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Trophic Structure of Macrozoobenthos in Permanent Streams in the Eastern Balkans

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Abstract: The present study provides data on the trophic structure of the benthic macroinvertebrate communities in mountainous and semi-mountainous small streams and river sections belonging to Mesta, Struma and Vardar River catchments from 7th Ecoregion. The benthic macroinvertebrates were assigned to seven Functional Feeding Groups. We analyzed their trophic structure and the dynamics in different seasons. The level of similarity between the sampling localities was analyzed in the context of both the river typology and the water catchment. A comparison between the two trophic indices was conducted in order to analyze the advantages of the application of these indices for assessment of the ecological status at the studied sites. We found that the trophic structure of the benthic macroinvertebrate communities in ostensibly typologically similar river sections differs at the undisturbed vs the impacted sampled sites. To a large extent, these differences were also determined by the presence of anthropogenic influence that resulted in the predominance of deposit feeders amplifying on higher disturbance on some of the studied rivers. Long-term negative pressure has led to changes in microhabitats that affect the structure and functioning of the aquatic ecosystem by transformation of the trophic structure of the macrozoobenthos.

Keywords: benthic macroinvertebrates; functional feeding groups; Rhithron Feeding Type Index (RETI); Index of Trophic Completeness (ITC); ecoregion

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1. Introduction

Macroinvertebrates play a fundamental role in the transfer of energy in freshwater ecosystems, as they have a considerable influence on the processing of autochthonous organic matter [1,2]. Therefore, feeding groups have been introduced in hydrobiology to characterize the macrozoobenthos morpho-behavior capacity and to indicate the location and role of aquatic invertebrates for the functioning of the lotic ecosystems [3–5]. The composition of functional feeding groups (FFGs) reflects the stream ecosystem conditions through adaptation of communities to stream habitat and food resources, including those associated with check-dam construction [6] or inorganic drainages [7]. As ecosystem function can be altered by a diversity of environmental factors, changes in FFG composition could also be used as an indicator of ecosystem health and recovery after disturbances [8]. Looking at the functional or trophic structure of communities is an essential step to better understanding the effects of environmental perturbations on biodiversity and ecosystem functions [9].

Studies have shown that the trophic structure of macrozoobenthos changes along the river continuum as a result of both natural factors [10] and anthropogenic impact [7,11–13]. This demonstrates the trophic structure of macroinvertebrates' communities as an indicator for the conditions of the aquatic environment as well [14].

The achievements of German hydrobiologists [15], which tested different functional groups' based indices, supported the strong indicative abilities of the trophic structure of the macrozoobenthos in lotic ecosystems. In addition, the Index of Trophic Completeness

(ITC) bioassessment approach [16] had good reliability and confirmed degradation in benthic communities caused by different types of anthropogenic impact such as alluvial gold mining [17].

Nowadays, most European freshwater ecosystems are impacted by human activities that lead to losses of taxa and/or discontinuities in the distribution of the benthic fauna [18]. Moreover, the local site-specific conditions contribute to the shaping of the FFG composition [19]. A great deal of research on FFGs from various watercourses across Europe has been conducted [19–21] but still there is a little information on the species and trophic structure of macroinvertebrates in the small mountainous and semi-mountainous rivers and streams from Eastern Balkans (e.g., [22–25]).

In Bulgaria, the trophic structure of the freshwater macrozoobenthos was studied for the first time in detail for Mesta River covering a 30-year period [26] regarding its designation as a site for a long-term research network within the European Network LTER. The Rhithron/Potamon Feeding Type Index (RETI-PETI) and its adapted version used in Bulgaria [27,28], is among the most frequently applied trophic indices in hydrobiological/benthological studies in the country. Later, the bio-indicative potential of the trophic structure and the application of different trophic indices in ecological status assessment of the benthic communities were analyzed on the example of representative lotic water bodies from the upper, middle and lower streams stretches of the Mesta, Tundzha, Veleka, Vit and Maritsa Rivers [29–31].

This study is the first to present data on the FFG of the macrozoobenthos in river stretches flowing through the territory of North Macedonia. In addition, it is an attempt to analyze the trophic structure of bottom macroinvertebrate communities in the poorly studied small, mostly 1st and 2nd order mountainous and semi-mountainous permanent river sections from the Eastern Balkans Ecoregion [32]—Mesta, Struma and Vardar River catchments.

The previous performed work on the same small rivers was focused on the ecological status assessment and general degradation due to hydromorphological stress, habitat loss or organic pollution [33]. In order to analyze how these processes reflect on the FFG of the studied bottom communities, our main objectives were: (i) to characterize the trophic structure of benthic macroinvertebrate communities in small 1st and 2nd order permanent streams in the studied area; (ii) to track the changes of the basic trophic groups in different periods and to determine the degree of similarity between the sampling sites, river basins and river types; (iii) to assess the ecological status and communities' functional completeness based on trophic indices.

2. Materials and Methods

2.1. Study Site

Field studies were conducted in the permanent small streams and rivers in the cross-border territory of Bulgaria (abbreviation code-BG) and North Macedonia (abbreviation code-MKD) belonging to the Mesta (MW—1_ to 6_BG sites), Struma (SW—7_ to 22_BG; 1_ to 9_MKD sites) and Vardar (VW—10_ to 16_MKD sites) River watersheds (Figure 1).

Sampling sites were selected from river sections that correspond to semi-mountainous (R5 river type) and mountainous (R3 river type) stretches in the 7th Ecoregion, in accordance with the current river typology of Bulgaria [34,35]. These river types are characteristic of the three studied river watersheds. The R3 group included 14 sites (>800 m a.s.l.) and the R5 group consisted of 24 sites (<800 m a.s.l.). The sites were mainly on 1st and 2nd order streams. For some of the sampling sites there was evidence of hydromorphological degradation and/or organic pollution, but for the rest of the sites no substantial sources of disturbances were noted [36]. Additionally, the degree of shading, assessed as a percentage of shade of the mirror from riparian vegetation, was determined in situ (Table A1). More details for site codes, altitude, stream order, stream type, predominant substrate, main pressures, the level of disturbances and anthropogenic alterations, were described within previous publications [33,36].

