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Effects of Environmental Factors on the Distribution and Diversity of Aquatic Oligochaetes

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Abstract: The aim of our study was to detect the actual distribution of oligochaete species and to identify their ecological differentiation with respect to environmental factors: altitude, temperature, oxygen concentration, conductivity, total organic carbon, and waterbody type. Although widespread, differentiation of oligochaete communities in four waterbody types and altitudinal groups can be observed through alpha and beta diversity. Their differences were analyzed using MANOVA, while the ecological preferences of species were presented with logistic Gaussian regression analyses. The highest number of the species of Oligochaeta was recorded in oligochaete communities in medium and large rivers. Total beta diversity decreased with the decreasing of waterbody size, the increasing of size of the substrate particles, river flow velocity, as well as altitude. Communities from small mountain rivers and streams and large and medium rivers with coarser substrate differed from other oligochaete communities. When coarser substrate was prevalent in smaller and medium rivers, a domination of a certain family was observed: Lumbriculidae (>800 m a.s.l.), Propappidae and Enchytraeidae (500–800 m), and Naididae (<500 m a.s.l.). Common species of Oligochaeta, with significantly overlapping ranges in running waters in Serbia, still show a clear grouping with respect to preference for certain types of waterbodies.

Keywords: aquatic worms; communities; diversity; waterbody types; Serbia

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1. Introduction

According to Limnofauna Europea [1], there are around 190 species of aquatic oligochaetes in Europe. Due to their potentially high densities, wide distribution, and indicator value, aquatic oligochaetes may be important for water management [2] but may also be indicative of a variety of environmental conditions other than pollution. The influence of stream hydrology and physical and chemical factors on aquatic Oligochaeta has been studied by many authors [3–6].

The diversity of the Oligochaeta fauna in Serbia is in accordance with research in European countries: the Netherlands, Belgium, Germany [7], Poland [8], the Czech Republic [9], and Estonia [6], as well as in the region: Slovenia [10], Bulgaria [11], Croatia [12], Montenegro [13], and Albania [14]. Generally, in these countries the number of species is around 100–150, depending on the amount of examined material, habitat types, and detailed determination.

Previous investigations of oligochaete fauna in Serbian freshwaters included community structure, composition, and species distribution in different waterbody types [15]. The first comprehensive species list with oligochaete fauna of the large lowland rivers, the Danube and Sava, as well as hilly and mountainous rivers south of the Danube corridor was presented. The high diversity of aquatic oligochaetes in Serbia was noted, with the largest participation of potamal and rhithral species, and running waters were divided into four groups based on the oligochaete community. An obvious distinction based on the dominant taxa in the community was as follows: naidids (naidins and tubificins), enchytraeids, and lumbriculids. The Danube basin was distinguished by a high species diversity and dominance of *Limnodrilus hoffmeisteri*. Lower river stretches of the Danube and Sava that flow through Serbian territory could be compared to lake ecosystems, representing the typical potamal. Generally, the diversity and relative abundance of the macroinvertebrate fauna is significantly influenced by substrate type and river current [16], so the qualitative composition of oligochaete assemblages in Serbian freshwaters had a clear pattern—lower diversity in tributaries, and an increased diversity in the main watercourses. Of course, due to anthropogenic factors, discrepancies in the distribution of oligochaetes can be observed in waterbodies with increased sedimentation, slower river current, and increased organic pollution of tributaries, allowing habitation by cosmopolitan species. Under these conditions, Schenková and Helešic [17] observed that substrate type does not have a crucial role, and the normal distribution of Oligochaeta can change in response to organic pollution, in the way that lower diversity could be observed in the polysaprobic zones of river stretches and higher diversity in oligosaprobic zones. On the other hand, saprobic conditions are important abiotic factors for the distribution of Oligochaeta, but the substrate type can reduce the indicator value of some taxa, which has been shown in previous research [15].

The aim of this work is to better understand the distribution and diversity of aquatic oligochaetes in smaller rivers and mountain watercourses, since they are usually neglected in these types of waters. The results presented should help to make oligochaetes more reliable in the biological validation of waterbody typology according to European best management practice.

2. Materials and Methods

2.1. Field and Labarotory Work

An investigation conducted in 2019 and 2020 (spring/summer) covered 119 watercourses (181 locations/sites) and included a variety of waterbodies from mountain streams, upper river stretches, and downstream to the large lowland rivers. Macroinvertebrate samples were collected using a combination of the kick and sweep and multihabitat sampling technique according to European Standards [18] using a FBA hand net (mesh size 500 µm and 250 µm). The samples were pooled, and the material was preserved in ethyl alcohol (70%). In total, 3600 oligochaeta individuals were collected. For species identification, appropriate keys were used [7,19]. Families Naididae (with subfamilies Naidinae, Tubificinae and Pristininae), Propappidae, Lumbricidae, and Lumbriculidae were identified to the species level, while family Enchytraeidae was identified to the lowest possible taxonomic level.

Water temperature (°C) at the moment of sampling, conductivity (µS/cm), and oxygen concentration (mg/L O_2) were measured with the Horiba W-23XD multiparametric probe (HORIBA Instruments Corporation, Irvine, CA, USA) in the field and total organic carbon (TOC; mg/L C; SRPS ISO 8245:1994) in the laboratory.

2.2. Data Analyses

The frequency of occurrence (F) for each species in oligochaete assemblages was calculated using the formula:

$$F = n/N,$$

where n is the number of samples in which a taxon was found, and N is the total number of samples.

