy for the benthic algal community [14]. Benthic diatoms are worldwide used in the ecological status assessment of aquatic ecosystems [15]. European Union Water Framework Directive [16] requires the ecological assessment of surface waters and recommends methods based on biological elements including benthic diatoms for this. This assessment is mainly based on indices calculated from the pollution sensitivity and relative abundances of diatom taxa in benthic communities [17].

Lately, more and more attention has been paid to traits that are "any morphological, physiological or phenological feature measurable at the individual level, from the cell to the whole-organism level, without reference to the environment or any other level of organization" [18] with the aim of linking them to their appearance along environmental gradients [19]. The most widely known and investigated traits of diatoms are the ecological guilds introduced by Passy [20]—modified and supplemented by Rimet and Bouchez [21]—and size (e.g., Reference [17]). Classification of diatoms according to these traits can be influenced by several environmental variables (e.g., Reference [19]). Other traits are more directly linked to physiological properties (e.g., traits proposed by van Dam et al. [22]) but are characteristic of the species level.

In the present study, we aimed to evaluate how the loading of a large city manifests in physical-chemical variables and what species composition and functional (trait) responses this loading results in the diatom communities. We carried out this study in the Danube River, which is Europe's second-largest catchment area and serves more than 80 million people by providing drinking water, industrial and agricultural water supply, hydroelectric power generation, tourism and fisheries among others [23]. We investigated the effect of Budapest with a population of 1.75 million, comparing

samples taken upstream and downstream of the city based on environmental variables and epilithic diatom assemblages. For comparison, we could use the data of the Joint Danube Surveys which have been conducted every six years since 2001 and which aim to investigate the status of the River Danube in various aspects, including hydromorphology, water chemistry and biological elements including phytobenthos [24].

2. Materials and Methods

2.1. Study Site

The left and right banks of two sections of the River Danube were designated for sampling—one section was 20 km upstream, the other was 12 km downstream of the center of Budapest (Figure 1).
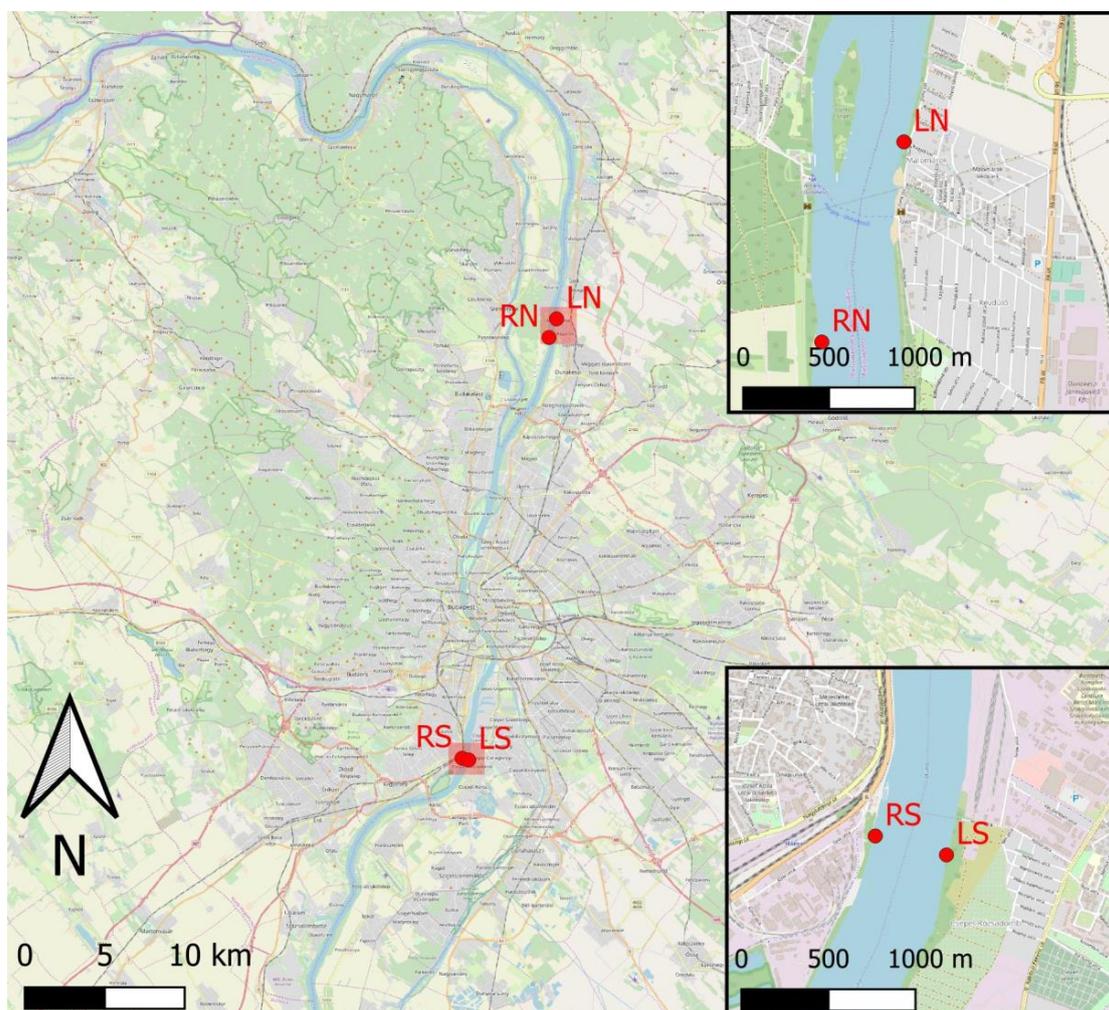
Figure 1. Map illustrating the location of sampling points. Markings: RN = upstream of Budapest, right bank; LN = upstream, left bank; RS = downstream, right bank; LS = downstream, left bank. The map was prepared with the data of Open Street Map (© OpenStreetMap contributors).

The right bank of the upstream section is on Szentendre Island, which is sparsely populated and is not characterized by intensive agriculture; therefore it can be regarded as a slightly impacted area. Moreover, this region involves a large protected area delivering about 90% of the drinking water demand of Budapest. The other three sampling points are regarded as impacted.

The left bank of the upstream section is affected by the town of Dunakeszi, which has an area of 31.06 km^2 and a population of 43,490 inhabitants (on 1 January 2018, [25]). Two waterworks and

one wastewater treatment plant belong to the town. Alagimajor, on the periphery of the town, is an agricultural area with farms [26].

The downstream section was impacted by the effects of Budapest.

Budapest, the capital of Hungary, is situated in the middle of the Danube basin on both banks of the river. The city area is 525.14 km^2 and its inhabitants number 1,749,734 (on 1 January 2018, [25]). Around 7% of the area (36.71 km^2) is protected (mainly in Buda). Mean annual total precipitation is 593 mm, with two wet periods (May–June and November–December) and two drier periods (February–March and September–October) which alternate [27]. Mean daily temperature is highest between July and August (22 °C) and lowest between December and February. The daily temperature fluctuation is highest from May to August and it can exceed 10 °C. The prevailing wind direction in the region is north-western [27].

The main factors affecting the quality of surface waters in the region of Budapest are—effluents of wastewater treatment plants (the three main plants are the North-Pest, the South-Pest and the Central Wastewater Treatment Plant located on the Csepel Island in the southern part of Budapest), the operation of combined sewer overflows during storms (in Budapest there are 35 combined sewer overflows), the introduction of extracted thermal waters after usage (both affecting chemical composition and temperature), industrial sewerage (service, manufacturing and energy industry) and hydromorphological interventions (e.g., flood defenses, two spurs, Kvassay Lock). A daily average of about 500–600 thousand m^3 of wastewater is produced in Budapest, 95% of which is biologically treated and introduced into the Danube and the Ráckeve (Soroksár) Danube arm. In December 2016, 97.3% of Budapest was connected to sewerage [27].