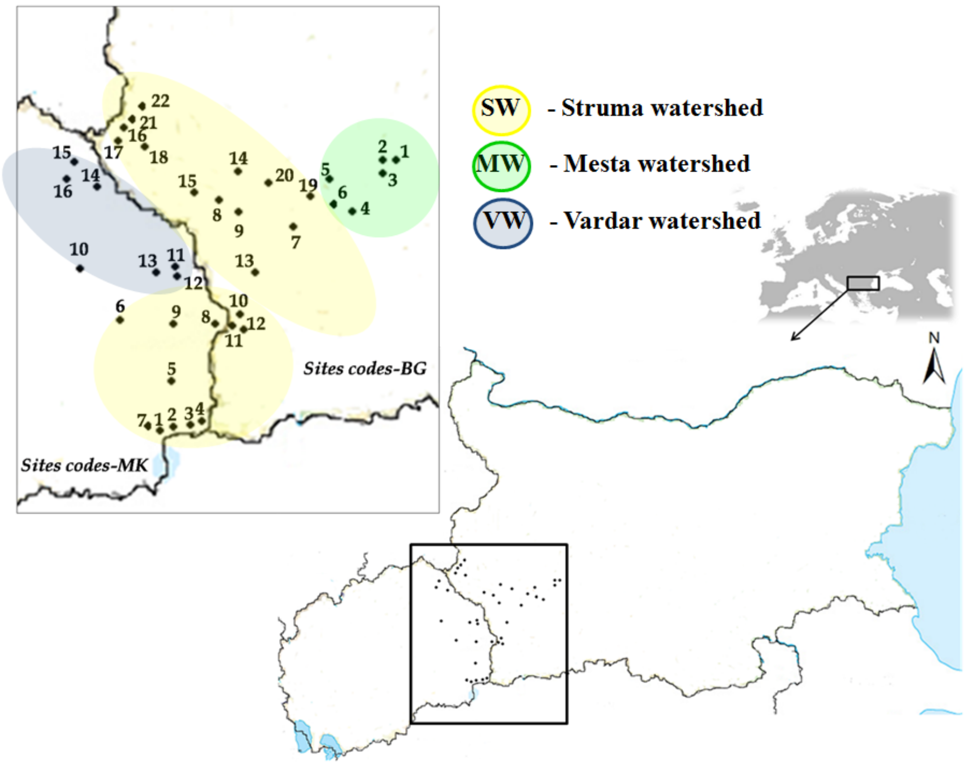

Figure 1. Map of the study area and location of the sampling sites per watersheds.

2.2. Benthic Macroinvertebrates Data Set

Within the study, a total of 38 sites were selected (Figure 1), with a total of 69 macroinvertebrates samples being processed (collected in autumn—October 2017 and in spring—April/May 2018). During the study periods, we observed in situ that the autumn sampling period had a lower flow regime compared to the following spring. The sampling was performed with a standard hydrobiological hand net (mesh size 500 µm) applying kick and sweep multihabitat procedure [37,38]. Laboratory treatment included the elutriation of the inorganic substrata and separation of the macroinvertebrates into benthic groups. All specimens were preserved in 70% ethanol identified to the possible lowest taxonomic level (Table 1). Concerning the established taxa within the separate river watersheds, all the details including the taxa list and FFG association are presented by Rimcheska and Vidinova [36].

Table 1. Level of identification of the systematic groups established during the study.

Systematic Group	Level of Identification
Turbellaria	genera, species
Oligochaeta	families, genera, species
Hirudinea	genera, species
Gastropoda	genera, species
Bivalvia	genera
Crustacea	genera, species
Ephemeroptera	genera, species
Odonata	genera, species
Plecoptera	genera, species

Table 1. *Cont.*

Systematic Group	Level of Identification
Coleoptera	genera, species
Heteroptera	genera, species
Megaloptera	genera, species
Trichoptera	genera, species
Diptera	families, genera, species
Nematoda	presence

2.3. Data Analysis

A total of 280 taxa identified for the entire survey [36] were used for the analysis. The classification of the taxa to FFGs and the calculation of the Rhithron Feeding Type Index (RETI) were performed in accordance with the conducted survey [33]. All the benthic macroinvertebrate taxa were assigned to the following FFGs: shredders (SH), scrapers (SC), collectors (CL), filter feeders (FL), deposit feeders (DF), predators (PR) and parasites (PA) [36]. The Index of Trophic Completeness (ITC) methodology was implemented [16]. Further, using the Macrozoobenthos Trophic Structure (MaTroS 2.0) specialized program, the ITC index was calculated (http://macro.nemi-ekb.ru/index.php?r=site/login&lang=en, accessed on 30 April 2020).

2.4. Statistical Analysis

The descriptive statistics function of MS Excel 2010 (min, max, median, range and interquartile ranges) was used for the analyses of the total abundance of taxa and trophic groups of R3 and R5 river types and indices values. A cluster analysis of the data set, based on the abundance of the trophic groups, was performed to assess the similarity level between the studied sites (Euclidean distance, Ward's method) with Statistica7 software. The Pearson correlation was used to determine the relation between the abundance of each trophic group and the degree of shading per site, and season and further multiple linear regression was applied through program package StatSoft (STATISTICA 7.0).

Using statistical software PRIMER-E v.6 [39], the multidimensional scaling (MDS) plot analysis was conducted to determine the level of similarity (Bray-Curtis) in the macrozoobenthos trophic structure between the R3 and R5 type sites with regard to the altitude and both river basin and river type affiliation.

