*Article*

# Cladocera and Geochemical Variables from Core Sediments Show Different Conditions of Hungarian Lakes

István Gyulai [1,2], János Korponai [3,4,*], Sheila Mumbi A. Wamugi [1,5], Jázmin Jakab [1,5], Umar Abba Kawu [1,5], Andor G. Soltész [1], Tamás Karches [3,4] and Uyanga Tumurtogoo [1,5]

[1] Department of Hydrobiology, Faculty of Science and Technology, University of Debrecen, Egyetem tér 1, H-4032 Debrecen, Hungary; istvan.gyulai@unideb.hu (I.G.); sheilla.mumbi@science.unideb.hu (S.M.A.W.); jakab.jazmin@science.unideb.hu (J.J.); kawuumar@mailbox.unideb.hu (U.A.K.); soltesz.andor2001@gmail.com (A.G.S.), uyanga.tumurtogoo@science.unideb.hu (U.T.)

[2] National Laboratory for Water Science and Water Security, Department of Hydrobiology, University of Debrecen, Egyetem tér 1, H-4032 Debrecen, Hungary

[3] Department of Water Supply and Sewerage, Faculty of Water Sciences, University of Public Services, Bajcsy-Zs. utca 12-14, H-6500 Baja, Hungary; karches.tamas@uni-nke.hu

[4] National Laboratory for Water Science and Water Security, Faculty of Water Sciences, University of Public Services, Bajcsy-Zs. utca 12-14, H-6500 Baja, Hungary

[5] Pál Juhász-Nagy Doctoral School of Biology and Environmental Sciences, University of Debrecen, Egyetem tér 1, H-4032 Debrecen, Hungary

*   Correspondence: korponai.janos@uni-nke.hu

**Abstract:** Studies on the sediments of lakes with varying trophic status are of particular importance when considering changes in the natural environment. In this study, our objective was to examine subfossil remains of Cladocera species and the relationship between the sedimental Cladocera assemblages and geochemical variables during 11 years of sediment records from northern Hungarian lakes. To achieve this, we compared sedimental cladoceran communities and the geochemistry of the sediment layers among lakes. Among the studied lakes, one was an intermittent lake (KMT: the Kis-Morotva Lake) which dried out in 2012 but was subsequently naturally refilled in 2013 by groundwater affected by the high-water level of the River Tisza. The other type consisted of permanent lakes (SZA: the Szabolcs oxbow lake, TI: the Timár Morotva Lake) that never dried out. The results of the beta diversity analysis show that the deposition of Cladocera communities was similar among the sediment layers of lakes, while the abundance differences contributed significantly to replacement. Subsequently, core sediment samples of the three lakes were compared based on the remains of Cladocera communities and geochemical variables using Adonis (PERMANOVA). The core sediment samples indicate variations in Cladocera communities alongside disparities in geochemical variables across the same lakes. In conclusion, the significance of sediment cores containing the remains of the Cladocera community has grown significantly in the reconstruction of historical ecological and climatic changes.

**Keywords:** short core; sediment; Cladocera remains; geochemical variable; paleolimnology

## 1. Introduction

Lentic waters are one of the most important water resources on earth, because they provide habitats for many rare species and play an important role in biodiversity conservation [1]. Therefore, it is important to understand the evolution pattern of lakes to be able to predict lake development trends and manage water resources. The variation in biological diversity and the evolution of hydrology in lakes have been valuable in assessing the impacts of both natural (runoff, precipitation, flooding, and snow meltwater) and human-induced disturbances (deforestation, urbanization, soil use, agriculture, and fishing) [2–5]. Floods are important sources of water recharge and refill for most surface water systems globally. Rivers can add suspended matter and nutrients to the lakes during flood events.

In the flooding period, subject to flood pulses, species interactions are complex due to periodic changes in lakes' physical and chemical characteristics [6,7], whereas dry periods can lead to increased isolation of lakes and an increased differentiation of aquatic fauna among lakes as they respond to the changes in environmental conditions [8,9]. In addition to these direct effects, the flood pulse also modifies the habitat heterogeneity that vegetation in the littoral zone provides, which in turn affects zooplankton communities [10,11]. Specifically, variations in vegetation features also affect water chemistry, which increases the beta diversity of zooplankton [12].

In general, sediments from lakes contain organic and inorganic remains that are useful for tracking the history of a lake or its catchment, as well as determining paleoecology and climate changes [13]. Moreover, single-core sediments from the deepest part of the lake provide a good representation of the dynamics of aquatic communities for the entire lake. On the other hand, Kattel et al. [14] reported that littoral species are underrepresented in the deep core sample [15]. Fossils are formed in sediment after taphonomic processes modify the remains of organisms after death.

Aquatic fauna primarily affiliated with cladocerans is found in most freshwater bodies. Cladocerans inhabit a wide variety of aquatic zones (pelagic, littoral, and benthic). Remains of cladocerans (shells, headshields, and claws) are well preserved in the sediment, unlike those of other aquatic microorganisms [16,17], allowing for species identification. Paleolimnological approaches are widely used to evaluate ecosystem functions and the resilience of aquatic ecosystems. In sediments, Cladocera species indicate historical changes in the environment, including global climate change, eutrophication, acidification, and differences in water levels [18–21]. In addition, there are many environmental factors that affect the distribution and abundance of cladoceran remains, including the movement of sediments and bioturbation, lake morphology, water depths, and water movements. Subfossil remains of cladocerans can be used as indicators of environmental changes [22].