According to the hydromorphological assessment during the Joint Danube Survey 3 at the upstream site, the left floodplain was extensively modified, the left bank, the channel and the right bank and floodplain were moderately modified, while at the downstream site both floodplains were severely modified and both banks and the channel were extensively modified [28].

We estimated the land use at the sampling points based on the method developed by Belletti et al. [29] by fitting a 500 m shore side buffer zone. This also showed an urbanization gradient from the right bank upstream that could be regarded as a reference point through the more affected left bank upstream of the most impacted downstream points (Table 1).

Table 1. Classification of sampling points according to land use, following Belletti et al. [29]. Rankings: 1—undisturbed/natural, 2—disturbed-modified, 3—artificial-heavily modified.

Sampling Point	Vegetation Cover	Bank Modification	Land Cover in 500 m Buffer	Sum
Upstream right bank	1	1	1	3
Upstream left bank	2	2	2	6
Downstream right bank	3	3	3	9
Downstream left bank	2	2	3	7

In the further part of the paper, we use the following markings for sampling points and the samples: RN—upstream (northern) to Budapest, right bank, LN—upstream (northern) to Budapest, left bank, RS—downstream (southern) to Budapest, right bank, LS—downstream (southern) to Budapest, left bank.

2.2. Sampling

Sampling was carried out weekly from 20 June to 30 July 2018. Epilithic samples were taken from five randomly chosen cobbles at each sampling point, scraping the biofilm with a toothbrush into tap water. Samples were preserved with formaldehyde (4% final concentration) until preparation [30].

2.3. Environmental Variables

Physico-chemical parameters: temperature, pH, conductivity, dissolved oxygen and turbidity were measured by a portable Hanna Multi Meter (Hanna Instrument, Woonsocket, RI, USA) and a Turbidity meter (Lovibond, Dortmund, Germany), respectively. Alkalinity, total hardness, orthophosphate total phosphorous and nitrite concentrations were determined by standard titrimetric and spectrophotometrical methods [31]. Anion (F^-, Cl^-, SO_4^{2-}, NO_3^-) and cation (Na^+, K^+, Mg^{2+}, Ca^{2+}, NH_4^+) concentrations were measured by applying DIONEX dual channel ion chromatography (Thermo Fisher Scientific, Waltham, MA, USA). Total organic carbon and total nitrogen concentrations were determined by using a Multi N/C 3100 TC-TN analyzer (Analytik Jena, Jena, Germany) according to the valid international standards (EN ISO 5667-3:1995 and MSZ EN 12260:2004). Elemental concentrations were measured applying a Plasma Quant Elite inductively coupled plasma mass-spectrometer (Analytik Jena, Jena, Germany) [31]. Water discharge data measured at Budapest were obtained from the webpage of the Hungarian Hydrological Forecasting Service [32].

2.4. Diatom Sample Processing

Diatom valves were cleaned with hydrogen-peroxide and hydrochloric acid. Cleaned valves were mounted with Naphrax mountant. Diatom taxa were identified under an Olympus IX70 inverted microscope equipped with differential interference contrast (DIC) optics at a magnification of 1500×. A minimum of 400 valves were identified to species or genus level.

2.5. Diatom Index and Trait Data

In order to assess the ecological status of the sampling points, the Specific Pollution Sensitivity Index (IPS, Coste in Reference [33]) was calculated using OMNIDIA 6.0.2 [34].

Eight diatom groups were assigned for each taxon: nitrogen uptake, oxygen requirement, trophity, saprobity, pH adaptation, ecological group and size. We used the ecological guilds proposed by Rimet and Bouchez [21] under the term ecological groups, following the suggestion by Tapolczai et al. [19]. For cell size, the categories determined by Lange et al. [35] were applied. For the other traits, the classifications of van Dam et al. [22] were applied. Values of these traits were obtained from the OMNIDIA 6.0.2 database [34].

2.6. Data Analysis

Two kinds of environmental variables were measured: general physico-chemical variables (chlorophyll *a*, temperature, pH, conductivity, turbidity, total hardness, total organic carbon, total nitrogen, total phosphorous, PO_4^{3-}-P, F^-, Cl^-, SO_4^{2-}, NO_3^-, NO_2^-, HCO_3^-, Na^+, K^+, Mg^{2+}, Ca^{2+}) and element (Ti, Fe, As, Se, Li, B, Al, Rb, Sr, Zr, Mo, Cd, Sn, Sb, I, Cs, Ba, Hg, Tl) concentrations. We analyzed these two groups separately. Two-way analysis of variance (two-way ANOVA) with Tukey's pairwise post hoc test was used to study whether environmental variables showed differences between either sites or banks.

The coefficient of variation was calculated to illustrate the homogeneity of variables based on the following formula:

$$CV\% = \frac{s}{\bar{x}} \times 100\%,$$

where s is standard deviation and is mean.

We applied two-way permutational multivariate analysis of variance PERMANOVA to compare samples based on the measured environmental variables): one factor was the site (upstream and downstream) and the other was the bank (right and left). One-way PERMANOVA was used to compare sample groups according to water regimes. Cluster analysis performed with Ward's method based on Euclidean distance was applied to illustrate the grouping of samples.

Relative abundance values of species and trait categories were calculated and used in statistical analyses. Samples were compared based on the composition of species as well as trait categories

with two-way PERMANOVA using the site (upstream and downstream) and the bank (right and left) as factors. Non-metric multidimensional scaling (NMDS) with the Bray-Curtis dissimilarity index was used for visualizing the separation of samples. The Similarity percentage (SIMPER) method was applied for visualizing taxa which were responsible for the observed difference between groups of samples [36].

Analyses were performed with the PAST version 3.0 software [37].

Boxplots were prepared with R version 3.5.3 [38]. The map was prepared using QGIS [39] with the data of Open Street Map (© OpenStreetMap contributors).

## 3. Results

3.1. Environmental Variables

Coefficient of variation was highest (above 50%) in the case of chlorophyll *a* concentration (121%), turbidity (52%), nitrite (52%), total phosphorus (52%), orthophosphate (61%), iron (78%), cadmium (197%), tin (294%), mercury (73%), lead (230%), manganese (61%), copper (59%) and zinc (232%). Minimum, mean and maximum values of all parameters are collected in Table S1.

