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Evaluating the Ecological Status of Fluvial Networks of Tropical Andean Catchments of Ecuador

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Abstract: In the tropical high mountains, human activities have strongly intensified in recent decades. Agricultural frontier movement toward higher elevations, river channel modifications, mining, and urban waste discharge threaten river ecosystem health, which is even more alarming when drinking water supply comes from surface water. The aim of the current study was to evaluate the ecological status of high mountain fluvial networks of tropical Andean catchments based on the definition of different river types. Physical–chemical variables and macroinvertebrate communities were sampled in 90 stations of seven tropical high mountain catchments. River habitat and riparian vegetation quality were further evaluated. K-means classification, using physical and hydro-morphological characteristics, identified six different river types. This classification was further refined to five river types by the analyses of macroinvertebrate communities through multidimensional scaling and analysis of similarity. The anthropogenic pressure gradients, present in the different river types, were inorganic (i.e., conductivity, turbidity), organic (i.e., fecal coliforms), river habitat, and riparian vegetation quality. Macroinvertebrate communities responded to different environmental variables in the páramo, mountain forest with humid shrub, urban, and Tarqui river types. Heterogeneous fluvial habitats and high altitude favored taxa such as *Atanatolica*, *Mortoniella*, *Helicopsyche*, *Anacroneuria*, *Paltostoma*, *Helicopsyche*, *Paltostoma*, *Atopsyche*, *Pheneps*, and *Maruina*. Chironomidae and *Psychoda* dipteran were associated with higher biochemical oxygen demand, lower oxygen concentration, high fecal coliforms, and total dissolved solids, while *Haitia* was linked to elevated nitrate concentrations. Integrated watershed management could benefit from a well-established biomonitoring network, considering different river types, which represents the natural variability of the ecosystems, as well as anthropogenic pressure gradients.

Keywords: tropical Andean rivers; river typology; anthropogenic pressure gradient; macroinvertebrates

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1. Introduction

Mountain regions provide water resources to downstream populations for agriculture, industrial activities, and human consumption [1,2]. In tropical mountain regions, water is mainly taken from surface and subsurface ecosystems. In mountain watersheds, water can be stored and released by a combination of different hydrological components including snowpack, glaciers, groundwater, and lakes [3,4]. Further, in the tropical high mountains, the páramo ecosystems [5] are characterized by having special properties for storage and regulation of the hydrological cycle, since they have a high content of organic matter, high porosity, and low apparent density, which allows most of the water to be retained in the soil and slowly be delivered to the watercourses [6], becoming the main regulator of the hydrological cycle in this type of ecosystems [7].

In the tropical high mountains, human activities have strongly intensified in recent decades. Agricultural activities have moved upward to higher elevations, modifying forested areas into pasture and agricultural lands, resulting in a severe loss of biodiversity [8] and increasing contamination by fertilizers and pesticides, which affect river water quality [9]. The lack of sanitation systems in most tropical high mountain settlements contributes to the release of a high number of diverse contaminants, such as pathogens of fecal origin [10].

In addition to the increasing contamination of tropical high mountain rivers, the modification of their channels also represents an alarming problem. Riverine vegetation is strongly affected or modified and, most of the time, eliminated [11]. Uncontrolled construction of houses, roads, and/or other civil infrastructures up to the riverbanks is common in this region. Riverine vegetation is key for healthy biological communities and, as such, for ecosystem health [12]. Different characteristics of riverine vegetation (i.e., percentage of vegetation cover, vegetation stratification, presence of native/introduced species, etc.) should be evaluated for ecosystem health assessment [13,14]. Further, the evaluation of fluvial habitat quality (i.e., occurrence of rheophile zones, sediment types, presence of shade, presence of natural elements and aquatic plants, etc.) also needs to be considered in biomonitoring programs and ecosystem health assessments [15].

In addition to the above-mentioned anthropogenic impacts, climate change is likely to affect tropical high mountain river systems through the possible change of rainfall patterns, which would have an effect on water availability for human use [16]. Cuenca, the third largest city of Ecuador with 505,585 inhabitants [2], obtains its drinking water from the surface resources of seven surrounding catchments. Owing to the fast-increasing population and the present anthropogenic activities, this city will face drinking water availability problems by no later than the year 2050. The Cajas National Park (CNP) is the most important páramo ecosystem in the Cuenca canton and is responsible for a large part of the current and future water supply for the city [5,17].