This study focused on Cladocera communities from two types of lakes (intermittent and permanent) in Hungary. We aimed to investigate two main aspects: (i) determining the representativeness of the core sediment sample from the deepest part of the lakes, both in comparisons between the types of lakes and in relation to the sediment layers along the lakes; and (ii) discussing the influence of environmental variables on the sediment layers and any differences between the types of lakes.

## 2. Materials and Methods

The core sediment samples were collected from three oxbow lakes (the Kis-Morotva lake (KMT), the Szabolcs oxbow lake (SZA), and the Timár Morotva Lake (TI)) in October 2021 (Figure 1). The lakes are located in the Rakamaz region, Northeast Hungary, on the floodplain of the River Tisza. The River Tisza flows through East Hungary and is the second largest water course in the country. Its total catchment area is 157,186 km$^2$, and the length of the Hungarian section of the river is 597 km out of a total length of 946 km [23]. The water regime of the river is highly variable, and large floods can occur in the river basin at any time of the year due to rainstorms, snowmelt, or both of these. Large floods are generated more frequently in the late winter and early spring. The warm period from May to October accounts for nearly 35% of the total floods, and the cold period from November to April accounts for 65%.

The oxbows are shallow, naturally formed, and eutrophic. The maximum depth of the KMT oxbow is 1.2 m, that of the SZA is 2.5 m, and that of the TI is 2.2 m. Their surface areas are 14 ha, 5.60 ha, and 9.15 ha, and their lengths are 1223 m, 957 m, and 1058 m, respectively [24]. The KMT oxbow is a nature conservation area and does not have any utilization or land use, being protected by the Natura 2000 program, while the SZA oxbow is used intensively for fishing (especially for angling). In terms of fish, the SZA oxbow is home to a variety of fish species, including grass carp, bream, carp, pike, perch, and wels. Dense reed belts surround the shoreline. Additionally, the lake is also surrounded by cattails, bulrushes, and sedges. The TI oxbow is a natural lake without any specific

utilisation; it too is protected by the Natura 2000 program. More than fifty percent of the lake is covered by emergent, submergent, and floating leaf macrophytes.
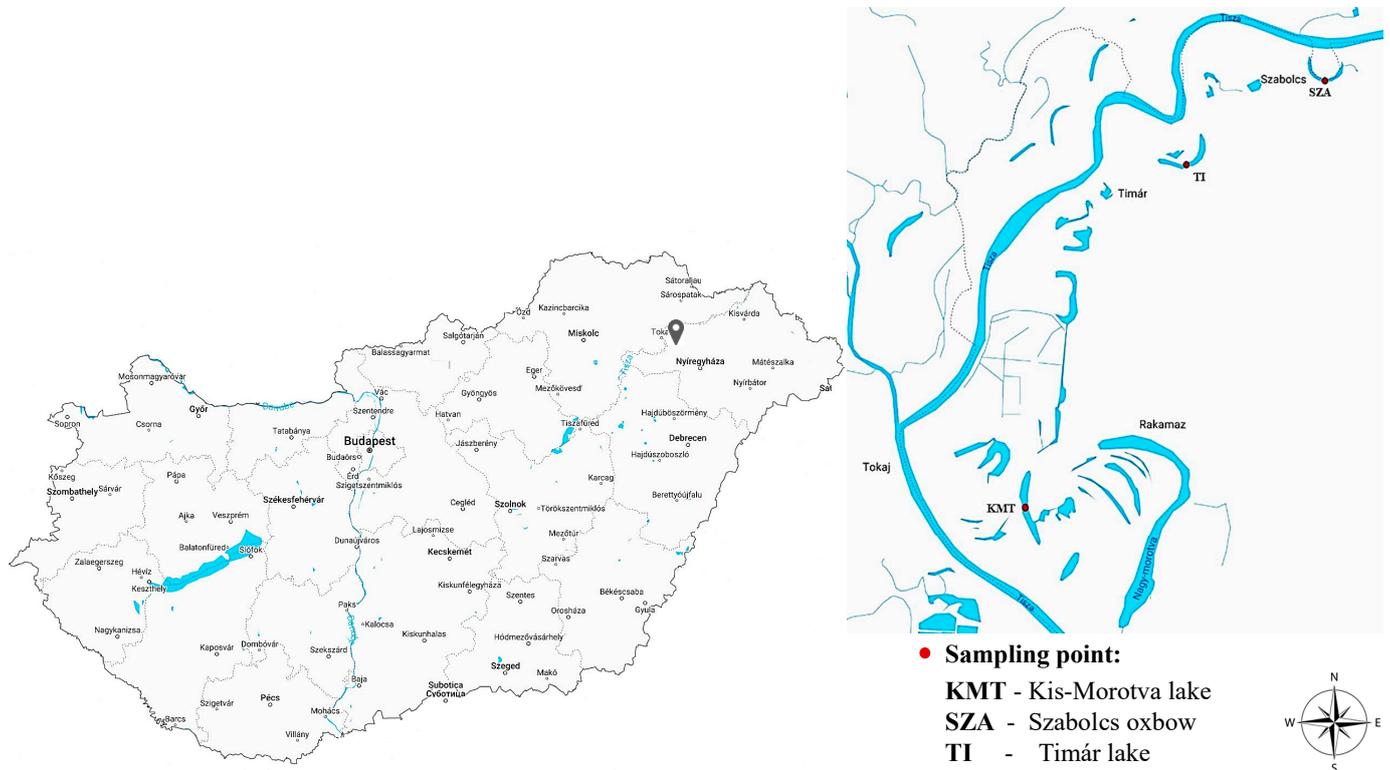


**Figure 1.** Areas of study and sampling points in lakes.

The study was conducted in two different types of lakes: the KMT oxbow is considered intermittent (dried out in 2012). Although all lakes can be recharged from the River Tisza, directly from floods and groundwater, the river was only able to recharge the KMT oxbow in the spring of 2013. The SZA and TI oxbows are permanent (i.e., they do not dry out). All of the lakes can be recharged from the River Tisza, directly from the floods and ground water.