Based on environmental variables, samples did not show significant separation according to either sites or banks. Instead, they tended to group according to sampling date, based on both general variables (chlorophyll *a*, temperature, pH, conductivity, turbidity, total hardness, total organic carbon, total nitrogen, total phosphorous, PO_4^{3-}-P, F^-, Cl^-, SO_4^{2-}, NO_3^-, NO_2^-, HCO_3^-, Na^+, K^+, Mg^{2+}, Ca^{2+}) and element (Ti, Fe, As, Se, Li, B, Al, Rb, Sr, Zr, Mo, Cd, Sn, Sb, I, Cs, Ba, Hg, Tl) concentrations (Figure 2A,B).
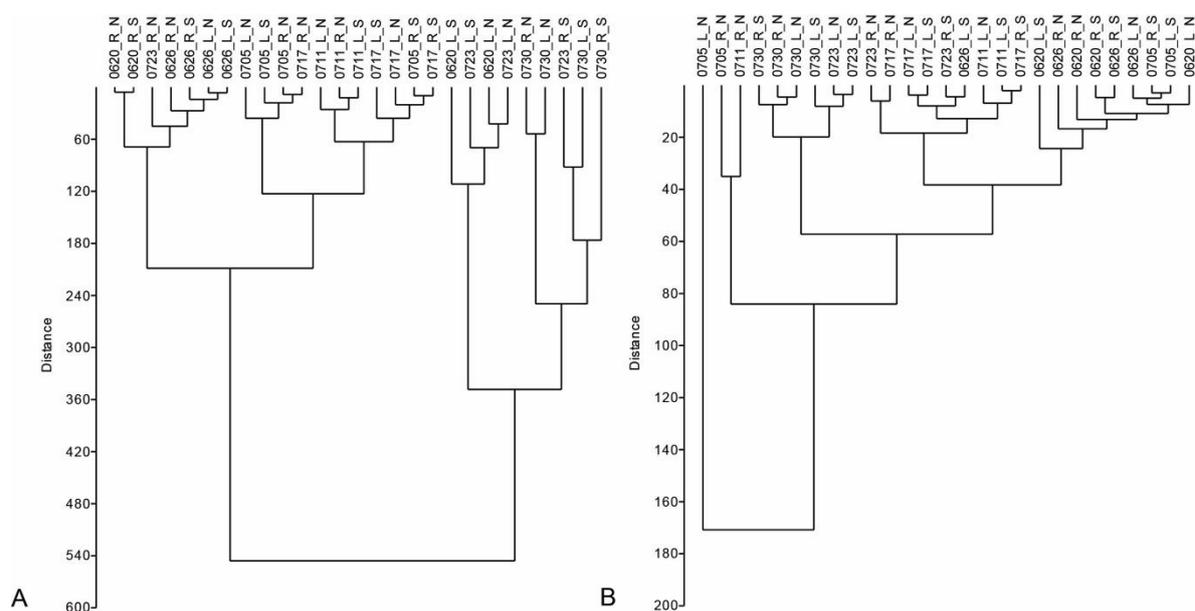
Figure 2. Euclidean distance-based cluster analysis (using Ward's method) of general physical-chemical variables (**A**) and element concentrations (**B**).

In the case of general variables, two main groups could be distinguished (Figure 2A) which approximately coincided with water regimes that could be separated based on water discharge. Water discharge values before and after 18 July significantly differed (ANOVA, *p* < 0.05, Figure 3); the former period (June and the first two-thirds of July) could be regarded as a medium water period, while the latter (end of July) as a low water period (which continued during August, data not shown). In the case of element concentrations, similar groupings could be observed; however, three samples formed separated lineages (Figure 2B).
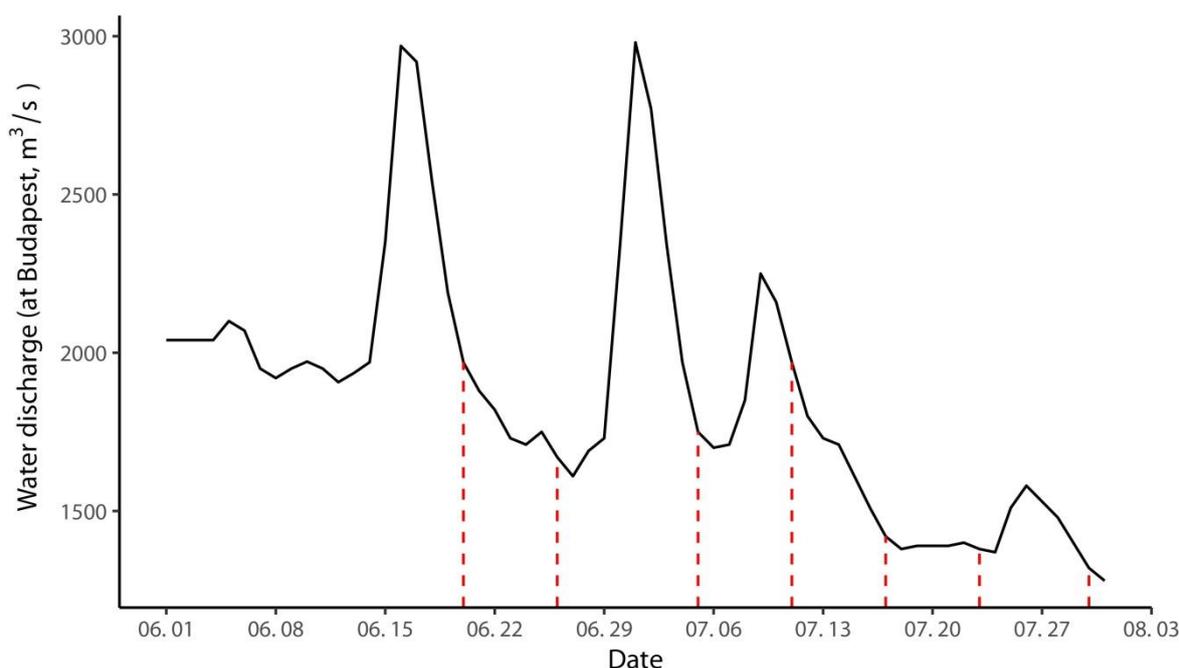
Figure 3. Water discharge measured at Budapest during the study period. Red dotted lines indicate water discharge values on the days of samplings.

Bray-Curtis dissimilarity based PERMANOVA did not show significant differences between the sampling points based on the measured environmental variables; neither the general variables nor the elements.

Nevertheless, some of the variables showed significant differences (two-way ANOVA, $p < 0.05$) between either the banks (pH, K^+, Ca^{2+}, Mg^{2+}, Na^+, As, Ba) or the sites (Mn).

Samples showed significant differences between the two water regimes (Bray-Curtis dissimilarity index-based PERMANOVA, $p < 0.05$), based on both general variables and element concentrations.

3.2. Diatom Assemblages

3.2.1. Species

Overall, 174 diatom taxa were identified, of which only 85 reached higher than a 1% proportion in at least one sample. Fifty-seven taxa occurred in only one sample, most of them (50 taxa) contributing less than 1%.

The most abundant species were *Amphora pediculus* (Kützing) Grunow and *Cocconeis euglypta* Ehrenberg which dominated (i.e., their relative abundance exceeded 5%) in most of the samples. *Achnanthidium delmontii* Pérès, Le Cohu and Barthès, *Amphora inariensis* Krammer, *Nitzschia inconspicua* Grunow, *Navicula cryptotenella* Lange-Bertalot and *Navicula antonii* Lange-Bertalot were dominant in at least the quarter of the samples. In some samples, *Navicula tripunctata* (O.F. Müller) Bory and *Discostella pseudostelligera* (Hustedt) Houk and Klee could reach a significant level of contribution.