Within the CNP, the main economic activities developed are tourism and fishing [18]. However, while human intervention in the headwaters of the CNP rivers is relatively scarce and is quite controlled, the impact in the middle and lower sectors of the rivers is substantially greater, threatening the ecological integrity of the catchments [2]. This is despite the fact that the sanitation control of the city of Cuenca has a combined sewage system and a network of sanitary interceptors and wastewater treatment plants, particularly the Ucubamba plant that treats about 95% of the wastewater of the city, and perimeter areas whose sewer networks are intercepted [19,20]. The remaining wastewater from the urban areas is treated in small plants. Nevertheless, there are important perimeter areas that have developed recently, the sewer networks of which are not intercepted and conveyed to treatment plants [20]; thus, an additional treatment plant and supplementary sanitary interceptors are projected [19].

Therefore, the assessment of ecosystem health through an extensive biomonitoring network, which characterizes the anthropogenic pressure gradient and natural variability of the ecosystems, is a fundamental tool in integrated watershed management. This is particularly relevant in the seven catchments supplying water to the city of Cuenca, where natural variability is linked to elevation and topographical characteristics. The use of the community of benthic macroinvertebrates present in water bodies is a fundamental tool to recognize natural or anthropogenic changes. Macroinvertebrates are widely used in various integrated management plans applied in other parts of the world, such as the Water Framework Directive (WFD) of the European Union, due to the demonstrated sensitivity of many species to different types of pollution [21–23]. Biological monitoring is based on the possibility of using structural and functional characteristics of the different levels of biological organization to comparatively evaluate the state of the biota, whose condition reflects the ecological state of the water body [24].

In view of the increasing anthropogenic activities currently occurring in the Andean region and the envisaged future water resources scarcity, evaluation of river ecosystems health is necessary to ensure the sustainable management of water resources in this region. Hereafter, to contribute to the achievement of this goal, the general objective of the study was to evaluate the ecological status of high mountain fluvial networks of tropical Andean catchments in southern Ecuador. The specific objectives were as follows: (i) defining the river types in the study catchments by means of multivariate methods; (ii) validating the previously defined river typology through the analyses of representative macroinvertebrate communities; (iii) assessing the anthropogenic pressure gradient on the different river types; and (iv) identifying the key environmental variables for benthic communities present in the different study river types. The novelty of the study included the development of a river typology in a highly heterogeneous (i.e., encompassing natural as well as human-induced variability) high mountain region using hydro-geomorphological characteristics. Further, this typology was refined by considering biological characteristics of the sampled rivers; an approach that has not been used commonly. The study was executed in a strongly understudied region using an extensive sampling network for the evaluation of river ecosystem health.

2. Materials and Methods

2.1. Study Area and Location of Sampling Stations

The study area (Figure 1) is located at the western part of the Paute river basin and encompasses seven catchments: Tomebamba (17 sampling stations), Yanuncay (18 sampling stations), Machángara (17 sampling stations), Tarqui (15 sampling stations), Cuenca (3 sampling stations), Cañar (11 sampling stations), and Balao (8 sampling stations). The elevation of the sampling stations ranged from 862 to 4017 m above sea level (a.s.l.). The average annual temperature is 16.3 °C, while average annual rainfall is approximately 879 mm in the study area. Seasonal and inter-annual rainfall exhibits a bimodal pattern with wet periods from February to April and October to November [17]. Land use and land cover in the study area can be summarized by the classes "high montane evergreen forest" (of the western Andes), "lower montane evergreen forest" (of the western Andes), "semi deciduous forest" (of the eastern Andes), "montane cloud forest" (of the western Andes), and "páramo", where *Polylepis* sp. [25] is present at higher elevations than 3400 m a.s.l.

The sampling stations are situated in river reaches surrounded by different vegetation covers. Within the CNP, where human activities are limited, the study was carried out in the headwaters of the catchments of the Tomebamba and Yanuncay rivers (Atlantic slope), and in the catchments of the Balao and Cañar rivers (Pacific slope). Additionally, there were also evaluated other high elevation stations, located outside the CNP, in the upper and middle portions of the Machángara catchment, and middle portions of the Tomebamba, Yanuncay and Tarqui catchments. Some of these stations are subjected to human impacts such as cattle raising, fish farming, and tourism. Finally, there were also evaluated stations located in the lower portions of the seven study catchments, where human impacts are important, as well as stations situated in the urban limits of the city of Cuenca.

2.2. Biotic and Abiotic Monitoring

In total, 90 sampling stations were evaluated in the seven study catchments (Figure 1) during the low discharge period of July–September of 2014. A 100 m long longitudinal river segment was chosen at each sampling station. First, a visual evaluation was carried out to define all the microhabitats existing in the river segment. Microhabitats were determined by evaluating the types of substrates, i.e., inorganic (blocks, stones, pebbles, gravel, sand, clay, and silt) and/or organic (leaf litter, macrophytes, bryophytes, filamentous algae, exposed roots, branches, and trunks). The area occupied by the different microhabitats in the river section was estimated through visual assessment in the field.