Oligochaeta were analyzed according to waterbody type by classifying each locality according to its characteristics (hydromorphological properties) into the following: Type 1—large rivers with fine substrate (silt, clay mud, and sand); Type 2—mix of large and medium rivers with coarser substrate (gravel, stones, and rocks); Type 3—small watercourses (up to 500 m a.s.l.); Type 4—small mountain rivers and streams (above 500 m a.s.l.);

and Type 5—slow flowing/stagnant waters (artificial canals and reservoirs), and in relation to the elevation gradient: 1—localities up to 500 m a.s.l.; 2—localities from 500 to 800 m a.s.l.; 3—localities above 800 m a.s.l.

A range of waterbody types represents a complex hydromorphological gradient. Environmental factors (flow velocity, bottom properties, temperature, oxygen concentrations) change predictably through waterbody types (5→1→2→3→4) and with increasing altitude. The logistic Gaussian regression [20,21] was used to detect the response and ecological preferences of the analyzed species, along the altitudinal gradient and gradient of waterbody types. The logistic Gaussian regression was performed using FLORA software version 2013 [22].

Within each group of the Oligochaeta community, components of alpha and beta diversity were investigated. Alpha diversity was assessed using Species Richness, the Shannon Index, and the Equitability Index. Components of beta diversity were analyzed using the procedures described by Baselga [23].

MANOVA [24] was utilized to find a combination of species that maximally discriminates groups of communities.

3. Results

Of the total number of macroinvertebrate samples, the Oligochaeta were found in 70 samples from 56 waterbodies. The Oligochaeta were represented by 34 taxa, belonging to 21 genera within 5 families. The distribution of the taxa recorded and the frequency of their occurrence are presented in Table 1.

Table 1. Distribution of taxa recorded with name abbreviations and the frequency of their occurrence.

		Waterbody Type					**Altitude**		
Taxon	Abb.	Type 1	Type 2	Type 3	Type 4	Type 5	Alt 1	Alt 2	Alt 3
f. Naididae									
subf. Naidinae									
Branhiodrilus hortensis (Stephenson, 1910)	Bra hor	0.2				0.09	0.04		
Chaetogaster diaphanus (Gruithuisen, 1828)	Cha dia		0.1				0.02		
Dero digitata Müller, 1773	Der dig					0.09	0.02		
Dero dorsalis Ferronière, 1899	Der dor					0.09	0.02		
Dero obtusa d'Udekem, 1835	Der obt					0.18	0.04		
Nais alpina Sperber, 1948	Nai alp				0.17			0.14	
Nais barbata Müller, 1773	Nai bar			0.03					0.1
Nais bretscheri Michaelsen,1899	Nai bre		0.2	0.37	0.17	0.27	0.3	0.29	0.2
Nais communis Piguet, 1906	Nai com			0.03	0.17		0.02		0.1
Nais elinguis Müller, 1774	Nai eli		0.4	0.16	0.17		0.13	0.29	0.2
Nais pseudobtusa Piguet, 1906	Nai pse		0.2				0.02	0.14	
Nais variabilis Piguet, 1906	Nai var		0.1				0.02		
Ophidonais serpentina (Müller, 1773)	Oph ser	0.2		0.05		0.27	0.11		
Uncinais uncinata (Ørsted, 1842)	Unc unc					0.09	0.02		
Stylaria lacustris (Linnaeus, 1767)	Sty lac	0.4		0.05		0.64	0.21		
subf. Pristininae									
Pristina aequiseta Bourne, 1891	Pri aeq			0.03			0.02		
subf. Tubificinae									
Bothrioneurum vejdovskyanum Štolc, 1888	Bot vej		0.1	0.03			0.04		

Table 1. *Cont.*

				Waterbody Type				**Altitude**	
Branchiura sowerbyi Beddard, 1892	Bra sow	0.2	0.1	0.05		0.09	0.09		
Ilyodrilus templetoni (Southern, 1909)	Ily tem		0.1				0.02		
Limnodrilus claparedeanus Ratzel, 1868	Lim cla	0.4	0.2	0.11			0.13	0.14	
Limnodrilus hoffmeisteri Claparède, 1862	Lim hof	0.8	0.6	0.55	0.33	0.55	0.62	0.43	0.3
Limnodrilus udekemianus Claparède, 1862	Lim ude	0.2	0.1	0.13		0.09	0.15		
Potamothrix hammoniensis (Michaelsen, 1901)	Pot ham	0.2	0.6	0.39	0.33	0.09	0.36	0.43	0.3
Potamothrix vejdovskyi (Hrabě, 1941)	Pot vej				0.17				0.1
Psammoryctides albicola (Michaelsen, 1901)	Psa alb			0.08	0.17		0.04		0.2
Psammoryctides barbatus (Grube, 1861)	Psa bar		0.1	0.05			0.04	0.14	
Lophochaeta ignota (Štolc, 1886)	Lop ign			0.03			0.02		
Tubifex tubifex (Müller, 1774)	Tub tub	0.2		0.08			0.08		
f. Propappidae									
Propappus volki (Michaelsen, 1916)	Pro vol		0.1	0.08			0.04	0.29	
f. Enchytraeidae									
Enchytraeidae gen. sp.	Enc	0.2	0.1	0.18	0.17	0.18	0.15	0.29	0.2
Fridericia sp.	Fri sp.			0.03		0.09	0.04		
Henlea ventriculosa (d'Udekem, 1854)	Hen ven			0.08			0.06		
Cernosvitoviella sp.	Cer sp.		0.2				0.02	0.14	
f. Lumbriculidae									
Stylodrilus heringianus Claparède,1862	Sty her		0.2	0.26	0.5		0.15	0.14	0.6
f. Lumbricidae									
Eiseniella tetraedra (Savigny, 1826)	Eis tet		0.2	0.05	0.17		0.04	0.14	0.2

Five localities stood out due to the high participation of oligochaetes in the macroinvertebrate community: Vrla—Vladičin Han (57.14% of individuals), Bjelica—Lučani (87.5%), Jablanica—Leće (88.46%), Krivaja—Bačka Topola (96.76%), and Tamnava—Koceljeva (98.60%).