Short (11 cm) sediment cores were retrieved from each lake using a gravity corer [25]. The cores were wrapped in plastic and aluminium foil and were stored at 4 °C until processing. The sediment cores were sliced at 1 cm intervals, and each sediment slice was prepared for the subfossil Cladocera and laboratory analysis. Samples of 1 cm$^3$ of sediment was taken from each layer of all sediment cores and treated in 100 mL of 10% KOH (potassium hydroxide) solution heated to 100 °C for 30 min. in a water bath. The samples were sieved through a 35 μm mesh, then dyed with safranin, following Frey [26]. The subfossil cladoceran remains were counted according to the method recommended by Korhola and Rautio [16]. A total of 33 quantitative slides were prepared by pipetting 100 μL volume of samples and making a cover slip for each sediment layer. The remains were examined at 100 and 400 magnifications using an Olympus BX53 light microscope. At least 100 cladoceran individuals, identified from approximately 200–250 remains (shells, headshields, and claws) were counted in each sample. For identification and nomenclature, keys from Gulyás and Forró [27], Szeroczynska, Sarmaja-Korjonen [28], and Błedzki and Rybak [29] were used.

Laboratory analysis of the core sediment samples was performed to estimate the organic matter and calcium carbonate (CaCO$_3$) content, as well as the elemental composition. Each of the sediment samples was burnt at 550 °C for four hours and 950 °C for two hours to estimate the organic matter and calcium carbonate content of the samples. The calcium carbonate (CaCO$_3$) and organic matter contents were determined using the loss-on-ignition (LOI) method, following Dean [30], complemented by Heiri [31].

To analyse the geochemical composition, 0.2 g of dried material from the core samples was weighed using an analytical balance (Precisa ES 225SM-DR). Dried weighted samples were digested with the ETHOS UP (Milestone Sorisole, Italy) microwave digestion system using a mixture of 5.0 mL 65% (m/m) $HNO_3$ (reagent grade, Scharlau, Barcelona, Spain) and 1.0 mL 30% (m/m) $H_2O_2$ (reagent grade, Merck, Darmstadt, Germany) [32]. Digested samples were transferred without loss into volume-calibrated plastic centrifuge tubes and diluted up to a final volume of 25.00 mL with ultrapure water (Synergy UV Millipore, Darmstadt, Germany). Before further elemental analysis, the solutions were maintained at room temperature.

The elemental concentration of the sediment samples was determined by inductively coupled plasma optical emission spectrometry (ICP-OES 5110 Vertical Dual View, Agilent Technologies, Santa Clara, United States) An autosampler (Agilent SPS4), a Meinhard® type nebulizer, and a double pass spray chamber were also used (ICP VI, Merck). Standard solutions of the macro elements (Al, Fe, Ca, K, Mg, Na) were prepared from the mono-element spectroscopic standard of 1000 mg $L^{-1}$ (Scharlau), while for the micro-elements (Ba, Cd, Co, Cr, Cu, Li, Mn, Ni, Pb, Sr, Zn), a solution was prepared from the multi-element spectroscopic standard solution of 1000 mg $L^{-1}$ (ICP IV, Merck). In both cases, a five-point calibration process was used for which standard solutions were diluted with 0.1 M $HNO_3$ prepared in ultrapure water. Besides geochemical parameters, the distance of each oxbow from the River Tisza was also determined. The distances and geochemical compositions of sediments were used as environmental variables in ordinations.

In the statistical analysis, a series of principal component analyses (PCA) was applied to evaluate the distribution of cladoceran species and geochemical elements among oxbows [14]. Prior to PCA, the cladoceran data were Hellinger transformed (square root of relative abundances) [33], while geochemical variables were standardized (z-transformed).

To evaluate the similarity between the oxbows regarding their cladoceran communities and environmental variables, we performed a PERMANOVA using scores for the first two PCA components in each sediment core. The PERMANOVA was based on Euclidean distance with 999 permutations and performed in an R environment through the adonis2 function of the vegan package [34]. We used the betadisper function from the vegan package [34] to verify the average distance from each point to the group median as a measure of multivariate dispersion.

Subsequently, a series of redundancy analyses (RDA) was used to identify the most important environmental variables responsible for the structures of the cladoceran community. First, RDA analyses were run without conditional variables as null models. Then, a three-RDA model was built with conditional variables: (a) type (intermittent, permanent); (b) oxbows; and (c) depth. In order to choose the geochemical variables explaining the maximum variation in the species data, highly colinear geochemical variables were identified by their variance inflation factors (VIF). Since variables with a high VIF (VIF > 20) have a strong impact on the multicollinearity of data, deleting these highly correlated variables increases the predictive power of the multiple regression. Monte Carlo permutation tests were applied in order to test the significance of each geochemical variable used in the RDA. Environmental variables which did not explain a significant portion of species variance after Monte Carlo permutations ($p < 0.05$; 999 random permutations) were removed from the RDA. A stepwise selection was run to determine the subset of significant environmental parameters.