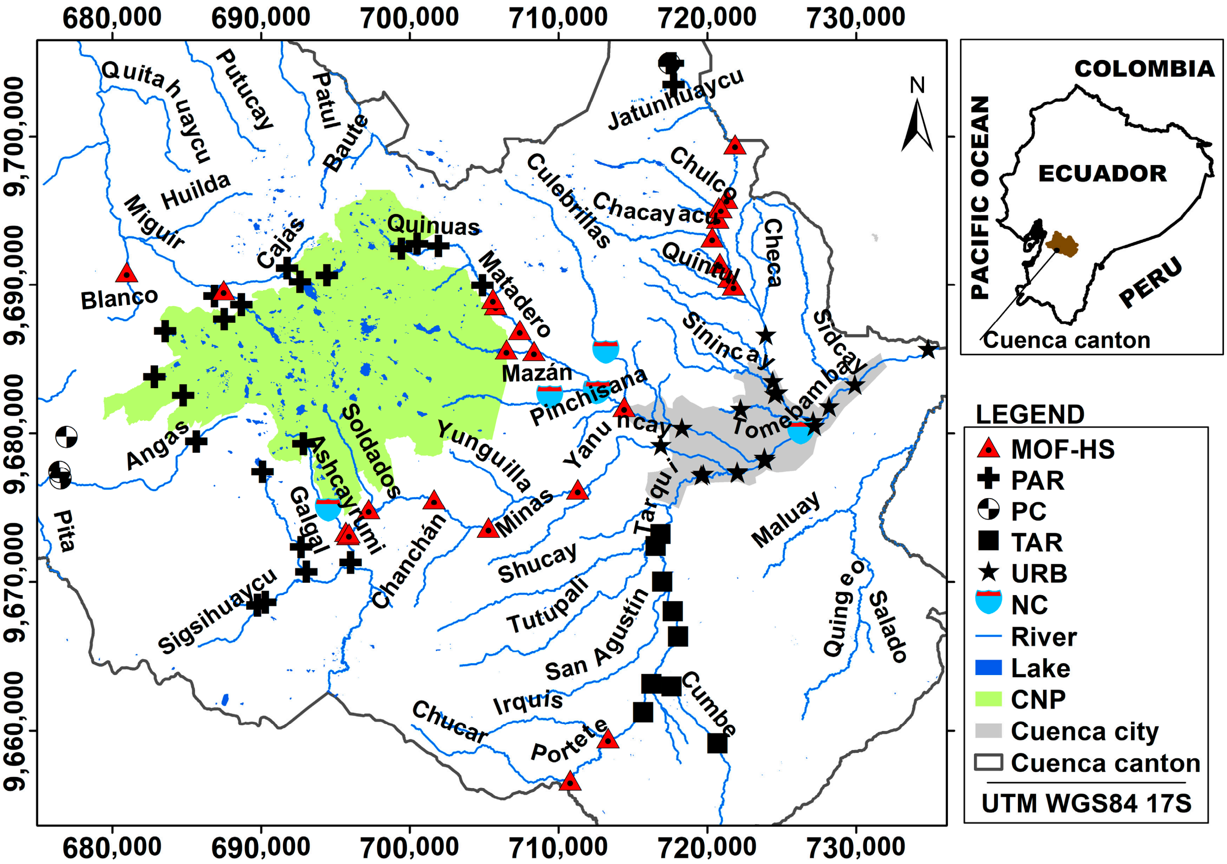

Figure 1. Distribution of the 90 sampling stations in the context of the Cuenca canton that is located at the southern Ecuadorian Andes and grouped as a function of the 5 different river types: PAR = páramo; MOF-HS = mountain forest and humid shrub; PC = Pacific coast; TAR = Tarqui; URB = urban. Very low abundances of macroinvertebrates were observed in five stations, whilst no biological sampling took place in another one; these six stations were grouped in class NC (i.e., "not classified"). CNP = Cajas National Park. Coordinates system: UTM (17S) WGS84.

Macroinvertebrate sampling was carried out with a square net (opening area: 25 cm side, mesh size: 500 µm) using the kick technique on an approximate area of 1 m^2, using 2 min per sampling [14]. A total of 8 replicates per sampling station was collected according to the representativeness of the different microhabitats. The eight replicates were grouped together, resulting in one compiled sample per sampling station. This compiled sample was preserved in a plastic recipient after adding to it 96% alcohol and some drops of glycerin. In parallel to the biological sampling, physical–chemical variables were measured using a multi-parametric probe (WTW MultiLine®Multi 3620, Xylem Analytics, Weilheim, Germany). These were temperature (°C), dissolved oxygen (mg L^{-1}), oxygen saturation, (%), pH, and electric conductivity (µS cm^{-1}). Moreover, a water sample was taken at each sampling station and transported to the laboratory for further analyses.