In the oligochaete assemblages, *Limnodrilus hoffmeisteri* (Claparède, 1862) was the dominant species in most waterbody types (68.70% in Type 1, 59.48% in Type 2, 45.40% in Type 3, and 31.18% in Type 5), with exception of Type 4, where *Stylodrilus heringianus* (Claparède, 1862) had the highest participation (75.55%). Regarding altitudes, *L. hoffmeisteri* had the highest percentage participation in localities below 500 m a.s.l. (52.03%). In localities from 500 to 800 m a.s.l. the dominant species was *P. hammoniensis* (27%), and at altitudes above 800 m it was *S. heringianus* (67.9%).

The most frequent species in our investigation was *L. hoffmeisteri* (F = 0.55), followed by *Potamothrix hammoniensis* (Michaelsen, 1901) (F = 0.36), *Nais bretscheri* (Michaelsen, 1899) (F = 0.29), and *S. heringianus* (F = 0.21). Other species were recorded with frequency of occurrence less than 0.2. *Limnodrilus hoffmeisteri* was the most frequent in waterbodies Type 1, 2, and 3; *S. heringianus* was the most frequent in waterbodies Type 4, while *Stylaria lacustris* (Linnaeus, 1758) was the most frequent in waterbodies Type 5. Regarding altitudes, the most frequent species below 500 m a.s.l. was *L. hoffmeisteri* (F = 0.62), followed by *P. hammoniensis* (F = 0.36) and *S. lacustris* (F = 0.21). At altitudes from 500 to 800 m a.s.l., the most frequent species were still *L. hoffmeisteri* and *P. hammoniensis* (F = 0.43), but also naidins (*N. bretscheri*, *N. elinguis*), propappids (*Propappus volki*), and enchytraeids were frequent; F = 0.29 each. At altitudes above 800 m, the most frequent species was *S. heringianus* (F = 0.6).

A few species were recorded only in certain types of waterbodies with low frequencies (Table 1): *Chaetogaster diaphanus* (Gruithuisen, 1828), *Nais pseudobtusa* Piguet, 1906, *N. variabilis* Piguet, 1906, *Ilyodrilus templetoni* (Southern, 1909) (Type 2), *N. barbata*, *Pristina aequiseta* Bourne, 1891, *Lophochaeta ignota* (Štolc, 1886), *Henlea ventriculosa* (d'Udekem, 1854), *Cernosvitoviella* sp. (Type 3), *Nais alpina* Sperber, 1948, *Potamothrix vejdovskyi* (Hrabě, 1941) (Type 4), *Dero* sp., *Uncinais uncinata* (Ørsted, 1842) (Type 5). *N. alpina* was found only at one locality at an altitude of 750 m a.s.l.

Tubificines, naidines, and enchytraeids were recorded in all waterbody types, but the tubificines were the most diverse in Type 3 and the naidines in Type 5 (Figure 1). Most of the families were recorded in all altitude groups of localities, except Propappidae and Pristininae (Figure 2). In altitudes below 500 m, the highest number of species was detected for Naidinae and Tubificinae.

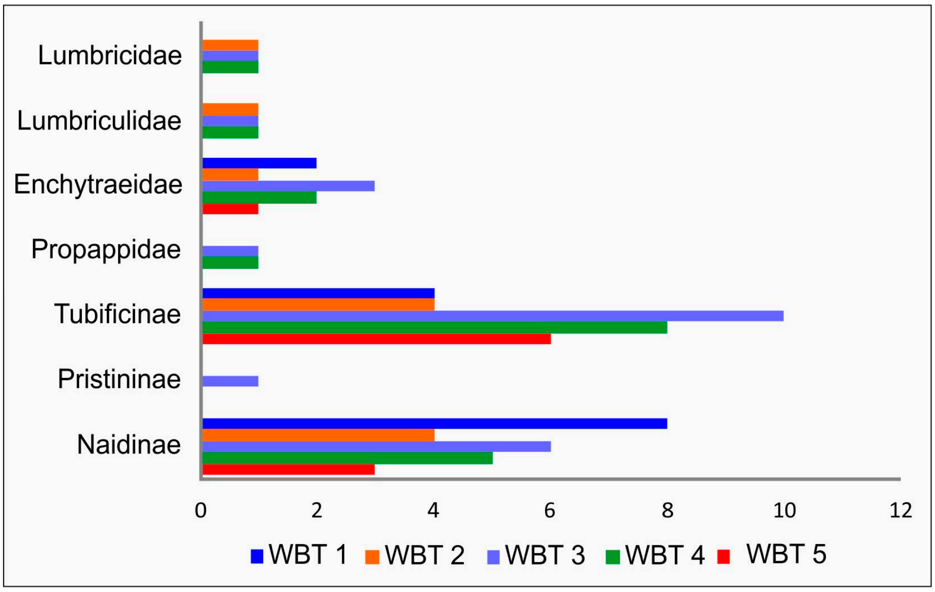

Figure 1. Number of species in different waterbody types (WBT).