3. Results

3.1. Trophic Structure of the Macroinvertebrates Communities, Dynamics of Abundance and Changes of the FFGs

Based on the summarized relative abundance of FFGs (in %), the studied macroinvertebrate communities were dominated by SC, DF and SH (42%, 26% and 20%, resp., Figure 2a). PR, CL and FL were represented with much smaller partitions (6%, 4% and 3% resp.), while the share of the PA was practically neglected (0.07%). The percentage share of SC, PR and CL were higher in autumn, while those of SH, DF and FL were higher during spring (Figure 2b). Furthermore, in both seasons, stenobiont species belonging to the groups of SH (e.g., Plecoptera) and SC (e.g., Ephemeroptera genera-*Rhithrogena* and *Epeorus*) were numerically dominant.

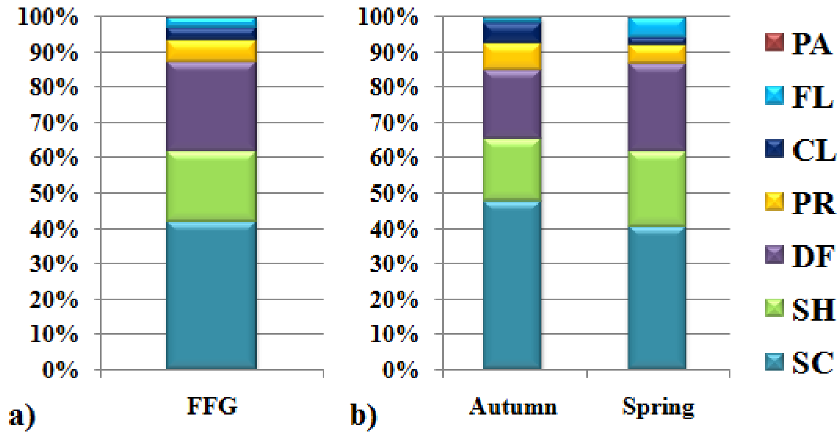

Figure 2. Relative abundance of the FFGs (in %): (**a**) for the whole survey period; (**b**) per sampling season. Legend: x-axis, FFG composition (**a**) and FFG composition per sampled season (**b**); y-axis, relative abundance of the trophic groups (For trophic groups abbreviations, see Materials and Methods).

Analyzing the studied watersheds, we established different proportions within the trophic groups and in each river basin (Figure 3). With the exception of CL, the closest structure with regard to the share of the FFG was observed between the Mesta and Struma Rivers (Figure 3a,b). In Vardar River basin, the sampled sites showed narrow ranges of the variation of CL and PR (Figure 3c). Compared to other watersheds, DF in Vardar have the smallest share, FL in Struma were with highest numbers, while SH occurred in roughly equal share in all studied river watersheds (Figure 3).

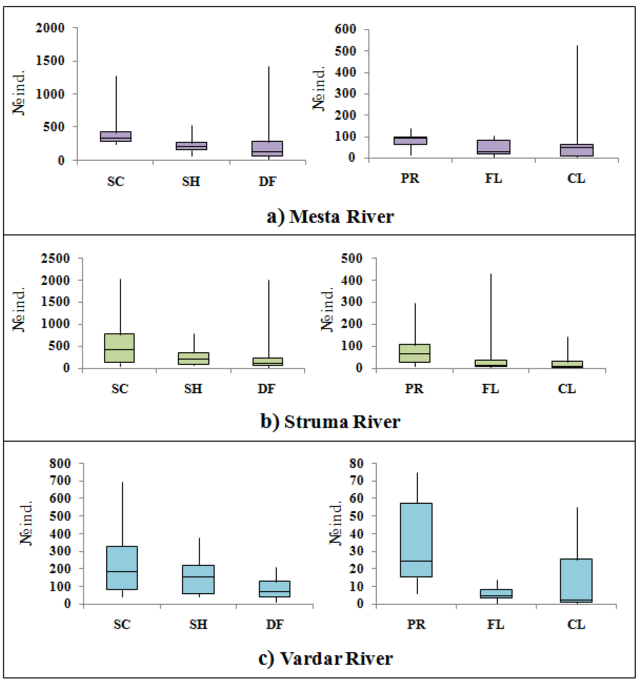

Figure 3. Box plots (median, range and interquartile ranges) of total abundance of the FFGs within the studied watersheds. Legend: x-axis: FFG (for abbreviations, see Materials and Methods); y-axis: total number of individuals.

Regarding the proportions of each trophic group, different patterns were registered between the two river types in the studied watersheds, and slight changes were evident (Figure 4). Primarily, compared with R3 sites, the R5 sites had higher FFG abundance and lower median range variability. Herein, SH (in R3 river type), SC and DF (in R5 river type) were the most abundant trophic groups. Thus, in R3 sites, the SC were more abundant during the spring, while in the autumn they were decreasing in numbers. The abundance of SC in R5 sites was lower in the spring season (Figure 4b). We found more pronounced dynamics of the SH, which were the most numerous for R3 sites during autumn (Figure 4a).