The evaluation of the quality of the river habitat was based on the River Habitat Index (IHF) developed by Acosta et al. [14] and Pardo et al. [15]. The IHF is made up of seven components, and each one has a maximum score. The final score of the IHF is given by the sum of all seven individual scores; its maximum possible value is 100. Table 1 provides a summary of the components of the IHF. In the case of the IHF3, which evaluates the composition of the mineral substrate (blocks, stones, pebbles, gravel, sand, clay, and silt) in the riverbed, partial scores were obtained for each of the eight replicates (the same number of replicates as for the macroinvertebrates) that were summed up at the end to give the final IHF3 score.

Table 1. Components of the River Habitat Index (IHF) used in the present study based on Acosta et al. [14] and Pardo et al. [15].

Components of River Habitat Index (IHF)	Definition	Explanation of Definition	Ecological Meaning of Score	Maximum Score
IHF1	Substrate inclusion and limitation	The amount of compacted sand present between the larger blocks in the rapid zones	Compacted sand limits the colonization of macroinvertebrates; hence, lack of it represents a higher score	10
IHF2	Frequency of riffles	Indicates the frequency of riffles in the riverbed	Riffles are particularly suitable habitats for macroinvertebrates; hence, their presence increases the score value	10
IHF3	Substrate composition	Evaluates the composition of the mineral substrate in the riverbed	Macroinvertebrates benefit from the diverse substrate; hence, the assigned score is higher when more types are present	20
IHF4	Speed/depth regimens	Evaluates the presence of four combinations of velocity (fast or slow) and depth (shallow or deep) of the water column	Maximum score is obtained when all the four combinations of velocity and depth are present in the river section	10
IHF5	Shade on the riverbed	Assesses the coverage of shadow on the riverbed	When shadow is present with some sunny openings, the assigned score is the highest	10
IHF6	Riverbed heterogeneity	Estimates the presence of leaf litter, exposed roots, trunks/branches, and natural dams	Presence of natural elements favors macroinvertebrates by creating physically more complex habitats; hence, the assigned score is higher	10
IHF7	Aquatic vegetation cover	Estimates the presence of different types of aquatic vegetation (bryophytes, benthic stream algae, filamentous algae, macrophytes)	Presence of aquatic vegetation provide food sources and an increased surface for colonization; hence, the assigned score is higher	30

The riparian vegetation quality was evaluated by using the Riparian Vegetation Quality Index (QBR) based on Munné et al. [13] and Acosta et al. [14], considering two basic types: páramo and forest (Table 2). The QBR is composed of four components, each one having a maximum possible score of 25 points; the final value is obtained by summing up the individual scores of the four components. In the QBR of páramo, the QBR2 was not considered due to the lack of stratification and less complex vegetation structure than forest; hence, the maximum possible score of the QBR for páramo was 75 points instead of 100, as in the other case. Nevertheless, to compare the QBR of páramo to the values of the QBR of forest, the values of the QBR páramo were linearly rescaled to a 100-point scale.

At each sampling station, hydro-morphological features were evaluated, such as altitude (m a.s.l.), geographic location (coordinates system: UTM 17S WGS84), discharge (L s^{-1}), channel width (m), and average water column depth (m). At a representative river cross section, the average velocity was determined through the integration of several water velocities measured by means of a propeller flow meter (Global Water Flow Probe-FP111, Xylem Analytics). Water depth was measured at the same points as the velocity (throughout the cross section), and averages were calculated to represent the water depth at the surveyed cross section. The stream discharge was estimated at each sampling station by employing the velocity (times area) integration method [26] at the representative cross section. Further, slope (%), river order [27], catchment area (km^2), and geological composition (8 types) were defined for each sampling station. The values of some of the geomorphological features, such as slope (%) and catchment area (km^2), were defined from the available digital elevation model (DEM) of the study catchments, a LIDAR product of the SIGTIERRAS project (http://www.sigtierras.gob.ec) of the Ecuadorian government [28,29], by using the geographical information systems (GIS) software ArcGIS® version 10.3 and TerrSet® version 18.21.

Table 2. Components of the Riparian Vegetation Quality Index (QBR) used in the present study based on Munné et al. [13] and Acosta et al. [14].