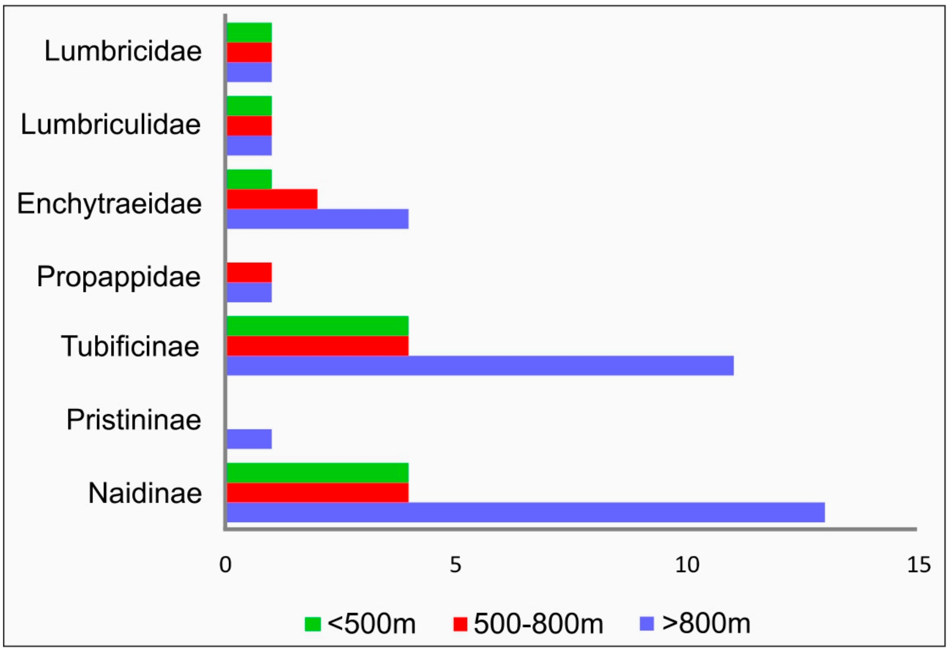

Figure 2. Number of species in different altitudes (Alt).

The components of alpha diversity within the oligochaete community were analyzed in relation to altitude (Figure 3) and waterbody types (Figure 4). Three groups of oligochaete communities differed significantly in relation to altitudes, where all three components of alpha diversity (Shannon entropy, Species richness, and Equitability) showed similar trends. Communities at altitudes of 500–800 m showed the highest values of alpha diversity components, while communities at the highest altitudes showed the lowest values. Differentiation of oligochaete groups in relation to different waterbody types is not so obvious. Species richness and equitability showed similar trends. The highest species richness was observed in WBT 2.

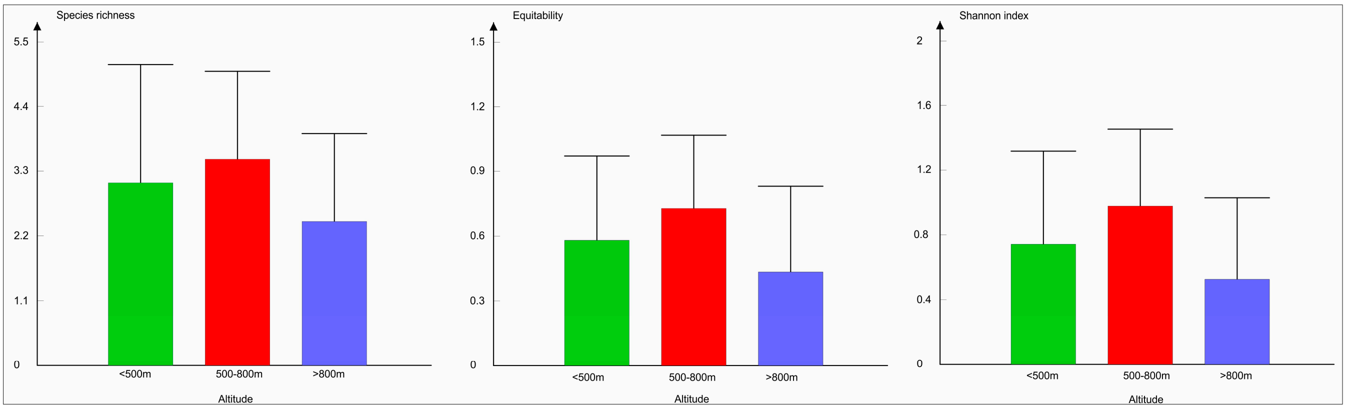

Figure 3. Alpha diversity components within oligochaetes communities in relation to altitude.

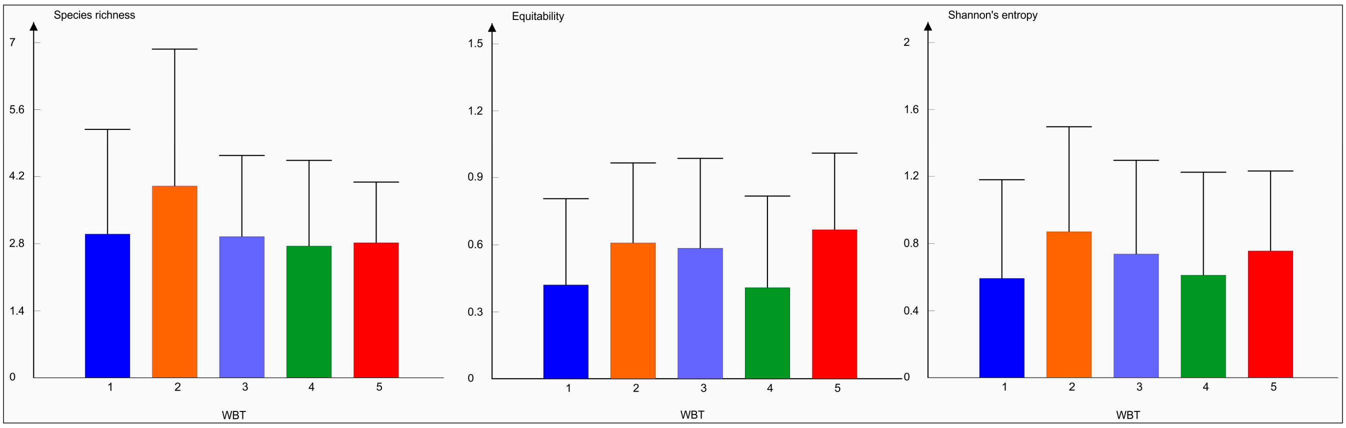

Figure 4. Alpha diversity components within oligochaetes communities in relation to the waterbody types (WBT).