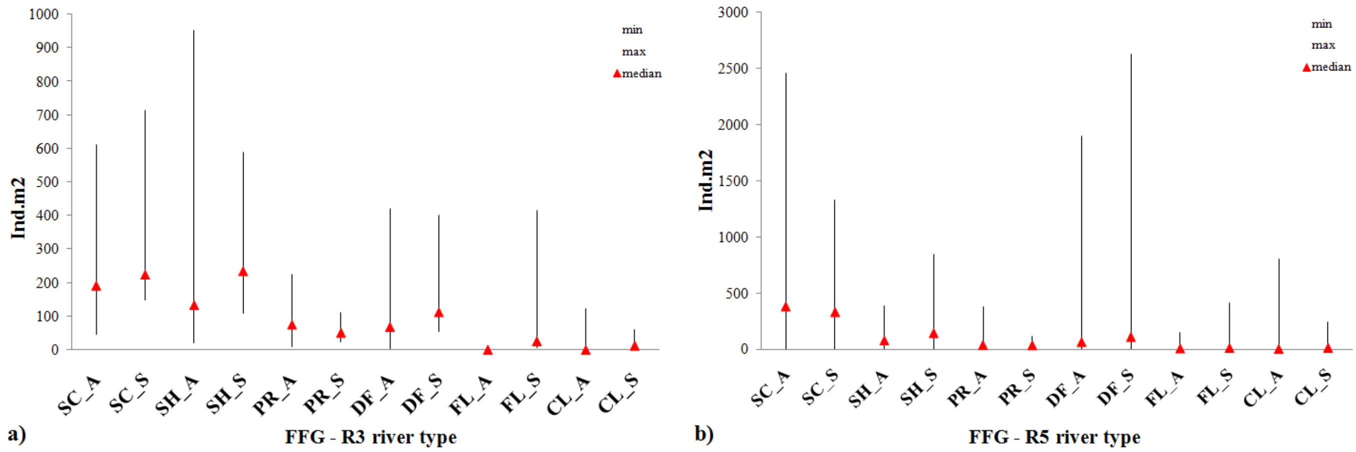

Figure 4. Total abundance of the trophic groups (min, max, median values) per season: (**a**) for the R3 sites and (**b**) for the R5 sites. For abbreviations, see Materials and Methods. Legend: A—autumn, S—spring.

The group of FL at R3 sites was more abundant in spring, while in autumn it had a negligible share regarding the total number of specimens. Downstream, at R5 sites, FL prevailed also in spring (Figure 4b) but with a less pronounced change in the abundance. Concerning the dynamics of DF, we found them with more indicative numbers for R5 sites in both seasons. Simultaneously, DF represent the most abundant group at this river type during spring. In R3 sites, DF did not show a different seasonal pattern (Figure 4a). CL characterized the R5 sites with the highest abundance in autumn, while at R3 sites their numbers were much lower, especially in spring. The PR had similar patterns for both river types having higher numbers in autumn and reduced in spring (Figure 4).

The performed linear correlation analysis between the degree of shading and abundance of the FFG by river types and seasons showed several significant negative correlations ($p < 0.05$) for R3 river type–SC in autumn (r = −0.70) and FL in spring (r = −0.68) and for R5 river type–DF in both seasons (r = −0.42 and r = −0.41 resp.) (Table 2). In general, the shading negatively affected the numbers of individual trophic groups to a greater extent at the R5 river type sites (albeit with lower values of 'r') (Table 2). The regression analyses of SC and FL distributional patterns (both having higher significant values of 'r') clearly pointed out the tendency for diminishing abundance with increasing percentage of shading (Figure 5).

Table 2. Linear correlation coefficients between the degree of shading and abundance of each trophic groups for both river types and sampling season ($p < 0.05$; n—number of cases). The (*) asterisk indicates the significant correlations.

River Type/Season	n	SC	SH	PR	DF	FL	CL
R3_A	10	−0.70 *	−0.38	−0.12	0.02	−0.15	−0.35
R3_S	9	0.32	0.46	−0.55	0.51	−0.68 *	0.10
R5_A	23	−0.02	0.28	−0.16	−0.42 *	−0.24	−0.40
R5_S	27	−0.11	−0.19	0.37	−0.41 *	−0.36	−0.26

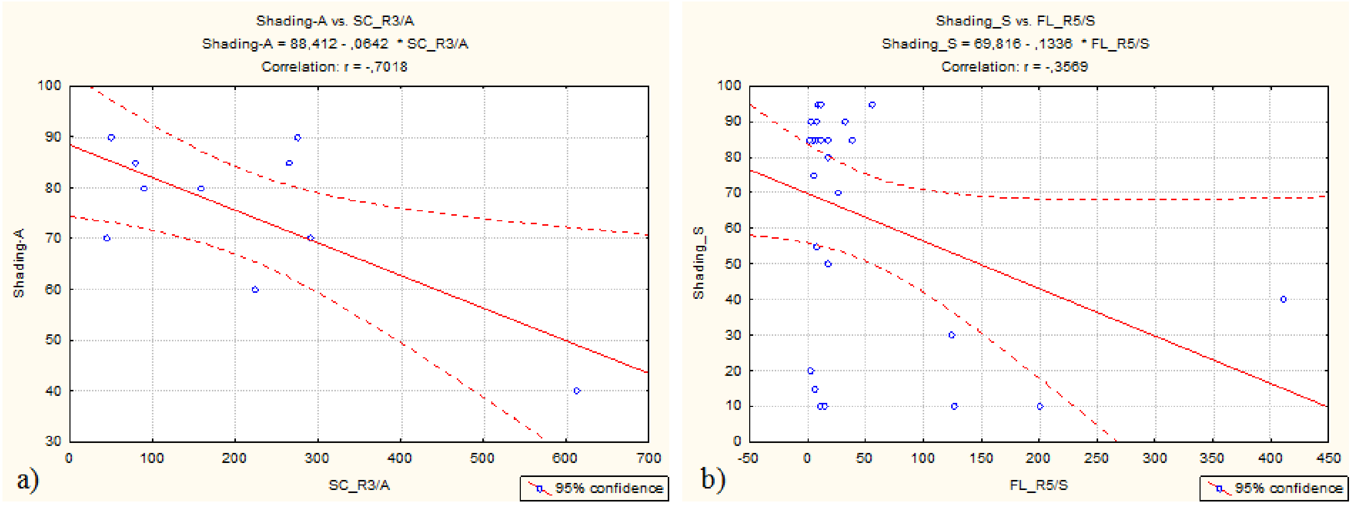

Figure 5. Scatterplots (Multiple linear regression) of the total number of individuals: (**a**) SC from R3 sites in relation to the % of shading during the autumn season (R2 = 0.,5402; F = 4.11, p = 0.06); and (**b**) FL from R5 sites in relation to the degree of shading during the spring season (R^2 = 0.2045; F = 3.08, p = 0.06). Legend: A—autumn, S—spring.