Components of Riparian Vegetation Quality Index (QBR)	Definition	Explanation of Definition	Explanation of the Score	Maximum Score
QBR1	Coverage of the riparian zone	Evaluates the degree of vegetation cover on each of the riverbanks	Larger areas covered by vegetation on the riverbanks represent higher scores	25
QBR2	Vegetation structure of the riparian zone	Assesses the type of species that form the riparian cover and the natural stratification of the plant community	Presence of trees and more developed vegetation stratification receive higher scores	25
QBR3	Quality of the riparian cover	Evaluates whether the tree species are native or introduced and whether anthropogenic activities are present	Native vegetation receives the highest score, and presence of anthropogenic activities reduces the score	25
QBR4	Degree of naturalness of the river channel	Evaluates if the river channel is natural or to what degree it has been modified	The natural river receives the maximum score, and the different channel modifications reduce the score	25

2.3. Analyzing Water Quality Parameters and Benthic Macroinvertebrates

In the laboratory, macroinvertebrate samples were washed, the organisms were sorted out, and, whenever possible, the specimens were identified to their most specific taxonomic rank through the specialized literature [30–33]. Higher taxonomic divisions were used for organisms belonging to the Chironomidae family or the Oligochaeta subclass.

Standard methods for water chemical analyses were followed [34] to determine the following variables: biochemical oxygen demand (BOD5) in the water (mg L^{-1}), total dissolved solids (mg L^{-1}), turbidity (NTU), concentration of nitrates (mg L^{-1}), total phosphorus (mg L^{-1}), and fecal coliforms (MPN $(100\ mL)^{-1}$).

2.4. Statistical Analyses

To assign the sampling stations to different river types, first a database was constructed for each sampling station using the following variables: altitude (m a.s.l.), geographic coordinates (longitude and latitude, expressed in the UTM 17S WGS84 system), slope (%), river order [27], catchment area (km^2), geological compositions (eight types), vegetation types (páramo, high montane evergreen forest of the western Andes, lower montane evergreen forest of the western Andes, semi deciduous forest of the eastern Andes, montane cloud forest of the western Andes), average water temperature (°C), and precipitation (mm $year^{-1}$). Spearman correlation was carried out to detect highly correlated variables. Water temperature was strongly correlated with altitude ($r = -0.85$); hence, it was eliminated from further analyses. Due to the different types of variables, i.e., quantitative (continuous), qualitative (nominal), and ordinal (ranges), principal component analysis was not adequate for the purposes of the current study; hence, principal coordinate analysis (PCoA) was employed instead through the use of the program PAST version 3.0, which can deal adequately with this issue of different variable types [35]. Prior to the application of the PCoA, the quantitative variables were logarithmically transformed. PCoA assigned a new score value to each monitoring station with respect to each axis considered in the analysis. Every new score value represents a "coordinate" in the multidimensional space in the scope of the PCoA. The first axis of PCoA explained the major part of the variability of the data. For each site, the scores of this axis were corrected for the variability percentage explained (the eigenvalues) by simple multiplication [36]. Consequently, more discriminant power was given to each sampling station.

The newer score values of each monitoring station were used in the context of a K-means classification (implemented with PAST version 3.0) to group the monitoring stations according to river types. This method allows the number of groups (K) to which the different monitoring stations are assigned through the clustering process to be predetermined [11,37]. The groups should be (internally) as homogeneous as possible, differing from each other, and without a hierarchical structure [37,38]. In the current study, 4, 5, 6, and 7 different classification groups (i.e., predetermined K values) were tested using K-means clustering.

To validate the results of river typology generated by the K-means algorithm, macroinvertebrate communities were analyzed in two steps using the statistical software Primer version 6.0. First, multidimensional scaling (MDS) analysis was employed using the fourth root transformed relative abundances of macroinvertebrates. This analysis serves to locate the sampling stations in a spatial diagram according to their degree of similarity measured through the Bray–Curtis Index [39]. Further, the non-parametric analysis of similarity (ANOSIM) was used to verify the degree of similarity between the macroinvertebrate communities of each river type [40]. The dissimilarity between river types is measured through the R statistic (ranging from -1 to 1); R-values closer to 1 indicate greater differences [40]. From the 90 initial sampling stations, six of them were discarded for the MDS and ANOSIM analyses, namely, TOM-MIL-CONTROL that did not present any macroinvertebrates due to its high level of contamination; TOM-CU-010, TOM-MZ-CONTROL, TOM-TOM-005 and TOM-MZ-010 because they exhibited very low abundances of macroinvertebrates; and YAN-IZH-005, because only environmental variables were measured as, unfortunately, no biological sampling was performed. Thus, a total of 84 biological sampling stations was finally used in this study.