The highest number of oligochaete species was recorded in the group of oligochaete communities in medium and large rivers (WBT 2) and at altitudes of 500 to 800 m.

The total beta diversity, and its components, was analyzed in relation to different waterbody types and altitudes (Figure 5a,b).

The lowest values of nestedness with the highest species turnover were in WBT 1, with the highest total beta diversity as well. Total beta diversity decreases with decreasing waterbody size, increasing substrate size, and higher river flow velocity, as well as with increasing altitude. The oligochaete communities in small mountain rivers and streams, at altitudes above 800 m, showed the highest values for nestedness with the lowest species turnover.

A comparison of oligochaete communities' composition using MANOVA (Figure 6) showed differences in relation to altitudes. Three groups of communities were separated: those at altitudes above 800 m were characterized by a representative of the family Lumbriculidae, *S. heringianus* (followed by Lumbricidae, *E. tetraedra* and Naididae, *N. barbata*, *N.*

communis); communities at altitudes of 500–800 m were characterized by family Propappidae, *P. volki* (followed by Enchytraeidae, *Cernosvitoviella* sp. and Naididae, *Psammoryctides barbatus*); and communities at altitudes below 500 m were distinguished by the species *L. hoffmeisteri* (family Naididae).

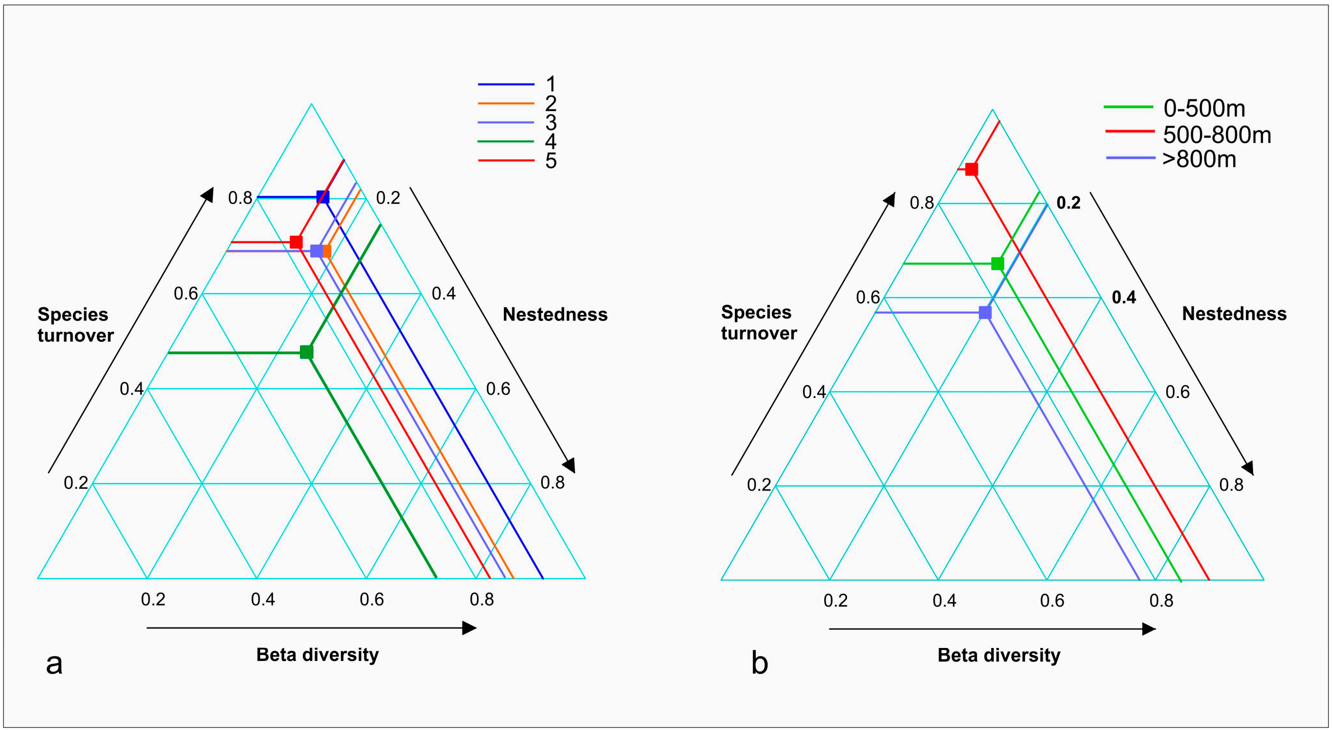

Figure 5. Beta diversity components in (**a**) different waterbody types (1–5) and (**b**) altitudes.