Species composition showed the separation of samples according to both the site and the bank (Figure 4A; two way-PERMANOVA, $p < 0.05$). We used SIMPER analysis to investigate which taxa were responsible for the differences. Contributions of *Achnanthidium delmontii, Cocconeis euglypta, Amphora pediculus, Nitzschia inconspicua* were the highest (Table 2). *Achnanthidium delmontii* was characteristic at the reference point (right bank upstream), while *N. inconspicua* reached a higher proportion at the left bank upstream. *C. euglypta* was common in the downstream samples, while *A. pediculus* was more abundant upstream. The OMNIDIA database only contains a few ecological data about *A. delmontii*: this is a nano-sized, low profile taxon. More information is available on the other three species which

contributed considerably to the separation. All of them are alkaliphilic and eutrophic. *Cocconeis euglypta* and *A. pediculus* share in their nitrogen uptake strategy (tolerant *N*-autotrophic), salinity and saprobity adaptation (oligohalobic and beta-mesosaprobic) and ecological group (low profile); only their oxygen requirements and sizes are different. *Nitzschia inconspicua* has moderate oxygen requirements as does *C. euglypta* and it is nanosized as *A. pediculus* but it differs from them in the nitrogen uptake strategy (facultative *N*-heterotrophic), salinity and saprobity adaptation (halophilic and alpha-mesosaprobic) and ecological group (motile).
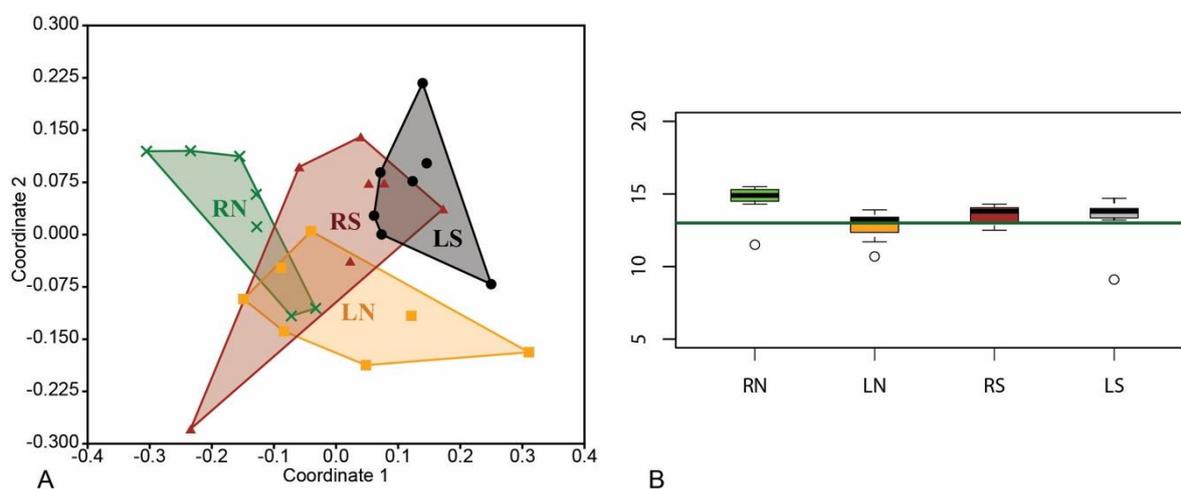
Figure 4. (**A**) Non-metric multidimensional scaling (NMDS) with Bray-Curtis dissimilarity index illustrating the separation of samples based on the relative abundances of species, Markings: green cross—samples from upstream, right bank (RN), orange filled square—samples from upstream, left bank (LN), brown filled triangle—samples from downstream, right bank (RS), black dot—samples from downstream, left bank (LS). (**B**) Boxplot illustrating values of Specific Pollution Sensitivity Index (IPS). The green line shows the good/moderate boundary value of the index.

Table 2. List of species contributing most to the separation of samples according to sites and banks, as shown by the similarity of percentage analysis.

	OMNIDIA Code	Average Dissimilarity	Contribution %	Cumulative %	Mean RN	Mean LN	Mean LS	Mean RS
Achnanthidium delmontii Pérès, Le Cohu & Barthès	ADMO	9.278	14.28	14.28	33.8	7.56	1.26	5.62
Cocconeis euglypta Ehrenberg	CEUG	8.342	12.84	27.12	6.02	7.12	24.5	24.7
Amphora pediculus (Kützing) Grunow	APED	5.854	9.013	36.14	17.1	20.8	13.8	11.8
Nitzschia inconspicua Grunow	NINC	4.11	6.327	42.46	3.51	15.4	1.66	0.861
Discostella pseudostelligera (Hustedt) Houk & Klee	DPSG	2.426	3.734	46.2	0.833	1.33	6.4	3.91
Navicula cryptotenella Lange-Bertalot	NCTE	2.1	3.232	49.43	2.19	2.08	7.14	4.42
Amphora inariensis Krammer	AINA	1.963	3.022	52.45	6.03	4.77	3.85	4.33
Nitzschia paleacea (Grunow) Grunow	NPAE	1.731	2.665	55.12	2.62	0.829	3.18	1.13
Navicula tripunctata (O.F. Müller) Bory	NTPT	1.58	2.432	57.55	0.224	1.25	4.69	2.64
Navicula antonii Lange-Bertalot	NANT	1.48	2.278	59.83	2.34	3.29	3.18	1.75

The diatom index (IPS) was highest on the right bank, upstream of Budapest, where the land use was the lowest (Figure 4B), while it was the lowest at the same site on the left bank.

3.2.2. Traits

The following traits showed the separation of samples according to both sites and banks: nitrogen uptake, pH adaptation, oxygen requirement, trophity, saprobity, halobity (Figures 5 and 6).
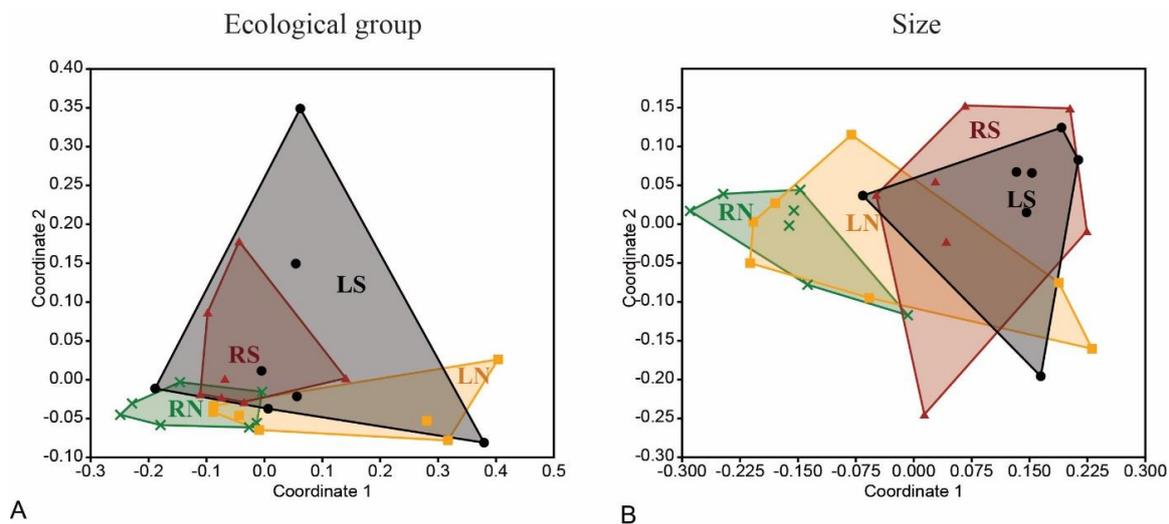
Figure 5. Non-metric multidimensional scaling (NMDS) with Bray-Curtis dissimilarity index illustrating the separation of samples based on the relative abundances of ecological groups (**A**) and size classes (**B**). Markings: green cross—samples from upstream, right bank (RN), orange filled square—samples from upstream, left bank (LN), brown filled triangle—samples from downstream, right bank (RS), black dot—samples from downstream, left bank (LS).

Based on size, the samples separated according to sites (Figure 5A); based on ecological groups, banks (Figure 5B) differed significantly (PERMANOVA, $p < 0.05$).

Separation of samples based on nitrogen uptake was caused by the differences of relative abundances of tolerant nitrogen autotrophic and facultative nitrogen heterotrophic taxa. Tolerant nitrogen autotrophic taxa occurred in a higher proportion in the downstream section than in the upstream section; in RN samples they were dominant and diatoms of other categories had very low contributions. The relative abundance of facultative and obligatory nitrogen heterotrophs increased in LN (Figure 7A).