To define the pressure gradients for each river type, first a database was constructed with the following environmental variables: dissolved oxygen (mg L^{-1}), biochemical oxygen demand (mg L^{-1}), oxygen saturation (%), conductivity (μS cm^{-1}), total dissolved solids (mg L^{-1}), turbidity (NTU), concentration of nitrates (mg L^{-1}), total phosphorus (mg L^{-1}), fecal coliforms (MPN (100 mL) $^{-1}$), River Habitat Index (IHF), and Riparian Vegetation Quality Index (QBR). Due to the fact that the pH variation was very low in all the monitoring stations (pH = either 7 or 8), representing low ecological significance, this parameter was excluded from further analyses. All quantitative variables were logarithmically transformed. Further, principal component analysis (PCA) was performed using the afore-mentioned environmental variables (with the statistical software CANOCO version 4.5 [41]). The PCA was carried out separately for the 4 river types that were defined by the K-means classification analysis, except for the stations belonging to the river type "rivers of the Pacific coast". This river type had a low number of stations (5) with minimal human impacts. Prior to the PCAs, a Pearson correlation matrix was constructed for each river type to rule out highly correlated variables (i.e., $r > 0.70$). The scores of the first axis of the PCA were normalized from 0 to 1 so that the stations closest to 0 would represent the least and those closest to 1 the most anthropogenically impacted.

To detect the important environmental variables, which influence the distribution of macroinvertebrate communities in each river type, redundancy analysis (RDA) was applied using the statistical software CANOCO version 4.5. The same sampling stations were used as in the case of ANOSIM and MDS (84 sampling stations). As in the case of PCA, sampling stations belonging to the river type "rivers of the Pacific coast" were excluded from this analysis due to the low number of stations located in rivers of this type. The relative abundances of the macroinvertebrates were fourth root transformed, and only taxa found in more than 10% of the stations were included in the analysis to rule out noise by taxa with a low frequency of occurrence. The physical–chemical variables that were also used in the PCA after correlation analyses were logarithmically transformed. Additionally, hydro-morphological variables, namely, elevation (m.a.s.l.), river order, channel width (m), channel average depth (m), discharge (L s^{-1}), and slope (%), were also considered in the RDA. A Monte Carlo multiple permutation test (499 permutations) was used to select the significant environmental variables by the forward selection method, which were included in the RDA model.

3. Results

3.1. Diversity of Macroinvertebrate Community at the Sampled Stations

The sampling stations in the western slope of the Andes (Cañar and Balao catchments) presented the highest average taxa richness (26.6 and 25.0, respectively), with maximum and minimum taxa richness values of 38 and 11 in the Cañar catchment and 48 and 8 in the Balao catchment, respectively. At the stations in the eastern Andean slope, the highest average richness was 23.8 in the Yanuncay catchment (with maximum and minimum richness values of 36 and 15, respectively) and the lowest in the Cuenca river (9 taxa). The greatest richness of macroinvertebrate taxa (48) was found in the Malacatos river in the Balao catchment (CHAU-MA-010).

3.2. Definition of River Typology

Principal coordinate analysis was used to summarize the variability of all analyzed parameters. The first, second, and third axes explained, respectively 34.6%, 12.6%, and 8.3% of the variability (eigenvalues 2.09, 0.76, and 0.50, correspondingly). As the first axis explained the highest variability, it was chosen as the new descriptive variable in the K-means analyses considering K values of 4, 5, 6, and 7. In the current case, K = 6 was finally selected since the grouping of the sampling stations presented the greatest ecological coherence among the group members. Correspondingly, the sampling stations were grouped into the following six different river types (Supplementary Materials Table S1): páramo rivers (PAR), mountain forest rivers (MOF), rivers with humid shrub (HS), urban rivers (URB), rivers of the Pacific coast (PC), and Tarqui river (TAR).

3.3. Validation of River Typology Using Macroinvertebrate Communities

The ANOSIM revealed a high degree of similarity (R = 0.05) between the sampling stations of MOF and HS rivers, which indicated that the macroinvertebrate communities in these two river types were practically indistinguishable (Table 3). The multidimensional scaling (MDS) analyses (Figure 2) showed similar results; hence, sampling stations from these two river types were grouped into a single type (MOF-HS). Further, both the MDS and ANOSIM analyses revealed that the main macroinvertebrate community differences were observed between the PC rivers and the other river types. In addition, four of the Tarqui river sampling stations (TAR-IRQ-CONTROL, TAR-PORT-CONTROL, TAR-SHU-CONTROL, and TAR-TAR-030) had the least modified channel morphology among all the Tarqui stations. These four stations maintained their original water course and had stone and pebble substrates, unlike the rest of sites of the Tarqui river with sand and silt substrates. Their macroinvertebrate communities were more similar to those of the sampling stations of HS rivers than to the rest of the Tarqui river stations (Figure 2); hence, they were incorporated into the HS river type.

Table 3. R statistic values of the analysis of similarity (ANOSIM) as a function of the river type. PAR = páramo river; MOF = mountain forest river; HS = humid shrub river; PC = Pacific coast river; TAR = Tarqui river; URB = urban river.