3.2. Similarity in the Trophic Structure of the Benthic Communities

The cluster analysis of the similarities based on the benthic FFG, distinguished two well-separated groups depending on river types, whether R3 or R5 clusters A, B_1, B_{2a} consisted of R5 sites, and B_{2b} contained R3 sites only (Figure 5). Considering the differences in benthic FFG composition according to the degree of the anthropogenic impact, the most polluted sites were grouped in cluster A_2 (8_MKD and 4_BG). The sites in subgroups B_{2a} and B_{2b} were the most similar regarding the trophic structure. Within these subgroups, the only distinction was the altitude as they were separated based on the affiliation R3 or R5 river type sites. Cluster B_1 consisted of samples with the most similar FFG composition (from SW) or in closest geographical distances-MKD sites (1_, 2_, 3_, 4_, 5_, 7_) and 14_BG site (Figures 1 and 6).

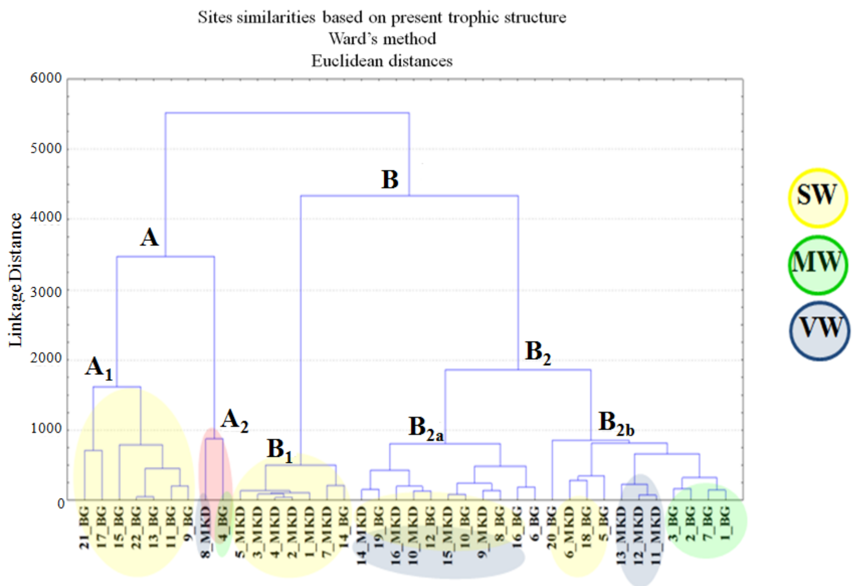

Figure 6. A cluster dendrogram of the similarity between the sampling sites based on the absolute abundance of the FFG (For abbreviations and site codes, see Materials and Methods). The red circle (subgroup A_2) represents the most polluted sites.

3.3. Ecological Quality and Classification of the Studied Sites by Different FFG-Based Indices

The RETI values varied from 0.21 (5_MKD, "bad" ecological quality, EQ) to 0.99 (17_BG and 12_MKD, "high" EQ) (Appendix A). Despite the wide ranges of the index values, most of them were higher than 5.9 in both seasons, which corresponded to good and high EQ (Figure 7a). The RETI values did not differ significantly between R3 and R5 river type sites (Figure 7a).