According to pH adaptation, alkaliphilic taxa were dominant; however, they were least abundant upstream on the right bank (Figure 7B).

Mainly oxybiontic taxa and taxa with a moderate oxygen requirement occurred in the samples. Oxybiontic diatoms dominated in upstream right bank samples in which the proportion of polyoxybiontic taxa also increased. At the downstream points, taxa with moderate oxygen requirement had the highest proportion (Figure 7C).

Among saprobity trait categories, beta-meso-saprobic taxa were the most abundant, with the proportion increasing from upstream of downstream. Alpha-meso-saprobic diatoms were more abundant on the left bank than the right bank. Oligosaprobic species reached the highest relative abundance in RN samples (Figure 7D).

Based on the trophity trait, samples were characterized by the dominance of eutrophic species and their proportion decreased on the right bank upstream (Figure 7E).

Nano-sized taxa became dominant upstream on both banks, while downstream points were characterized by a diverse community in terms of size, particularly with a significantly higher proportion of very large diatoms (Figure 8A).
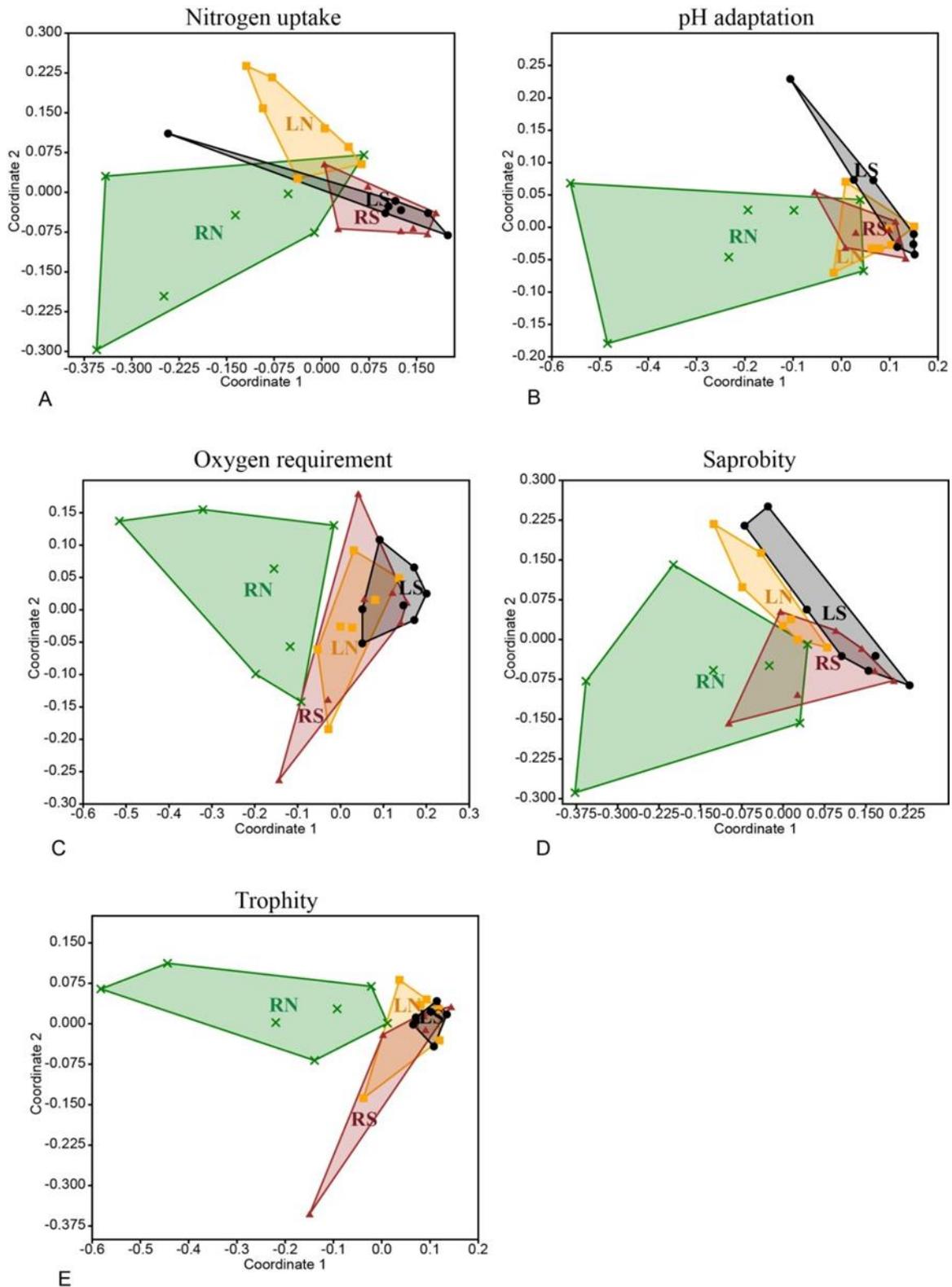
Figure 6. Non-metric multidimensional scaling (NMDS) with Bray-Curtis dissimilarity index illustrating the separation of samples based on the relative abundances of the categories of traits defined by van Dam et al. [22] in samples from the four sampling points. (**A**) Nitrogen adaptation strategy, (**B**) pH adaptation strategy, (**C**) oxygen requirement, (**D**) saprobity, (**E**) trophity. Markings: green cross—samples from upstream, right bank (RN), orange filled square—samples from upstream, left bank (LN), brown filled triangle—samples from downstream, right bank (RS), black dot—samples from downstream, left bank (LS).
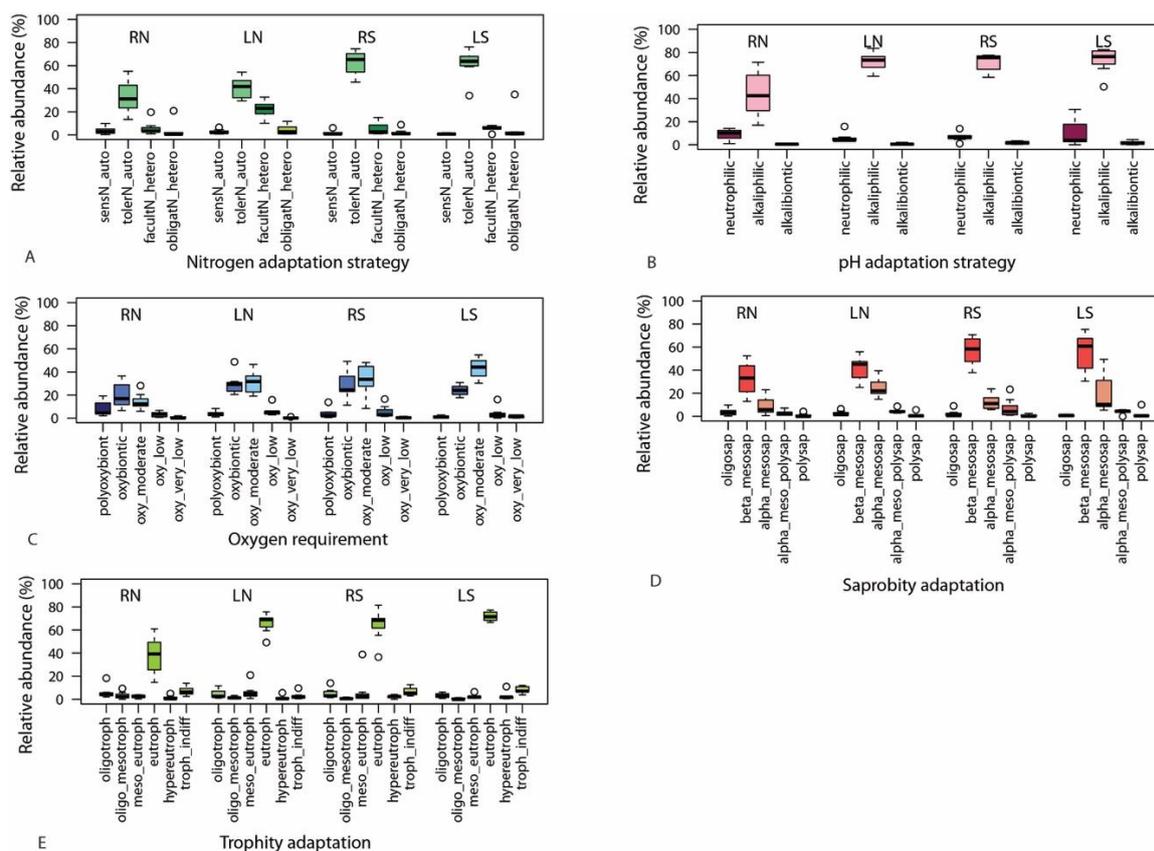
Figure 7. Boxplots illustrating relative abundances of the categories of traits defined by van Dam et al. [22] in samples from the four sampling points (RN = upstream, right bank, LN = upstream, left bank, RS = downstream, right bank, LS = downstream, left bank). (**A**) Nitrogen adaptation strategy, (**B**) pH adaptation strategy, (**C**) oxygen requirement, (**D**) saprobity, (**E**) trophity. Abbreviations: sensN_auto = sensitive nitrogen autotrophic, tolerN_auto = tolerant nitrogen autotrophic, facultN_hetero = facultative nitrogen heterotrophic, obligatN_hetero = obligatory nitrogen heterotrophic; oxy_moderate = moderate oxygen requirement, oxy_low = low oxygen requirement, oxy_very_low = very low oxygen requirement; oligo-, beta_meso-, alpha_meso-, alpha_meso_poly- and polysap stand for oligo-, beta-meso-, alpha-meso-, alpha-meso-poly- and polysaprobic; oligo-, oligo_meso-, meso-, meso_eu-, eu-, hypereutroph and troph_indiff mean oligo-, oligo_meso-, meso-, meso-eu-, eu-, hypereutrophic and trophic indifferent.