The species diversity of the subfossil cladoceran assemblages was estimated using Hill's numbers [35] on densities, in which N0 represents species richness (number of taxa); N1 the Shannon–Wiener diversity; and N2 the inverse Simpson diversity, which is the reciprocal of Simpson's diversity index [35–37]. The Shannon–Wiener index is sensitive to rare taxa, while the Simpson index is sensitive to dominant ones [36]. Hill's numbers were calculated using the renyi function of the vegan package. For the diversity ordering, we averaged true diversity values according to oxbows.

The total variance of species compositional data can be regarded as beta-diversity; thus, beta-diversity was calculated for all lakes separately. Beta-diversity is divided into three components: replacement, richness difference, and similarity, using the beta.div.comp function of the adespatial r-package. Replacement refers to the substitution of species among sediment layers, while richness difference indicates how much communities differ from each other in their number of species [38]. To calculate this, we used the SDR-simplex approach [38,39] based on the Jaccard index. The pairwise values could then be presented on ternary plots (i.e., simplices), where Replacement + Richness Difference + Similarity = 1. With the pairwise sums of the additive components, it was also possible to compare the contribution of beta diversity (Replacement + Richness difference) and nestedness (Richness difference + Similarity) to gamma diversity. The local contribution to the beta diversity (LCBD) of sediment layers for the oxbows was also determined by the beta.div function of the adespatial R package. All analyses were run in the R statistical environment [40].

## 3. Results

A total of 38 cladocera taxa were identified, belonging to five families (Bosminidae, Chydoridae, Daphniidae, Leptodoridae, and Sididae), in the sediment samples of the three lakes. Chydoridae was the most species-rich family, while Bosminidae, Daphniidae, Leptodoridae, and Sididae were represented by a few species. A total of 72% of the species pool (28 species) was common to all three lakes. The seven most frequently occurring species (*Acroperus harpae*, *Alona guttata*, *Bosmina coregoni*, *Bosmina longirostris*, *Chydorus sphaericus*, *Coronatella rectangula*, and *Graptoleberis testudinaria*) were dominant and collectively present in all lakes. The total abundance of Cladocera species varied from 715 to 4418 individuals per cm$^3$ (a mean of 2421 individuals per cm$^3$). Our results showed a high number of Cladocera communities in the SZA oxbow (taxa: 37) and a lower number in TI oxbow (taxa: 31). The abundance of Cladocera species was higher in the SZA and TI oxbows than in the KMT (taxa: 34) oxbow. *Alona rustica* was present only in the SZA oxbow. *Disparalona rostrata* was present in the sediment layer of the KMT, SZA, and TI oxbows.

Thirty-three Cladoceran taxa were present in the core sediment samples from the KMT oxbow, dominated by *Alona guttata*, *Coronatella rectangula*, *Bosmina coregoni*, *Bosmina longirostris*, *Chydorus sphaericus*, and *Graptoleberis testudinaria*. Those species inhabiting sediments were almost absent, with remains of *Monospilus dispar* (layer: 2–3), *Paralona pigra* (layer 4–5), *Chydorus gibbus* (layer 5–6), and *Alonella nana* (layer 9–10) being present in some core sediment layers. Additionally, some layers of the core sediment showed no remains of *Alonella exigua* (layer: 0–1) or *Acroperus harpae* (layer: 8–9). The total number of specimens in the first two layers was lower than that of the rest of the layers.

The highest species number (37) was found in the SZA oxbow. Additionally, the number of dominant species in this oxbow was also higher in comparison to the other oxbows. These dominant species were: *Acroperus harpae*, *Alona guttata*, *Alonella excisa*, *Bosmina coregoni*, *Bosmina longirostris*, *Coronatella rectangula*, *Chydorus sphaericus*, *Graptoleberis testudinaria*, *Pleuroxus trigonellus*, and *Sida crystallina*, which were present in all layers. Some species were absent in a few sediment layers, including remains of *Alonella exigua* (layer: 3–4), *Oxyurella tenuicaudis* (layer: 4–5), and *Alona intermedia* (layer: 10–11). *Alona rustica* was present only in the sediment layer (layer: 7–8) of the SZA oxbow. Moreover, *Camptocercus lilljeborgi* was almost absent in the sediment layer, and was present in only two layers (2–3) and (9–10). The lowest number of taxa (31) was present in the TI oxbow. In this oxbow, 10 taxa were dominant, but 8 taxa were also found as dominant species in the SZA oxbow. In addition, two species (*Alona intermedia* and *Picripleuroxus laevis*) occurred in all of the sediment layers.

The PCA analysis of both the cladoceran data and the environmental variables revealed the divergent characteristics of the three oxbows. The dispersion of each of the cladoceran and environmental variables was homogenous, and the oxbows differed in terms of both cladoceran communities and geochemical element composition (Table 1).

**Table 1.** PERMANOVA F and *p* values based on Euclidean distance for PCA scores and statistic parameters of the permutation test for homogeneity of multivariate dispersions (PERMDISP), based on PERMANOVA for functional composition. Numbers in bold represent significant values. In both tests, 999 permutations were made.

| Data Set | | R | F | *p* |
|---|---|---|---|---|
| Cladocerans | PERMDISP | | 1.4 | 0.268 |
| | PERMANOVA | 0.7145 | 37.529 | **0.001** |
| Environmental variables | PERMDISP | | 0.225 | 0.809 |
| | PERMANOVA | 0.591 | 21.639 | **0.001** |

The series of RDA analyses showed that each oxbow differed in terms of its cladoceran community (Figures 2–4). In the null model, significant environmental variables (distance from the Tisza, Cu, Zn, Ni, and $LOI_{950}$) explained 43% of the total variance in the cladoceran communities. The depth of the sediment layers as a conditional variable explained only 3.3% of the difference, and the variance explained by the conditional variables (Ba and $LOI_{950}$) decreased to 18%. When sites were introduced as conditional variables in the RDA, the sites explained 29% of the total variance and no variance remained constrained. The variance partitioning confirmed the results of the PCA, i.e., the species pools of these oxbows are different from each other (Table 2).