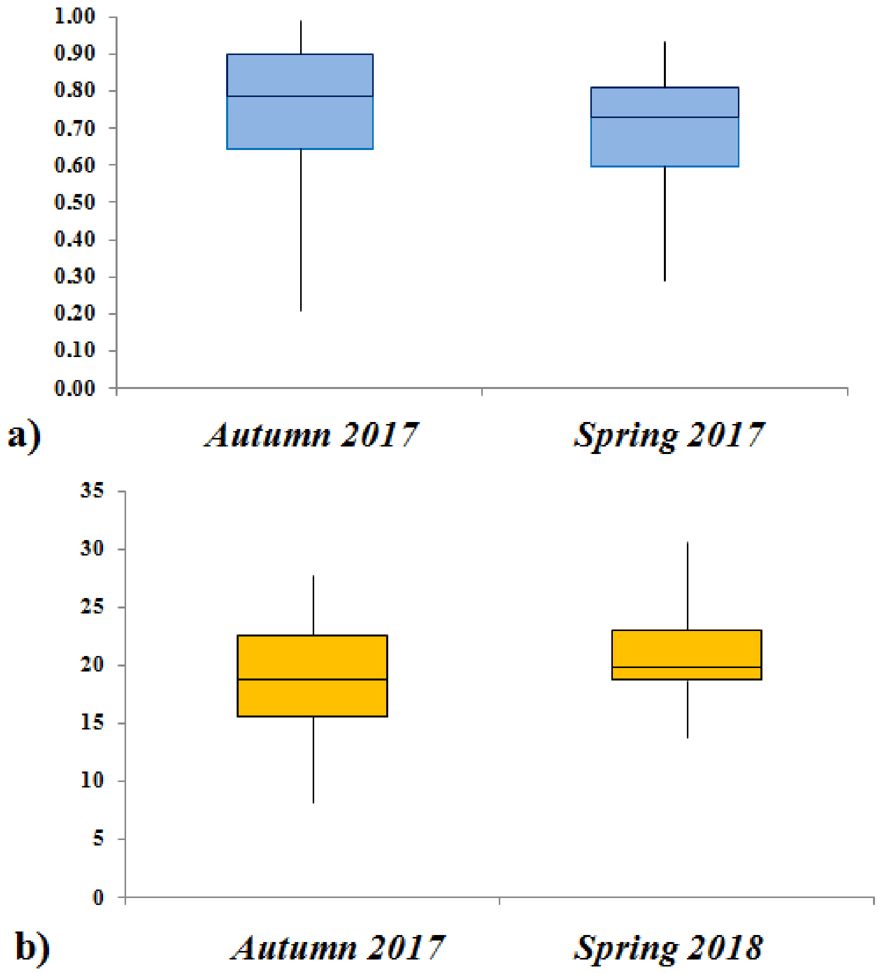

Figure 7. Box plot (median, range and interquartile ranges) of RETI (**a**) and ITC (**b**) index values during the study period. Legend: x-axis–sampling season; y-axis–indices values.

Compared to the RETI, the variation in the ITC values was much lower. The index varied from 8.27 (11_ and 13_MKD in autumn, poor EQ—IV class) to 30.6 (1_BG in spring, high EQ—I class) (Figure 7b, Appendix A). The lowest EQ was established during the autumn period. Moreover, a total of seven MKD sites had poor EQ (class IV) in the same season. Significant differences between the ITC scores of R3 and R5 river type sites were noted, with lower EQ of R3 river sections being strongly expressed during the autumn period (Figure 7b). The variations of both indices were more pronounced in autumn (Figure 7).

Within the MDS analysis, the most polluted sites (8_MKD, 4_BG) were clearly separated on the left side of the plot (Figure 8). The rest of the sites were grouped in the central part of the diagram, pointing out the higher similarity between the trophic structures of the most alike sites per river types. It also included the sites with the highest values of RETI and ITC indices as well. Herein, the separation by the river typology is observed, as most of the R5 sites were grouped at the lower left side of the plot and the R3 sites were spread more at the center and upper part of the diagram (Figure 8). Exceptions were the R3 (2_BG, 13_MKD) and R5 site (19_BG) whose trophic structure slightly differed from the other ones.

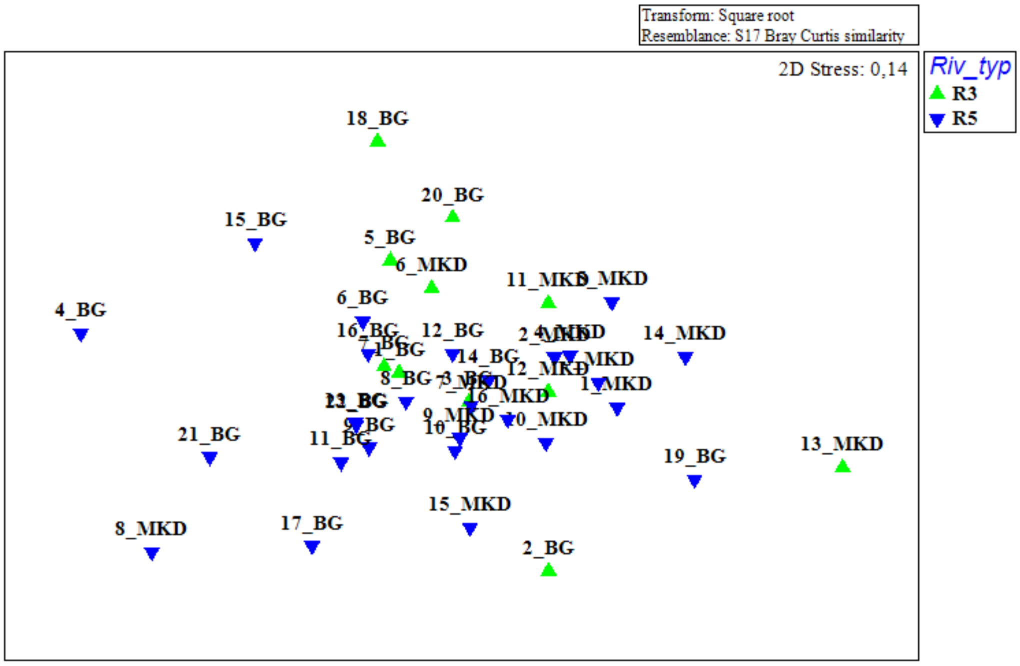

Figure 8. MDS plot of the similarity between the studied mountainous (R3) and semi-mountainous (R5) sites based on their trophic structure (For site codes see Materials and Methods).