River Type	R Statistic	River Type	R Statistic
PAR/PC	0.65	PC/MOF	0.76
PAR/URB	0.62	HS/TAR	0.73
PAR/TAR	0.51	HS/URB	0.60
PAR/HS	0.20	HS/MOF	0.05
PAR/MOF	0.08	MOF/TAR	0.88
PC/TAR	1.00	MOF/URB	0.71
PC/URB	0.95	URB/TAR	0.57
PC/HS	0.83		

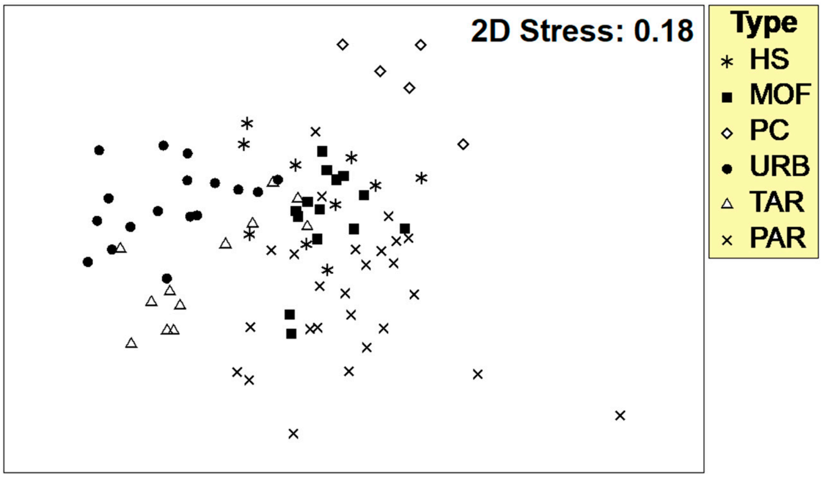

Figure 2. Macroinvertebrate communities at the 84 sampling stations of the six river types grouped by multidimensional scaling (MDS). HS = humid shrub river; MOF = mountain forest river; PC = Pacific coast river; URB = urban river; TAR = Tarqui river; and PAR = páramo river.

These results, from the composition of the macroinvertebrate communities (ANOSIM and MDS), were used as a criterion to correct the first typology generated by K-means and group the stations considering the ecological characteristics of the rivers. Hence, a corrected distribution of the sampling stations was obtained as a function of the river typology (Figure 1), whereby 26 sampling stations were classified as being situated in the páramo river type, 24 in the MOF-HS river type, 17 in urban rivers, 12 in the Tarqui river, and 5 in rivers draining toward the Pacific coast.

3.4. Anthropogenic Pressure Gradient of the Different River Types

Table 4 summarizes the mean observed magnitudes of the different physical and chemical variables in the four river types. Temperature, conductivity, total dissolved solids, BOD5, turbidity, and concentration of fecal coliforms increased toward the lower elevation stations. The measured nitrate and phosphorous values were in the concentration range of other studies carried out in the Paute river basin, such as that of Sotomayor et al. [2]. However, in the PAR river type there were two stations and in the MOF-HS river type three stations where anthropogenic impacts were strongly present, which resulted in much higher concentrations of nitrate and total phosphorus. This is reflected in the higher mean and standard deviation of the nitrate and total phosphorus concentrations in both river types (Table 4). Sampling stations of the PC river type were not considered in the anthropogenic pressure analyses, since there were very few stations in this type.

Table 4. Mean and standard deviation (STD) values of the environmental variables and components of the River Habitat Index (IHF) and Riparian Vegetation Quality Index (QBR) associated with the four river types: páramo (PAR), mountain forest and humid shrub (MOF-HS), Tarqui (TAR), and urban (URB).