The species number of cladocerans varied between 16 and 22 in the KMT oxbow. The N1 diversity ranged between 7 and 15 in the KMT oxbow, between 13 and 17 in the SZA, and between 9 and 13 in the TI. The N2 diversity ranged between 4 and 11 in the KMT, 9 and 13 in the SZA, and 5 and 10 in the TI (Figure 3). The contribution of the sediment layers to the beta diversity of the oxbows shows how environmental changes can alter cladoceran communities. Any changes in the environmental factors could increase the variances of the cladoceran communities, i.e., beta diversity. Thus, local distribution to beta diversity (LCBD) will be changed. There were only small changes observed in the LCBD along the depth in the oxbows generally, but the greatest contributions to these changes were made in the top layer in the KMT oxbow and between the 3–8 cm section and the bottommost layers in the SZA oxbow. Changes in the LCBD in Timár Morotva Lake were relatively smooth along the depth (Figure 3). The SZA oxbow presented the highest diversity, while the KMT was slightly more diverse in rare taxa and the TI was slightly more diverse in abundant species (Figure 4).

The decomposition of the beta-diversity of oxbows revealed that common cladoceran species determine species pools of sediment cores, since the similarity among the layers was high: 58% in the KMT, 61% in the SZA, and 64% in the TI (Figure 3). Replacement components, which indicate that these species are responsible for the dissimilarities of the sediment layers, were quite high (32%, 31%, and 28% in the KMT, SZA, and TI, respectively). Richness Diff, which refers to those species that contribute to the changes in richness, is given by the absolute difference between the numbers of species found at the sites (10%, 8%, 10% in the KMT, SZA and TI, respectively). All beta-diversity components were very similar among the oxbows (Figure 5).