It was mainly the low profile and motile group which occurred in the samples. The proportion of the low profile group (LPG) was much higher than the other groups on the right bank. Motile taxa became more abundant on the left bank. Planktic species had a higher contribution in the downstream section (Figure 8B).
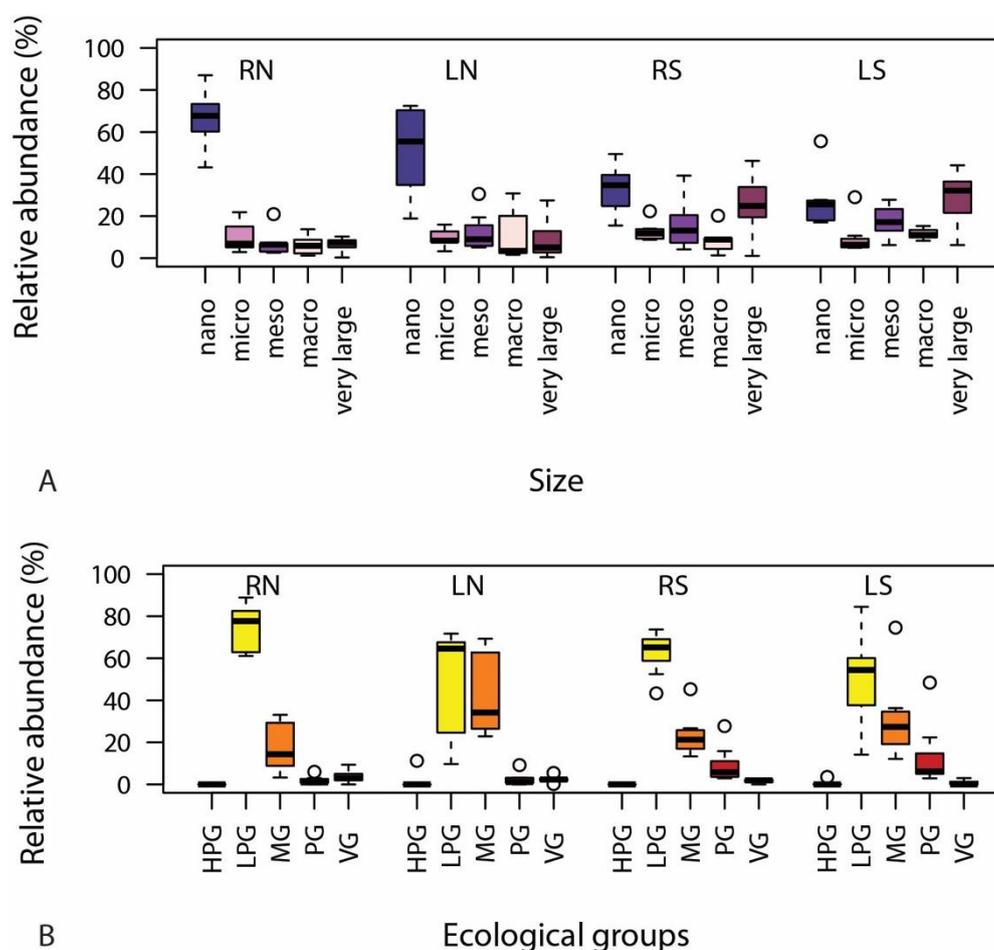
Figure 8. Boxplots illustrating relative abundances of size classes and ecological groups in samples from the four sampling points. Abbreviations: HPG = high profile group, LPG = low profile group, MG = motile group, PG = planktic group, VG = variable group.

4. Discussion

4.1. Water Quality Based on Environmental Variables

The effect of urbanization on water quality can vary more strongly than it does on hydrology and geomorphology, depending on the nature of urban land use (residential versus commercial/industrial), the presence of wastewater treatment plants, illegal discharge connections, effluent or combined sewer overflows, landfills, failing septic systems and the extent of stormwater drainage [1]. Many effects on water quality are transient, associated with discharges from a sewerage overflow or the first flush of stormwater; however, there are longer-term effects mediated through river sediments due to the particulate-associated forms of the contaminants (e.g., metals, phosphorus) [40].

Joint Danube Surveys (JDS) regularly assess the status of the River Danube in terms of hydromorphology, water chemistry and biological elements (phytoplankton, phytobenthos, macrophytes, macrozoobenthos, fish [24]). These data provide an opportunity to track the changes in water quality over the years and compare them with results of the present study. Total nitrogen and phosphorus concentrations decreased from JDS1 (in 2001) to JDS3 (in 2013) [41] and our measurements showed that these trends have continued since then. Nitrate, orthophosphate, chloride and sulphate concentrations and conductivity were also lower, while potassium, calcium and magnesium concentrations were higher compared to the result of JDS3. The water quality of the River Danube, assessed on basis of the nutrient concentration, has improved in the last few decades, presumably due to the installation of numerous wastewater treatment plants.

Currently, three main wastewater plants operate in Budapest: North- and South-Pest and Budapest Central Wastewater Treatment Plants. The oldest is the North-Pest Wastewater Treatment Plant, in operation since 1980. These WWTPs carry out mechanical (grit collector, screens, sand collector), biological (activated sludge basins) and chemical (expulsion of phosphorous) treatments [42,43].