4. Discussion

In natural conditions, environmental factors determine the trophic structure of the river ecosystem health [40]. Jiang et al. [41] noted the main structure-determining factors in the formation of the trophic composition of benthic communities to be altitude, bottom substrate, river order and river width. In this study, the ratio between FFGs at the undisturbed sites corresponded to the principles set in the River Continuum Concept [10], where this was further confirmed in the research conducted by the following studies [18,26,42,43]. SH inhabit predominantly gravelly and rocky bottom substrates, typical riverbed of high mountain river stretches [44]. This characteristic of SH dominance corresponds to our findings for R3 river type sites in autumn. SC are primary consumers associated with gravel bottom, open river stretches and rapids [45,46]. SH and SC are dominant in macrozoobenthic trophic structure at reference lotic sites [29], an observation that was also supported by our study. Under human pressure, depending on the type and strength of the impact transformation of the trophic structure of the macrozoobenthos and reorganization between FFGs was observed (e.g. [30]). The process of this restructuring primarily affected the sensitive taxa, which belong to groups of SH and SC. They decreased in numbers or even vanished from the community, at the expense of increasing the share of more tolerant groups of DF or FL. Barbour et al. [47] pointed out that the SH and SC, named obligate groups, respond more sensitively to anthropogenic stress, while generalist groups such as FL, CL exhibit considerable tolerance for various contaminations. According to some studies [48,49], SH are rare in impacted streams, which was also confirmed by our results. Moreover, the predominance of stenobiont oligosaprobic (most of the representatives from the order of Plecoptera: *Taeniopteryx schoenemundi*, *Brachyptera seticornis*, *B. risi*, *Dinocras megacephala*, *Leuctra pseudosignifera*, *L. hippopus*, *L. inermis*, *Protonemura montana*, *P. intricata intricata*, *P. praecox praecox*, *Nemoura flexuosa* [36]) benthic species which belong to the group of SH were associated with the presence of CPOM of natural origin in the water and indicate unaffected environmental conditions. Conversely, the group of DF was the dominant trophic group in muddy sediments [30,50] and indicative of the presence of a significant amount of organic matter (autochthonous or allochthonous) [44,45,51]. Within this study, these findings were observed at R5 sites (4_BG and 8_MKD). CL are relatively tolerant and occur in different habitats and under specific environmental conditions [46]. A high proportion of CL is often associated with downstream parts of rivers [52], similar to that found at site 14_BG. PRs are fed by actively pursuing their preys [50] and their density is relatively evenly distributed along the river [14]. We observed that the higher presence of PR usually was combined with the lowest presence/or total absence of the CL, findings supported by Kerakova et al. [29] as well. The group of PR does not have a pronounced seasonal character while the remaining trophic groups differ in abundance during different seasons, even within a single river type. FL representatives, which usually inhabit soft and muddy substrates, are fed by passive filtration of the FPOM and UFPOM from the water column [45,52]. Their abundance is associated with the presence of large quantities of water carrying suspended solids [53]. In our study, the FL proportion in the surveyed communities was negligible, findings observed also by Nicola et al. [23]. Only at site 5_MKD, in autumn the passive FL (Simuliidae) dominated the other feeding groups, probably as result of the hydromorphological/and anthropogenical processes expressed at this season that influenced the FFG composition. Concerning the following spring, the proportion of FL on this site was negligible and the SH, SC and DF took over the benthic community' trophic structure.

We stated that in unaffected conditions especially in the mountainous river sections, the trophic structure is type-specific and slightly differs between the studied, closely related rivers. At the sites, which are characterized as unaffected/undisturbed (1_, 13_, 17_BG; 1_, 9_, 11_, 12_MKD) [33], SH and SC dominate in the macrozoobenthos communities. According to Kerakova et al. [30] SH does not show differential grouping based on factors such as season, river type or river basin. Herein, at R3 sites SC and SH prevailed in spring, while at R5 sites SC decreased in VW and MW compared to SH, which were outnumbered by SC in SW and MW. Regardless of the river type, DF in SW were more abundant in autumn, in VW they increased in spring, while in MW DF did not show seasonal variation in abundance. Moreover, even we noted degradation at the sampled localities from VW, due to hydromorphological alterations [33]. These findings correspond to lower values of the RETI index as we detected bad and poor EQ at sites 8_MKD (autumn) and 14_MKD (spring). At these sites, DF prevailed in the macroinvertebrates communities, as a result of the anthropogenic interference [33,36]. The less anthropogenic disturbances (organic pollution) resulted in the lowest numbers of DF (e.g. sites 2_, 14_, 20_BG and 13_MKD).

The transformation of the trophic structure, expressed by the increase in DF as a result of mining activities on site 14_MKD (VW) was evident. The most polluted sites (as contained tolerant taxa, mainly aquatic worms and caddisflies) [33] 4_BG and 8_MKD (SW) were separated as a result of the highest dominance of the DF. At these sites, we found many tolerant taxa, mainly of the subclass Oligochaeta and less tolerant species from the order Trichoptera, which prevailed in the benthic community. Herein, the values of ecological quality indices (BMWP, BI, EPT taxa richness and ASPT) were also lowest [33]. Moreover, within the cluster analysis (subgroups B_1 and B_{2b}), we noted the longest linkage distances among the sites (with similar FFG distribution at the similar altitudes) with the highest water quality.

From the previously performed work, it was evident that the seasonality and site degradation had a determining role in the formation of the FFG's species composition [33]. Within this study, our results pointed out on a negative correlation between the abundance of SC (in autumn) and FL (in spring) and the degree of shedding in the mountainous rivers. Another authors also found that at lower altitudes and less shading, the proportion of FL increased [30].

In general, ecological assessment of the lotic water bodies is based on species richness that strongly depends on the diversity of the microhabitats, seasonal changes, the river typology, riverbed alteration and all aspects of anthropogenic disturbances [33,36]. The trophic index RETI is very sensitive toward hydro-morphological degradation [26]. Thus, according to the RETI values, only seven undisturbed sites kept the high EQ during both seasons. The gained RETI scores for site 5_BG pointed out on a transition from moderate (as the lowest) in autumn to high EQ the following spring, likely owing to the increased water level during the late winter period. We also noted the disturbances on eight sites where a lower EQ was recorded at the following spring. For these findings, we can summarize that not only natural factors (water level fluctuation during different seasons) but anthropogenic impact (hydro-morphological changes and organic pollution) can led to restructuring of the FFG. Moreover, the effect caused by global climate processes in high-mountain (free of human impact) vulnerable ecosystems, which are reflected in the local scale [26], should not be neglected. Further, we obtained the EQ scores at some of the undisturbed sites that corresponded with the lower RETI values at autumn. An example is the site 5_MKD (autumn), where the RETI and ITC values corresponded to the lowest EQ, results that differed from the gained biotic indices values, which correspond to high/good water quality, and this site was listed as referent/or near the natural site [33]. Kerakova et al. [29] found comparatively lower variations of the assessment within no more than two ecological classes. A probable reason for the above discrepancy between the biotic and trophic index estimates may be the fact that at this stage no type-specific scale has been developed for RETI.