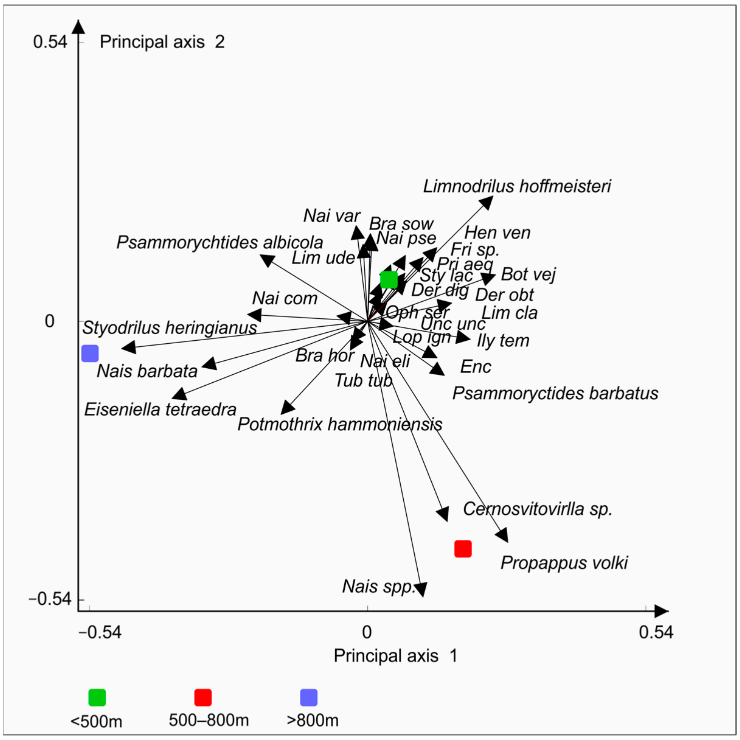

Figure 6. MANOVA analysis of oligochaete communities with respect to different altitudes.

MANOVA analyses with respect to WBT showed differences of oligochaete communities (Figure 7). Small mountain rivers and streams (WBT 4) and large and medium rivers with a larger substrate type (WBT 2) were distinguished from other communities. In WBT 2, enchytraeids (*Cernosvitoviella* sp.) and propappids (*P. volki*) dominated, while in WBT 4 lumbriculids (*S. heringianus*) and naidids (*N. communis*, *N. elinguis*, *P. hammoniensis*) were dominant. The communities in other waterbody types showed fewer differences (Figure 7).

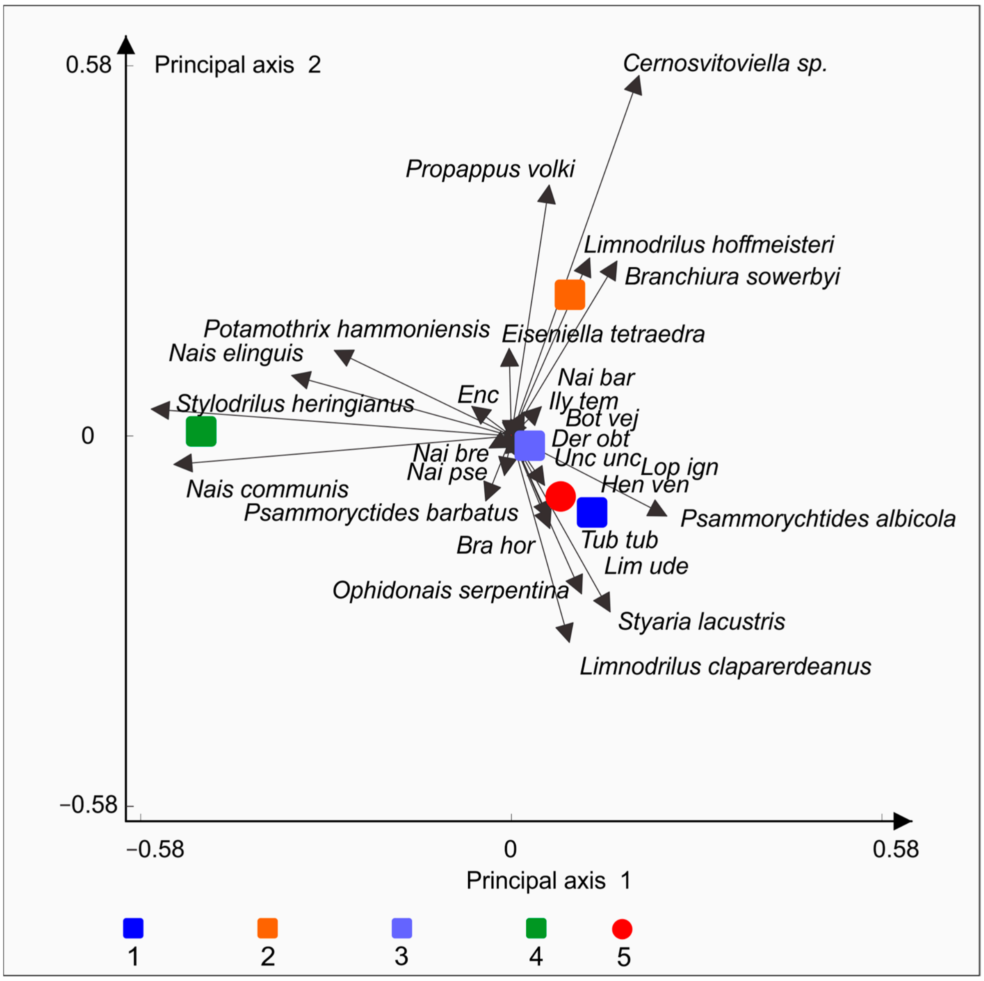

Figure 7. MANOVA analysis of oligochaete communities with respect to different waterbody types.

Response curves of selected species with respect to several environmental factors are shown in Figure 8. Gaussian logistic regression showed the ecological differentiation of the species which characterized oligochaete communities along the oxygen concentration, altitudinal gradient, and with respect to the waterbody type. The greatest ecological tolerance with respect to altitude was observed for *S. heringianus* and *E. tetraedra* (over 1200 m a.s.l.), while *B. sowerbyi*, *O. serpentina*, and *S. lacustris* were distinguished by their narrow range of distribution along the altitudinal gradient, with the optimal altitude up to 300 m and below.