A daily average of 500,000–600,000 m^3 arrives in these WWTPs, where 95% of the wastewater is treated biologically [44]. According to our data, sites upstream and downstream of Budapest differed significantly neither in nutrient concentrations (nitrite, nitrate, orthophosphate, total nitrogen and phosphorous) nor in heavy metal concentrations, indicating the efficiency of wastewater treatment in the removal of these components. Using their newly developed method, combined cluster and discriminant analysis of environmental variables (runoff, pH, conductivity, water temperature, chemical and biological oxygen demand, dissolved oxygen, Cl^-, SO_4^{2-}, HCO_3^-, Mg^{2+}, Ca^{2+}, Na^+, K^+, NH_4^+-N, NO_2^--N, NO_3^--N, total nitrogen, particulate, dissolved, soluble reactive and total phosphorous, total suspended solids and chlorophyll *a*) Kovács et al. [45] also found that sampling points upstream and downstream of Budapest formed a homogenous group probably due to the efficient wastewater treatment.

However, it has been recently recognized that organic micropollutants such as pharmaceuticals and personal care products pose a serious risk to aquatic ecosystems and conventional wastewater treatments are ineffective in the removal of such pollutants [46]. Unfortunately, currently applied metrics are not specified to indicate these compounds yet.

4.2. Ecological Status Indicated by Diatom Assemblages and the Usability of Traits

Although most of the environmental variables did not show differences between sampling points, epilithic diatom communities unambiguously reflected the effect of urbanization. Several studies (e.g., References [47,48]) have suggested that diatom assemblages can show no or an unexpected, statistical relationship with the measured environmental variables; however, they can better indicate water quality. This is because they are long term indicators adapted to a scale of values of environmental pressures within a certain period, thus they are more likely to represent the typical conditions than chemical values obtained from punctual water samples [47,49]. Szczepocka and Szulc [9] also considered biological analyses to provide more reliable information about water quality than physico-chemical measurements because the latter may be affected by pollution that was introduced just before sampling and such intermittent pollution events have a less pronounced effect on indicator organism communities.

4.2.1. Species Composition

Species composition of diatom assemblages significantly differed between the sampling points, indicating the effect of various level of land use.

Among the most dominant species, *Achnanthidium delmontii* and *Amphora inariensis* was characteristic at the reference point (RN).

Interestingly, these species along with *Navicula tripunctata* were not reported in the list of species reaching a relative abundance of at least 5% at a minimum of one site during JDS3 (see Reference [47]). The explanation for this may be: (1) the abundance of these species has increased since 2013, (2) the difference between the two research studies which performed the identification, then and now.

In previous research, we found *Achnanthidium delmontii* abundant in a sample, but it was difficult to identify and to distinguish from *Achnanthidium pyrenaicum* and *Achnanthidium rivulare* under a light microscope. Therefore we studied the sample under a scanning electron microscope, identified the species as *A. delmontii* and determined the features (valve shape, central area and stria density) that allowed the identification of the species under a light microscope. In contrast to the two most similar species mentioned above, on the raphe valve of *A. delmontii*, the central area forms a more or less rectangular fascia. Moreover, *A. delmontii* has less dense striae than *A. rivulare* and is wider than *A. pyrenaicum*, which has narrower apices. Using these features we were able to unambiguously identify *A. delmontii* in samples presented here. Although the methodology of the Water Framework Directive

does not require the use of a scanning electron microscope, based on our previous experiences we recommend its use for small-sized species that can be confused under a light microscope.

Achnanthidium delmontii was described from the Mediterranean river Cèze in 2012 [50]. It was characteristic of cobbles (diameter 6–40 cm) in an Argentina river [51]. Its invasive behavior was reported [50,51].

Lange-Bertalot et al. [52] wrote about *Amphora inariensis*, noting that "according to Krammer and Lange-Bertalot [53] it colonizes oligotrophic, anthropogenically undisturbed standing freshwater habitats with low to medium electrolyte content but it is found in a variety of environments, sometimes moderately impacted." Of its traits only a few are known: it is an oligotrophic, micro-sized, low profile species.

Left bank upstream of Budapest was a transition between the reference RN and the heavily impacted downstream points. Here species, like *Amphora pediculus*, *Nitzschia inconspicua* and *Navicula antonii*, more tolerant to trophic and/or saprobic conditions [52], became characteristic, (*Achnanthidium delmontii* and *Amphora inariensis* were also present but in smaller amounts). *Amphora pediculus* is a dominant species in the River Danube [47,54,55]. It was also found in downstream samples in a lower proportion. Besides, the most heavily impacted downstream points were dominated by *Cocconeis euglypta*, *Navicula cryptotenella* and *N. tripunctata*.

4.2.2. Traits

During this summer period, alkaliphilic, oligohalobous, beta-mesosaprobic, eutrophic, tolerant *N*-autotrophic, oxybiontic or moderate oxygen requirement, low profile taxa were dominant in the epilithic diatom community of the section of the River Danube near Budapest. On the right bank upstream of Budapest, where the extent of the land-use was the most negligible, there was the lowest ratio of eutrophic and highest ratio of polyoxybiontic, nanosized and LPG species. On the left bank at the same site, there was the highest ratio of facultative and obligatory *N*-heterotrophs. The species with lower oxygen requirement occurred in higher abundance downstream of Budapest than upstream.

Ecological groups [20,21] are among the most frequently studied traits and several studies have investigated their relationship with environmental variables. The low profile group (LPG) includes species of short stature, including prostrate, adnate, erect and slow-moving species that are adapted to high current velocities and to low nutrient concentrations [21]. It is beneficial for the LPG attached strongly to the substrate when the establishment of a three-dimensional biofilm is prevented; however, it still persists in the case of copious periphytic growth but in lower numbers and suffers resource limitation [20,21]. Tapolczai et al. [19] challenged the idea that because low-profile species are suppressed in thick biofilm this means that they have an advantage under low-resource circumstances. It has the ability to withstand abrasion and physical damage, therefore dominating in unstable habitats, like epipsammon [20,21].

The high profile group (HPG) includes species of tall stature, including erect, filamentous, branched, chain-forming, tube-forming, stalked and colonial centrics and large size taxa [21]. These taxa represent the canopy layer of the biofilm [19], having a more direct connection with the ambient water [54] and preferring high nutrient concentrations (however, the relationship between the HPG and nutrient concentrations was the weakest in Passy [20]'s study). They are adapted to low current velocities being more abundant in the more stable or more sheltered habitats, like epilithon and the epiphyton [20,21].

The motile group (MG) includes fast-moving species that are superior competitors for nutrients in nutrient-rich environments and can move to resource-rich microhabitats avoiding stress. The MG was significantly higher in the unstable epipelon, where it can escape from being buried [20,21]. This ability of motile diatoms was what Bahls [56] based his siltation index on (the sum of the percentages of *Navicula*, *Nitzschia*, *Surirella* and *Cylindrotheca* species and varieties). This index was used during the National Water Quality Assessment (NAWQA) Program in the United States, although the relationships between the silt index and indicators of stream siltation processes (e.g., substrate embeddedness and

suspended-sediment concentrations) were relatively weak, presumably because of the influence of nutrient conditions [57].

We observed that low profile diatoms were most abundant in almost all samples; the second most abundant group was the motile, which became more dominant mainly in LN samples. High profile species could reach relative abundance above 5% only in one sample (12.75% in 0730 LN). As trophic conditions were eutrophic during the whole study period (as was shown by the environmental variables and the high relative abundance of eutrophic taxa), the proportions of ecological groups can be explained by hydromorphological reasons rather than nutrient concentration. Most of the LPG taxa which occurred in our samples were eutrophic, this observation also contradicts the hypothesis on its adaptation to a too low nutrient concentration. The formation of the thick and complex biofilm may be prevented by the frequent moving and slewing-around of the small cobbles.