**Table 2.** Results of redundancy analyses. Numbers in bold represent significant values.

| | Null Model | | Depth Conditioned | | Site Conditioned | |
|---|---|---|---|---|---|---|
| | Inertia | Proportion | Inertia | Proportion | Inertia | Proportion |
| Total | 0.1697 | 1 | 0.1697 | 1 | 0.1697 | 1 |
| Conditional | | | 0.0056 | 0.0329 | 0.0487 | 0.2872 |
| Constrained | 0.0728 | 0.4288 | 0.0299 | 0.1760 | | |
| Unconstrained | 0.0969 | 0.5712 | 0.1342 | 0.7911 | 0.1209 | 0.7128 |
| F | 4.053 | | 3.2261 | | 0 | |
| *p* | **0.001** | | **0.001** | | 0 | |
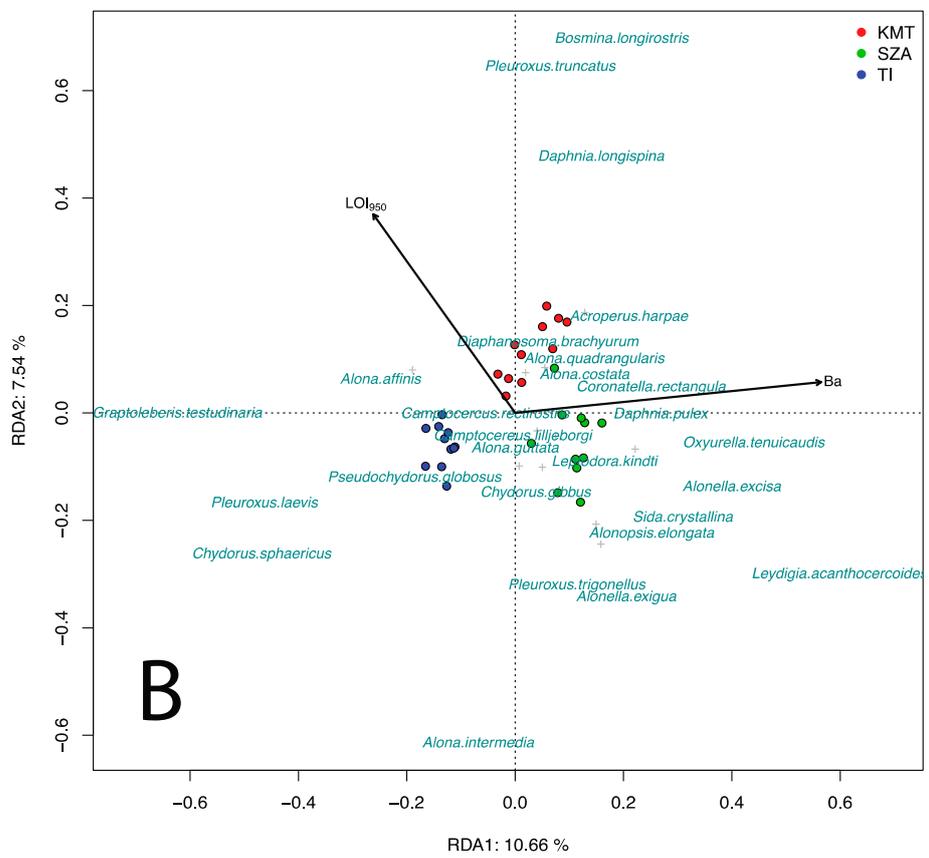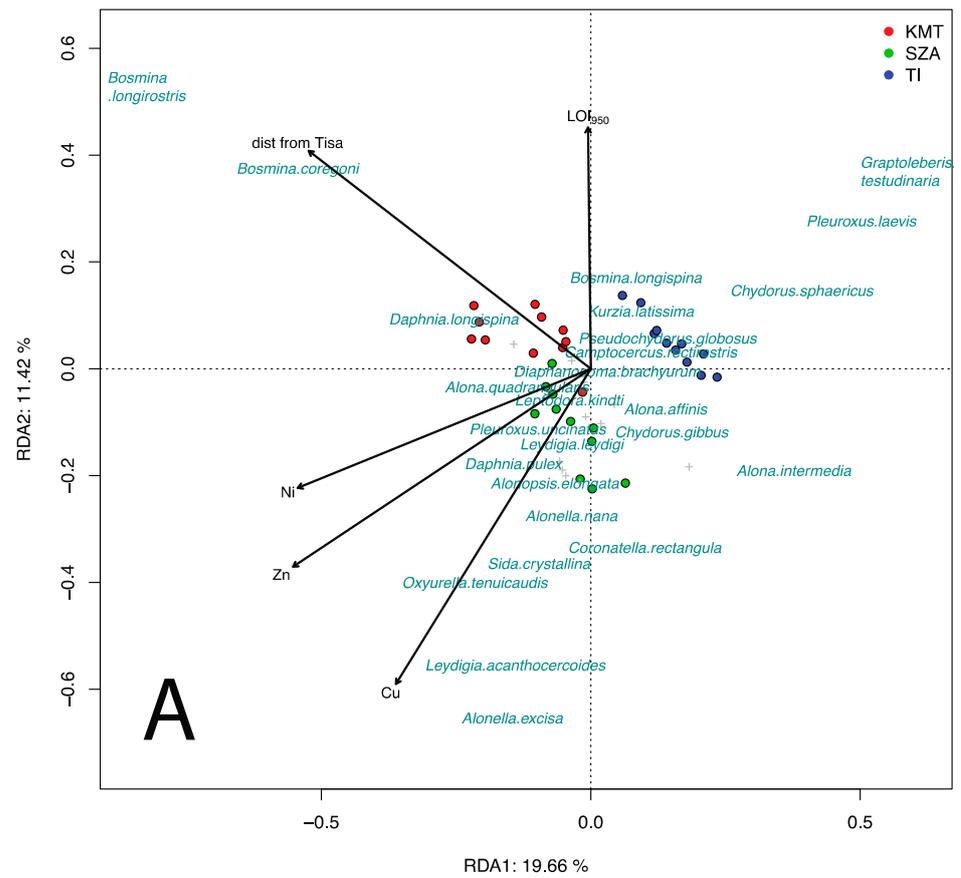
**Figure 2.** *Cont.*
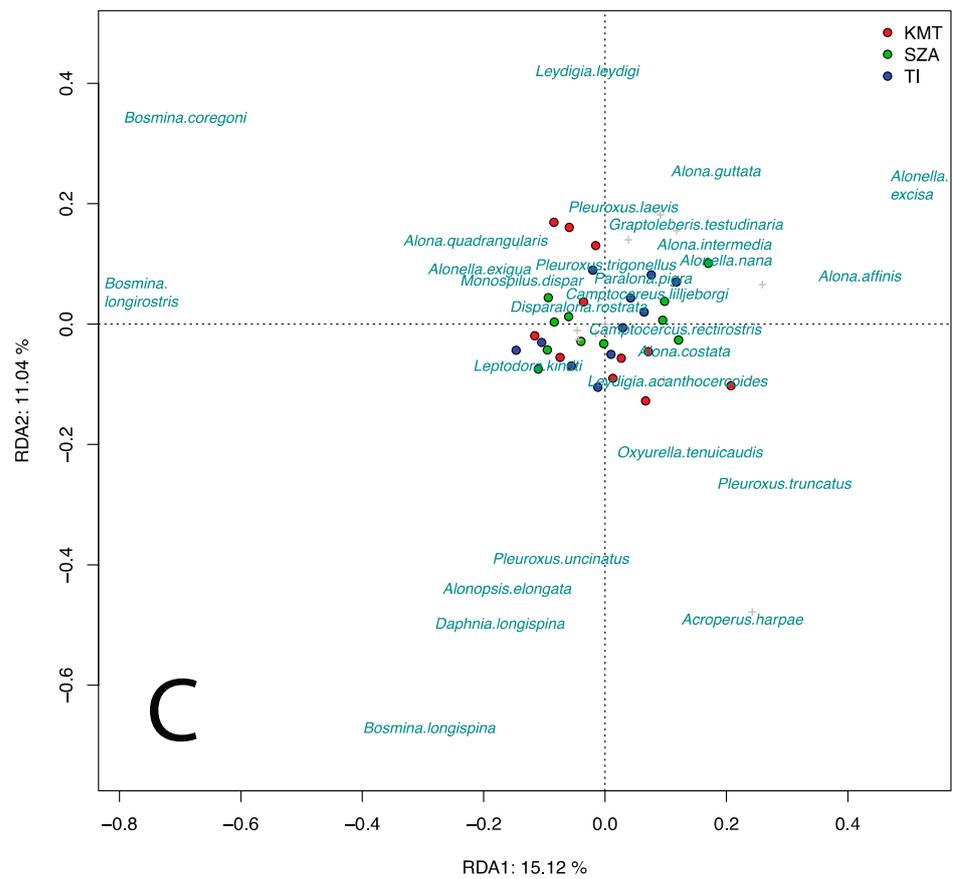
**Figure 2.** Biplot of redundancy analyses (arrows represent significant environmental variables. (**A**) null model; (**B**) depth-conditioned model, (**C**) site-conditioned model). (+ species with lower priority if labels overlap).
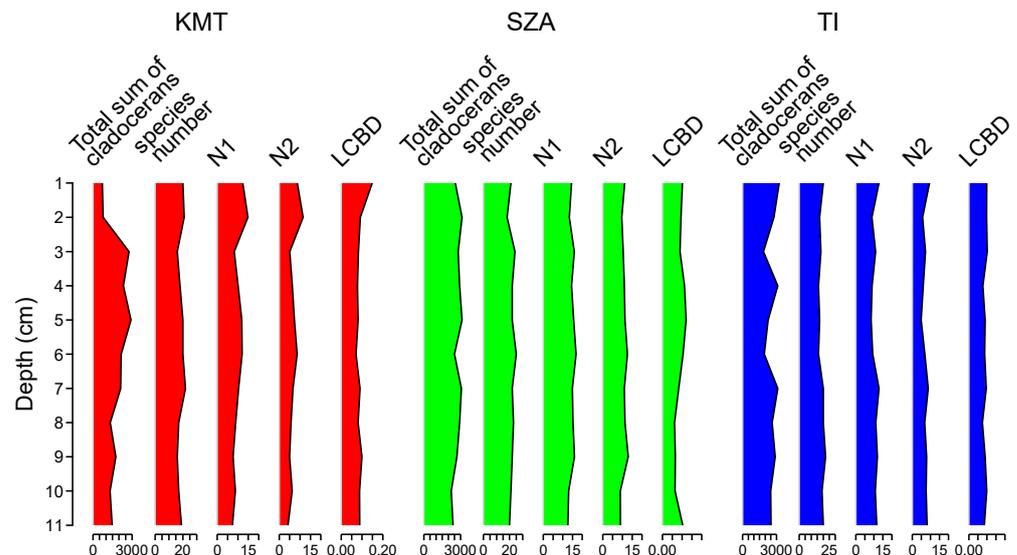