The ecological tolerance of analyzed species with respect to waterbody type was wide (most of the species occurred in all waterbody types). The ecological differentiation was obvious for *P. volki*, *P. barbatus*, and *S. heringianus*. These species avoid large rivers with fine substrate, while *Stylaria lacustris* preferred slow and stagnant waters with fine sediment (Types 1 and 5). Most species had a wide response curve when this gradient was observed. *Eiseniella tetraedra* showed the narrowest tolerance curve with respect to oxygen concentration. A high concentration of oxygen is preferred by this species, but also by *P. volki* and *N. elinguis*, which showed a wider range of tolerances. With respect to the temperature gradient, the narrow ecological tolerance was recorded only for *E. tetraedra* and *B. sowerbyi*. With respect to other analyzed ecological preferences, conductivity and total organic carbon, the ecological tolerances of the analyzed species were wide. The ecological optimum of all species ranged from 6 to 9 mg/L, except for *E. tetraedra*, which was recorded in assemblages with oxygen concentrations higher than 9 mg/L.

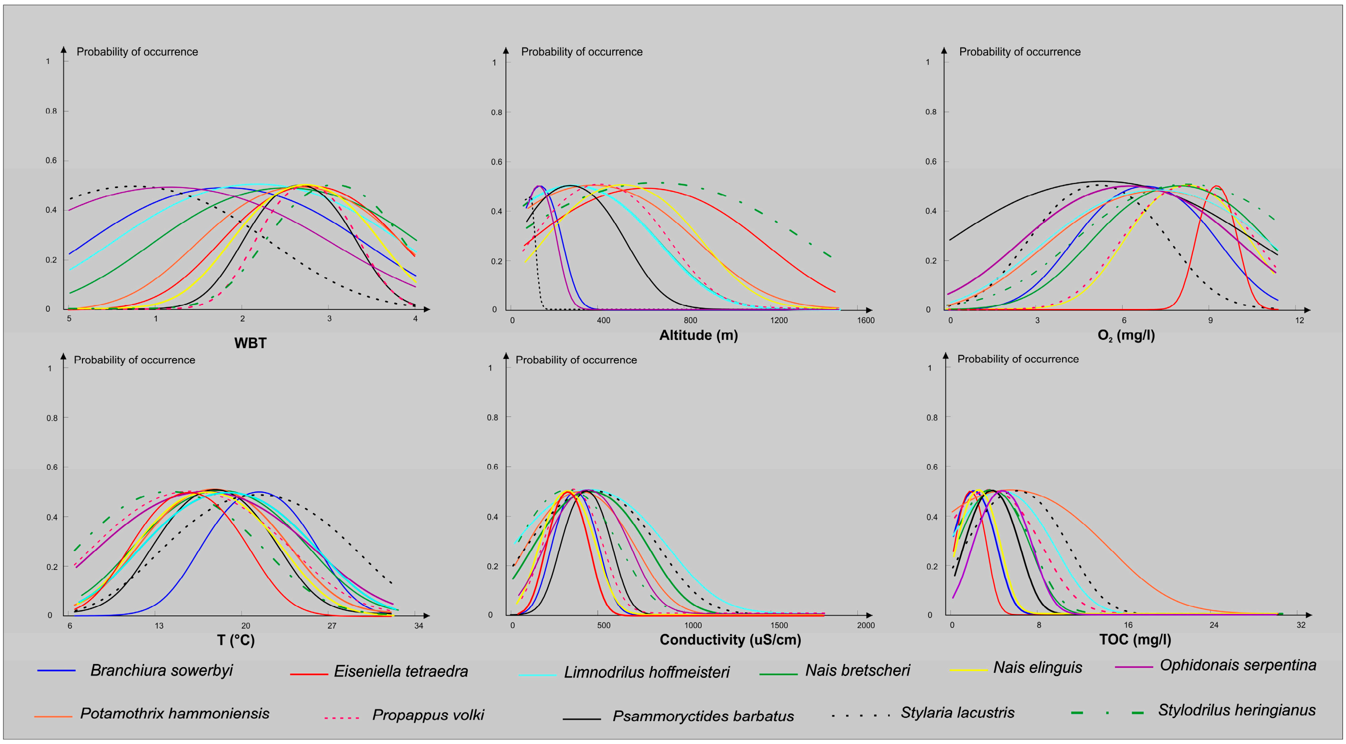

Figure 8. The ecological differentiation of the analyzed species with respect to selected factors.

The highest range of ecological tolerance with respect to conductivity and TOC gradient showed *P. hammoniensis* and *L. hoffmeisteri*.

4. Discussion

In the fauna of freshwaters of Serbia, a total of 97 species (45 genera from 8 families) have been recorded so far [8]. A third of the species (37) were recorded only in the main flow of the Danube River. The Danube is considered as the center of biodiversity and the main corridor for the spread of Ponto-Caspian oligochaetes species [25]. Since the Danube was not included in this study, many species typically found in this catchment area were missing from the results, especially the species from the genera *Potamothrix*, *Psammoryctides*, and *Isochaetides*. Oligochaete communities from this investigation include 34 taxa (21 genera from 5 families), which is comparable with previous investigations in Serbia and in the surrounding countries that belong to the same biogeographical territory. Oligochaete fauna of running waters in Serbia has been enriched by a new species. The record of *N. alpina* is interesting because it is a rare species; some consider it endemic to Europe [7]. It is a rheophil, stenotherm species, inhabits stony bottoms in the upper and middle courses of brooks and rivers, and prefers cooler waters [7]. The exotic, tropical species *B. hortensis*, which could be invasive, is rare in Europe. So far, it has been recorded only in seven European countries [26]. In Serbia, it has expanded from the main course of the Danube to the main tributaries and canals, and it has successfully established stable populations in the new environment and is regularly recorded during annual monitoring. The species is adapted to a wide range of substrate types, from small wetland pools to large ponds [27], and it seems to be tolerant to organic load and pollution. In Serbia, it is successfully expanding its range to the area north of the Danube (Pannonian Plain), while it has not yet been observed in the hilly-mountainous region south of the Danube. As van Haaren and Soors [7] pointed out, the specific ecological demands for this species remain to be determined.