Similarly, Trábert et al. [55] found that ecological groups can reflect the effect of substrate and differences between flow regimes rather than nutrient loads. In their study conducted in the River Danube, the HPG also had a low relative abundance in the River Danube compared to the LPG and the MG, due to its inability to withstand the disturbance of high current velocity and water-level fluctuations.

The HPG could not grow under decreased light intensity [58] which can be caused by high suspended matter content and turbidity [55]. In contrast, the LPG is more common in shaded environments [58].

The planktic group was introduced by Rimet and Bouchez [21] (as "planktic guild") who applied this term to taxa adapted to lentic environments with a morphological adaptation that enables them to resist sedimentation and to filamentous taxa that were included in the HPG in Passy [20]'s system (with the exception of filamentous taxa which are benthic). These diatoms could settle down for hydromorphological reasons rather than because of changes in water quality [59]. Planktic taxa can reach even a relatively high proportion in benthic assemblages in sections close to reservoirs, in late-successional stages of matured biofilm or when settling down due to a reduced flow rate [60,61]. In our study, these species (mainly *Discostella pseudostelligera*, *Stephanodiscus minutulus* (Kützing) Cleve and Möller, *Cyclostephanos invisitatus* (M.H. Hohn and Hellermann) E.C.Theriot, Stoermer and Håkasson and *Cyclotella atomus* var. *gracilis* Genkal and Kiss), reached a significantly higher proportion in the downstream samples.

Beside ecological groups, size is an also commonly used trait. It is a master trait that significantly influences physiological activities (e.g., growth, metabolism) [62] and determines the ability of a species to recover after disturbance [58]. In our study, nano-sized diatoms prevailed at the points upstream of Budapest, while their relative abundance decreased downstream in favor of other size classes, particularly the very large taxa. Lange et al. [58] also found that the proportion of small cell sizes decreased at a higher level of farming intensity, supporting their hypothesis that increased nutrient supply supported the growth of larger cells. Small sizes are advantageous under nutrient-limiting conditions because of the high surface to volume ratio and are more beneficial in competition for nutrients under nutrient-limited conditions, while large cell sizes may be advantageous under conditions of fluctuating nutrients because of their increased nutrient storage capacity in vacuoles that may be enormous, relative to cell volume [62].

Applying the same size classes that we used, Berthon et al. [17] found that the c2 and c5 size class (micro and very large in our study) significantly discriminated between the trophic and the organic pollution classes and the c3 (meso) between the trophic classes.

There are traits linked to physiological properties (e.g., nitrogen uptake strategy) which are more difficult to measure and are characteristic of species and not of genus; however, these can be more clearly linked to environmental variables and can be applied in ecological status assessments of waters (e.g., the percentage of nitrogen heterotrophic diatoms was used for the organic sources of nitrogen by Peterson and Porter [48]). Such metrics (e.g., the percentage of eutrophic, nitrogen-autotrophic,

nitrogen-heterotrophic, halophilic diatoms) were used in the evaluation of stream-water quality during the NAWQA Program in the United States [57]

In our study, the proportion of tolerant nitrogen autotrophs increased downstream of Budapest. These diatoms can use an inorganic nitrogen source but they can tolerate an elevated concentration of organically bound nitrogen [22] and their increased relative abundance may indicate a higher nutrient load. In Gore Creek (Colorado, USA) the increasing biovolume of nitrogen autotrophic diatoms was consistent with the increasing inorganic nitrogen (nitrate) concentration that was affected by the discharge from a wastewater treatment plant [63]. In LN samples, the relative abundance of nitrogen heterotrophs increased (tolerant nitrogen autotrophs remained dominant). Facultative nitrogen heterotrophs can process organic nitrogen to supply cellular energy requirements (in addition to photosynthesis), as well as acquiring sufficient nutrients required for nitrogen metabolism [22,48]. Thus, the abundance of nitrogen heterotrophs often increases in turbid rivers (or shaded streams) that are organically enriched with nitrogen [48].

Among the trophity adaptation strategies, the eutrophic one was dominant but its proportion was significantly lower in RN samples than in the other samples. In the case of the Yellowstone River basin eutrophic and nitrogen indicators appeared to better indicate trophic conditions than measured nutrient concentrations [48]. Nutrient concentrations generally were low throughout the length of the Yellowstone River (total nitrogen ranged between 0.3 and 0.4 mg·L^{-1} and total phosphorous varied from 0.016 to 0.038 mg·L^{-1}); however, indicators of algal biomass and the percentage of eutrophic and nitrogen indicator diatoms were relatively high in the middle segments of the river and near the mouths of major tributaries [48,57]. Instantaneous dissolved nitrate loads were high, which was reflected in the relatively high percentage of nitrogen autotrophs and this corresponded closely with the abundance of eutrophic diatoms [48].

Polyoxybiontic taxa and diatoms with moderate oxygen requirement showed opposite changes, indicating the effect of the land-use gradient. Saprobity was strongly associated with oxygen requirement. In cases in which both traits were known, oligosaprobic species were all polyoxybiontic, β-mesosapric ones were mainly oxybiontic and most of the α-mesosaprobic taxa had moderate oxygen requirements. This is due to the fact that the saprobity trait combines indicator properties for the presence of biodegradable organic matter and the oxygen concentrations [22]. Distribution of many taxa are not limited by decreasing saprobity but only by increasing pollution levels; therefore the presence in a sample of taxa which are not resistant to heavy loads of organic pollution can be used to differentiate this sample from samples which are taken from heavily polluted water bodies; the presence of taxa which are very tolerant to organic pollution does not always indicate the presence of organic pollution [22].

4.2.3. Diatom Index

We used the IPS diatom index to assess the ecological status of the studied section of the River Danube. Overall, it has reached a "good" status in most of the cases (applying boundary values provided by Ács et al. [64] on hydromorphological type 23, to which the studied section belongs, Table 3); however, there were differences between sampling points. IPS values reached the highest values on the right bank upstream of Budapest (sometimes almost reaching high status), a value below 13 occurred only in one case, when downstream points also reflected "moderate" status. IPS values were lowest on the left bank upstream. "Moderate" status could be experienced, especially at the LN and RS points. These results show that there has been an overall improvement in ecological status compared to the status assessed during JDS3 in 2013 when the ecological status was "moderate" in this section, with higher IPS values at the points upstream of Budapest than in those downstream [47].

Table 3. Boundary values of ecological states for hydromorphological type 23, based on IPS [64].

Ecological Status Boundary	IPS Value
High/good	15.6
Good/moderate	13.0
Moderate/poor	8.7
poor/bad	4.3

5. Conclusions

It can be clearly seen that after the introduction of the EU Water Framework Directive the nutrient loading significantly decreased in the studied section due to the installation of several efficiently working wastewater treatment plants upstream and downstream of Budapest. This conclusion was suggested by the observation that we did not find significant differences in the measured chemical variables between the upstream and downstream points. In contrast, variations in the species and trait composition of the diatom communities were detected, which are more likely to be related to land use. Benthic diatoms are sessile and show temporally integrative responses to environmental changes caused by land use, while chemical measurements reflect an instantaneous status [47,49]. Our results confirmed our earlier finding, that in such a large river as the Danube the diatom ecological groups reflect the effect of hydromorphology, rather than nutrient loads [55]. IPS, the diatom metric used, is robust enough: although it was developed for indicating nutrient load, our results suggest that it can be used for detecting the effects of land use, as well.

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