**Figure 3.** Stratigraphy plots of Cladocera abundances and diversities (number of species, Hill's N1, Hill's N2) and local contribution to beta-diversity (LCBD) of the cladoceran communities in the oxbows (KMT: Kis-Morotva oxbow, SZA: Szabolcs oxbow, TI: Timár Morotva oxbow).
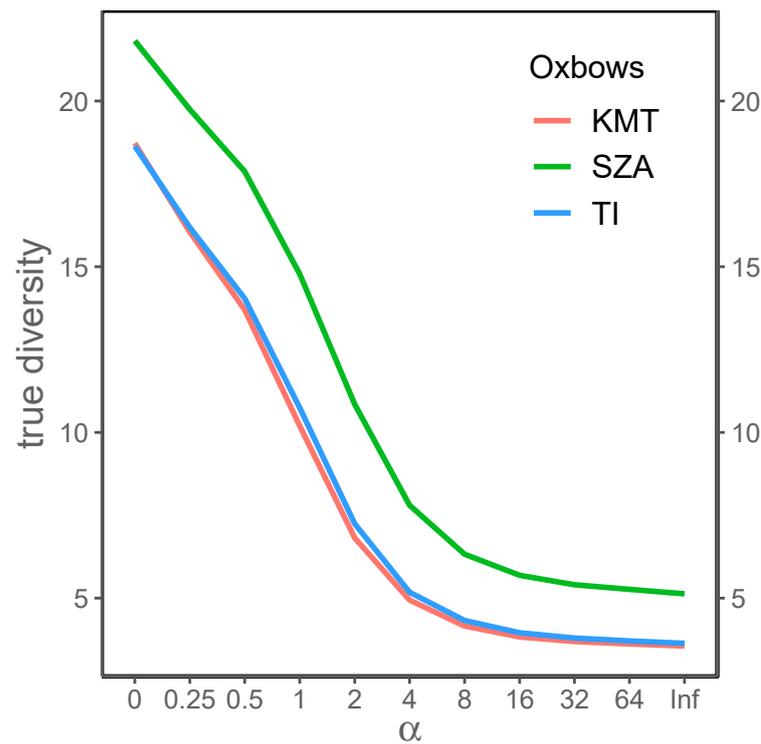
**Figure 4.** Diversity ordering of the oxbows.
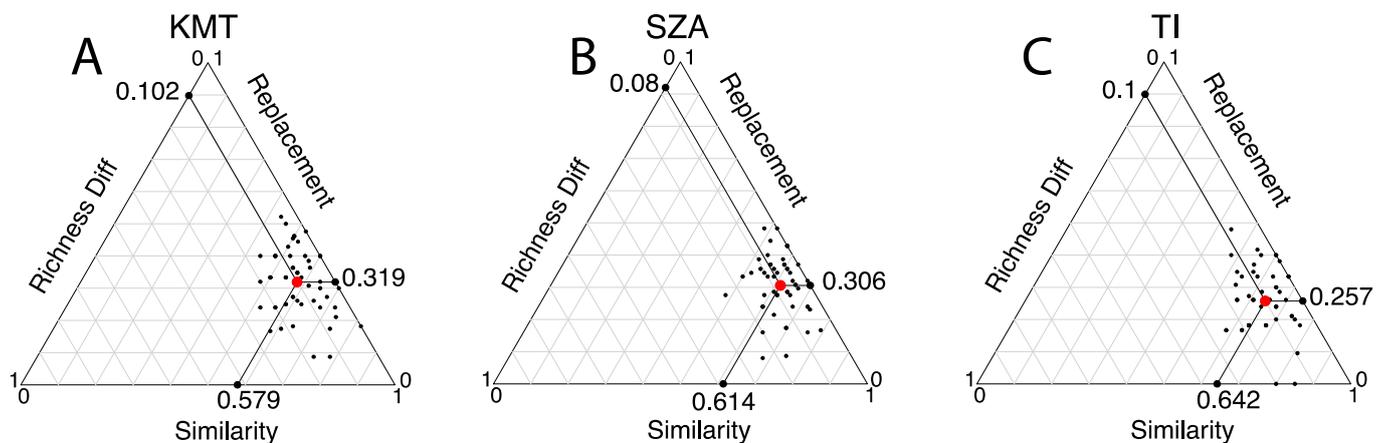