Unlike the RETI values, which do not differ between R3 and R5 river type sites, those of the ITC report larger differences, especially if the R3 rivers have a lower EQ during the autumn period. Testing the ITC index on our studied sites gave us a reason to suggest the following: When there are more than five random species [48] found in river sites of R3 and R5 type (without dominant representatives) and where more than 1/4 of the total species belong to a guild not presented in the sample itself, these species should be added at MaTroS 2.0 for calculation. This is very important especially if all the taxa in the sample belong to one/or two different trophic guilds only. Our result demonstrated that the incomplete list included in the software product was a reason why the EQ determined by the ITC is lower compared to the RETI value. In the R3 and R5 rivers that we studied, such cases with single specimens/species were commonly noted, depending on the water catchment and available water resources as opposed to the situations in big lowland rivers. Thus, the hypothesis expressed here has to be further tested and adjusted for other river types.

5. Conclusions

The FFG composition demonstrated a pronounced seasonal dynamics. Although at the undisturbed/reference R3 and R5 sites the close similarity was evident, the trophic structure of the macroinvertebrate communities was characterized as type-specific. Under anthropogenic influence, a transformation is observed in the composition of the trophic structure, which is associated with a decreasing in the abundance of the more sensitive groups (SH and SC) at the expense of an increase in the abundance of more tolerant ones (DF). The ecological status of the river ecosystems assessed by the FFGs of the benthic macroinvertebrates showed high sensitivity and vulnerability of the RETI to human impacts. Based on our results, we consider that once type-specific scales have been developed, the RETI could be reliable and applicable for ecological status evaluation of the mountainous and semi-mountainous rivers. As for the ITC, its application in this study showed that this trophic index needs further adjustment for small mountain rivers and streams.

Our results are also a contribution to the study of the processes of the functioning of macrozoobenthos communities as a key biological element in semi-mountain and mountain lotic ecosystems. In order to avoid the adverse impact of the factors with negative effects on these extremely valuable ecosystems, the authorized institutions should pay particular attention to conservation and take adequate measures aimed at preventing their pollution in semi-mountainous and mountain rivers.

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Appendix A

Table A1. Values of ITC and RETI indices, the corresponding ecological quality class (EQ-class) and the degree of shading (% SHA) per studied sites and sampled seasons.

	Autumn 2017					Spring 2018				
Site Code	**ITC**	**EQ-Class**	**RETI**	**EQ-Class**	**% SHA**	**ITC**	**EQ-Class**	**RETI**	**EQ-Class**	**% SHA**
1_BG	26.73	II	0.91	I	40	30.6	II	0.69	II	60
2_BG	14.25	III	0.96	I	90	/		/		/
3_BG	26.44	II	0.8	I	60	26.44	II	0.8	II	90
4_BG	21	II	0.41	III	10	23.64	II	0.46	III	10
5_BG	26.73	II	0.77	III	70	22.88	II	0.8	I	80
6_BG	26.44	II	0.57	III	30	23.64	II	0.53	II	30
7_BG	22.58	II	0.75	II	80	26.44	II	0.83	I	85
8_BG	26.75	II	0.84	I	70	26.75	II	0.83	I	85
9_BG	15.92	III	0.94	I	10	18.73	III	0.55	II	10
10_BG	19.79	III	0.9	I	80	17.15	III	0.75	II	85
11_BG	19.79	III	0.81	I	95	19.79	III	0.79	II	95
12_BG	26.75	II	0.66	II	20	13.85	III	0.7	II	20
13_BG	15.59	III	0.96	I	90	15.59	III	0.83	I	90
14_BG	20.8	III	0.64	II	70	23.94	II	0.81	I	70
15_BG	26.75	II	0.66	II	60	26.75	II	0.71	II	40
16_BG	27.75	II	0.8	I	90	19.79	III	0.53	II	95
17_BG	19.77	III	0.99	I	90	15.92	III	0.9	I	85
18_BG	16.34	III	0.5	III	80	/		/		/
19_BG	/		/		/	19.79	III	0.81	I	90
20_BG	/		/		/	22.58	II	0.47	III	50
21_BG	/		/		/	19.77	III	0.67	II	90
22_BG	/		/		/	19.79	III	0.9	I	50
1_MKD	16.64	III	0.76	II	90	22.58	II	0.68	II	95
2_MKD	15.92	III	0.5	III	70	22.59	II	0.67	II	85
3_MKD	15.92	III	0.82	I	75	15.59	III	0.49	III	85
4_MKD	18.73	III	0.55	II	70	19.79	III	0.59	II	75
5_MKD	12.95	IV	0.21	V	70	22.59	II	0.6	II	85
6_MKD	18.73	III	0.5	III	90	19.79	III	0.65	II	95
7_MKD	/		/		/	19.44	III	0.81	I	95
8_MKD	11.96	IV	0.25	V	30	19.79	III	0.35	III	10

Table A1. *Cont.*

Site Code	Autumn 2017 ITC	EQ-Class	RETI	EQ-Class	% SHA	Spring 2018 ITC	EQ-Class	RETI	EQ-Class	% SHA
9_MKD	19.79	III	0.79	I	85	19.79	III	0.93	I	80
10_MKD	15.59	III	0.85	I	85	26.44	II	0.75	II	75
11_MKD	8.27	IV	0.67	II	70	18.73	III	0.76	II	70
12_MKD	12.42	IV	0.99	I	85	22.88	II	0.81	I	95
13_MKD	8.27	IV	0.92	I	80	15.59	III	0.84	I	85
14_MKD	10.4	IV	0.82	I	10	20.08	III	0.29	IV	15
15_MKD	19.79	III	0.92	I	50	15.92	III	0.94	I	55
16_MKD	12.79	IV	0.7	II	10	17.4	III	0.71	II	10

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