This study confirmed previous conclusions [15] that *Limnodrilus hoffmeisteri* is the most important edificator of the oligochaete fauna in the Serbian waters, as it was recorded in all waterbody types, with the highest frequency of occurrence and percentage participation in oligochaete communities in waterbody Types 1, 2, and 3. It was typical for large lowland rivers, but also present in some small- and medium-sized watercourses at altitudes above 500 m a.s.l. Like in the research of [15], the characteristic species of hilly and mountainous types of watercourses (especially above 800 m) was *Stylodrilus heringianus*, the dominant species in waterbody Type 4. This species typically inhabits springs, preferring a lower water temperature and level of eutrophication, harder substrates, and faster currents [28]. Phytophilous *Stylaria lacustris* was dominant in waterbody Type 5, a typical lentic habitat type, with slow current or stagnant water and with the presence of detritus and macrophytes. In periphyton, the domination of naidines is expected, particularly the domination of *S. lacustris* [29].

Microhabitat complexity in large rivers enables the presence of euryvalent, a cosmopolitan species, such as most of the species from the Tubificinae subfamily, with the dominance of *L. hoffmeisteri*. Microhabitat complexity with altitude decreases and an increase in the number of taxa that prefer low organic load was observed. The same patterns are noted by Atanacković et al. [15]. Such significant reduction in microhabitat complexity is a selective pressure, which reduces the number of species, as concluded by Marinković et al. [30] for leeches, another group of Annelids. High beta diversity in communities dominated by *L. hoffmeisteri* (WBT 1, 2, and 3) is attributable to the difference in species composition (species turnover) and not to species richness (nestedness). These waterbodies differed in their habitat characteristics, providing a variety of microhabitats that offer a range of suitable conditions for different species. The opposite was observed in communities dominated by *S. heringianus* (WBT 4), with the highest values for nestedness and the lowest for species turnover. A plausible explanation for this phenomenon could be that these streams at higher altitudes have characteristics that are a limiting factor for most other oligochaete species, such as higher flow velocity and less organic matter and silt, resulting in each successive community being a subset of the previous community, while species substitution does not occur [30,31]. With harder substrate, sand and rocks, and a fast current, the lowest species turnover was expected due to the unfavorable environmental conditions for oligochaetes. In regard to oligochaete communities, the biodiversity of these habitats is generally low, the majority of species belong to the families Enchytraeidae and Lumbriculide, and the frequent occurrence of *Stylodrilus heringianus* is evident [28].

Environmental predictors can explain a relatively small part of total variability of oligochaete distribution. The altitudinal gradient affects substrate particle size and river current [32], so it had an effect on the community structure. *Eiseniella tetraedra*, *N. elinguis*, *S. heringianus*, *P. volki,* and *P. barbatus* preferred rivers with harder substrate and faster current and were absent in localities with fine substrate. This distinguishes them from *O. serpentina* and *S. lacustris,* which occurred only in rivers covered with fine substrate and with a slow-to-medium water current.

A higher abundance of enchytraeids and propappids is characteristic of rheo- and helocrene watercourses [33]. Communities in rivers with harder substrate (WBT 3 and 4) were distinguished by the presence of edificatory species from these families, while communities in small hilly and mountainous rivers (WBT 4) were characterized by edificatory species of lumbriculids. Representatives of the family Naididae showed a distribution pattern characterized by different preferences for flow velocity and substrate composition. *Nais bretscheri*, *N. barbata*, *N. pseudobtusa,* and *Bothrioneurum vejdovskyanum* preferred higher flow velocity and coarser substrate. When the water flow slowed down and the substrate was finer, these species were replaced by naidids that prefer almost stagnant waters (*S. lacustris*, *Chaetogaster* sp.). Martínez-Ansemil and Collado [34] reported that substrate and water velocity are the most important factors influencing the distribution of oligochaetes, while Marchand [35] noted that DO and organic matter affect the distribution of oligochaetes. Large rivers and lower river stretches could be compared to lake ecosystems (with high depth, slow flow, lower oxygen concentration) representing a typical potamal type, and as Atanacković et al. [15] showed, the slowing down of the river current contributes to more intensive sedimentation and in that way could significantly influencethe diversity and relative abundance of the oligochaete fauna. Thus, the oligochaetes in the tributaries are less diverse and abundant than in the main stream of the river [15]. The pelophilous group, which consists of the genera *Limnodrilus*, *Branchiura*, *Tubifex,* and *Pothamothrix,* was characteristic of a slow river current and fine substrate, and the psammophilous group (*Stylodrilus*, *Henlea*, *Nais* spp., and *Eiseniella*) was characteristic of habitats with harder substrates (sand, pebbles, and stones) and faster currents. Also, a lentic environment influenced the distribution of phytophilous *Ophidonais serpentina* and *Stylaria lacustris*. According to the present results, due to heterogeneous microhabitats, higher species richness was observed in oligochaete assemblages in these river stretches.

5. Conclusions

This study analyzed oligochaete communities with a focus on smaller rivers and mountain watercourses. It revealed a high diversity of oligochaetes at higher altitudes (500–800 m) and in rivers with coarser substrate. Also, it shows that substrate particle size and current velocity have a significant influence on the distribution and diversity of oligochaetes. Although the majority of aquatic oligochaetes prefer silt, clay, and slower water currents, some species such as *Stylodrilus heringianus* are typical inhabitants of mountain rivers and streams. The results also indicate that *Limnodrilus hoffmeisteri* is the most prominent edificator of the oligochaete fauna in the waters of Serbia. Further investigations that encompass all ecosystems, booth small and large rivers, as well as reservoirs, could give us the larger datasets necessary to answer the question of where the highest diversity of oligochaetes is. This study is an important step for using oligochaetes more reliably and effectively, as they are one of the necessary BQEs (biological quality elements) in the biological validation of waterbody typology in routine monitoring practice.

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