**Figure 5.** Triangular plots of beta diversity components (similarity, Richness Diff, and replacement) for the oxbows (**A**) KMT: Kis-Morotva oxbow, (**B**) SZA: Szabolcs oxbow, (**C**) TI: Timár Morotva oxbow. (Red dots are the means).

## 4. Discussion

The research examined a total of 39 species belonging to five families from three oxbow lakes in the Rakamaz region of Hungary. Chydoridae was the most species-rich family, as is similar to the other climatic regions [41–43]. Bosminidae, Daphniidae, Leptodoridae, and Sididae were subdominant families. In the studied lakes, the Chydoridae family is usually dominated by *Chydorus sphaericus*, which was also among the most abundant taxa in our core sediment samples, demonstrating adaptation to diverse resources of detrital food (detritus and attached microbes) and pelagic habitats under eutrophic conditions [44–46]. *Bosmina longirostris* (Bosminidae family) was the subdominant taxon in the lakes. This species has also been documented in many lakes and reservoirs in Europe's central, northern, and eastern areas. The dominant species in the oxbows included both littoral and planktonic species (littoral: *Acroperus harpae*, *Alona guttata*, *Chydorus sphaericus*,

*Coronatella rectangula*, and *Graptoleberis testudinaria*; planktonic: *Bosmina longirostris* and *B. coregoni*) [16].

The beta diversities (Richness Difference and replacement components) of the cores were lower (42%, 31%, and 36% in the KMT, SZA, and TI, respectively) than the nestedness (Richness Difference and similarity) (68%, 62%, and 74% in the KMT, SZA, and TI, respectively. In light of this result, we can conclude that the oxbows themselves supplied very similar environments for cladocerans, and their communities underwent similar changes. Site-conditioned RDA also confirmed this, since the site effect explained almost 30% of the total variance, while depth explained only 3%, i.e., the variances between oxbows were much larger than those within the oxbows.

Oxbows are shallow and eutrophic and are highly influenced by floods [21,47,48]. The SZA and TI are close to the River Tisza; thus, they receive water frequently from relatively small floods. The KMT is situated quite far from the river and is therefore only inundated during the highest floods. The community structure of aquatic invertebrates in floodplains is altered by hydrological dynamics [49,50]. During floods, cladocerans are washed out, and the new sediment buries all habitats. New populations can develop after being washed in by floods from other habitats [21]. The lotic and lentic statuses cannot be distinguished by species composition, but they can by quantity [21]. Cladoceran abundance increased from the bottom to the 3 cm depth in the KMT oxbow, indicating stable lentic phases, but the drop in abundance at the top could be due to drought. Cladoceran abundance showed minor changes due to the regular filling, while the changes in the abundance of Cladocera in the TI oxbow could be due to floods.

The SZA proved to be the most diverse oxbow due to it having the least dense macrophyte. The TI and KMT oxbows contained very dense macrophyte beds in which hypoxia could be developed near the sediment surface, preventing habitation by mud-dwelling Cladocera (*Leydigia* species, *Monospilus dipar*). Therefore, these species were found in the highest numbers in the SZA, with ten times more found in this oxbow than in the KMT and TI oxbows.

The cladoceran communities of oxbows differed significantly from each other (especially those of the SZA). Since the SZA is a fishing lake for anglers, it presented the lowest beta diversity, indicating a more homogenous habitat structure and high predation pressure of fish on cladocerans. Some sediment layers showed higher contributions to beta diversity, which probably reflects changes in the management of the oxbow.

The cladoceran community of the intermittent oxbow (KMT) gradually increased in complexity after re-entering in a natural way through groundwater affected by the high water level of the River Tisza. Due to the variable hydrological condition, this oxbow presented the highest variance in the cladoceran community (beta diversity). Its diversities have increased, and the topmost layers contribute more to its beta diversity, reflecting that the hydrological condition has become stable.

The cladoceran structure of the TI oxbow seems less variable, indicated by the high similarity and nestedness components of beta diversity. The almost constant LCBD values of its sediment layers prove that the hydrological conditions and fish pressure have not changed significantly.

## 5. Conclusions

To conclude, a study on the remains of cladocerans could reveal changes in their environment (hydrological condition, lake management, etc.). The reconstructed biodiversity of shallow lentic waters is determined mainly by littoral taxa, and pelagic taxa indicate the changes in open water patches in such shallow ecosystems.. The habitat preferences of the taxa can be used to reconstruct floods and to develop macrophyte cover. Our results confirm that subfossil Cladocera remains can be applied to reconstruct environmental and biodiversity changes in shallow waters.

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