	PAR		MOF-HS		TAR		URB	
Environmental Variables	**Mean**	**STD**	**Mean**	**STD**	**Mean**	**STD**	**Mean**	**STD**
Temperature (°C)	8.3	2.1	10.7	1.9	13.8	1.2	14.5	2.4
Conductivity (µS cm^{-1})	70.5	28.2	81.1	25.8	131.4	76.5	127.5	46.3
Dissolved oxygen (mg L^{-1})	8.0	0.4	8.1	0.4	7.2	1.4	7.5	0.5
Oxygen saturation (%)	104.9	3.0	102.2	9.4	93.6	17.3	98.5	4.1
pH	7.8	0.4	7.6	0.4	7.7	0.2	7.9	0.1
Total dissolved solids (mg L^{-1})	73.6	20.2	80.3	16.3	133.9	60.4	125.5	38.3
BOD5 (mg L^{-1})	0.7	0.5	0.6	0.3	2.2	1.6	5.9	5.4
Nitrates (mg L^{-1})	12.3	11.9	20.3	18.4	0.2	0.2	1.9	6.9
Total phosphorus (mg L^{-1})	8.1	5.4	12.7	11.1	0.2	0.1	0.9	2.7
Turbidity (NTU)	1.5	0.9	3.2	2.4	8.8	2.9	12.8	11.8
Fecal coliforms (MPN (100 mL)$^{-1}$)	49.0	68.8	1317.9	3428.4	187,073.8	241,871.0	423,954.9	893,572.2
IHF1	6.7	4.2	7.1	2.9	2.9	3.5	3.8	3.0
IHF2	9.6	1.3	9.7	0.9	6.5	2.1	9.4	1.2
IHF3	10.4	3.5	8.8	2.0	6.4	3.5	6.6	3.1
IHF4	8.3	1.7	9.1	1.3	8.3	3.6	7.5	1.5
IHF5	4.8	2.8	5.3	2.4	3.3	0.7	3.9	1.7
IHF6	6.8	2.5	7.3	1.7	4.3	2.0	6.0	1.9
IHF7	13.7	5.8	12.0	5.3	7.5	4.6	3.8	4.9
IHF	60.3	8.2	58.9	7.0	39.0	4.7	41.1	7.9
QBR1	19.2	9.2	6.4	10.0	0.0	0.0	0.3	1.2
QBR3	20.4	8.9	9.2	10.4	0.0	0.0	0.7	2.1
QBR4	24.0	3.2	14.5	10.4	1.3	3.5	0.3	1.2
QBR	82.4	25.2	18.4	6.5	10.0	8.0	8.8	8.8

To reduce the number of environmental variables, which were highly correlated (i.e., $r > 0.7$), Pearson correlation was employed in the remaining four river types. In the case of the PAR river type, the first axis of the PCA explained 26.9%, and the second axis explained 16.7% of the variability of the dataset. Fecal coliforms (FC) correlated with the first PC axis ($r = 0.51$) and conductivity and IHF1 correlated with the second axis ($r = -0.46$ and $r = 0.5$, respectively) (Table 5). Only two stations with slightly higher coliform concentration (283 and 124 MPN (100 mL)$^{-1}$) were related positively to the FC variable, while all the rest of the sampling stations, with very low FC values (on average, 49 MPN (100 mL)$^{-1}$), were situated in the opposite direction in the multidimensional space of the PCA. These two stations also had higher conductivity as compared to the rest of the stations. In the PCA of the MOF-HS river type, the first and second PCA axes explained 26.9% and 20.6% of the variability, respectively. Conductivity and biochemical oxygen demand (BOD5) correlated with the first PC axis ($r = 0.47$ and $r = -0.48$, respectively), and turbidity and the final value of the River Habitat Index (IHF) had a stronger correlation with the second axis ($r = 0.45$ and $r = -0.50$, respectively) (Table 5). Among the stations of this river type (PAR), there were reference stations (with no or little impact) having higher IHF and conductivity values, while stations with more anthropogenic impacts were related to higher BOD5 and turbidity and lower IHF (Table 4).

In the PCA of the sampling stations of the Tarqui river, the first and second PCA axes explained 52.5% and 22.9% of the variability, respectively. With regard to the first axis, the following variables had stronger correlations: turbidity ($r = 0.48$), the degree of naturalness of the river channel (QBR4) ($r = 0.49$), and the final value of the Riparian Vegetation Index (QBR) ($r = 0.45$), while FC had a strong correlation to the second PC axis ($r = 0.77$) (Table 5). In general, all stations had very low values of IHF (from 32 to 47) and QBR (from 0 to 20).

Only two stations reached values of 20 for QBR and QBR4; hence, these two stations were positively related to the afore-mentioned variables and also to (slightly) higher turbidity and lower FC values. Other stations exhibited low QBR and QBR4, higher FC, and lower turbidity (Table 4).

Table 5. Summary of the results of the correlation (loading) of the anthropogenic impact variables with the first two axes of the principal component analysis for the four river types included in the analysis.

Parameter	River Type							
	Páramo		Mountain Forest and Humid Shrub		Tarqui		Urban	
	Axis 1	Axis 2	Axis 1	Axis 2	Axis 1	Axis 2	Axis 1	Axis 2
Conductivity	0.11	**−0.46**	**0.47**	0.28	-	-	**0.59**	0.11
BOD5	0.31	0.39	**−0.48**	−0.05	-	-	-	-
N	0.39	−0.38	0.43	0.34	0.25	−0.43	−0.19	0.42
P	−0.05	0.14	−0.06	0.39	0.38	0.29	-	-
Turbidity	−0.07	0.15	−0.29	**0.45**	**0.48**	−0.07	**0.55**	−0.15
FC	**0.51**	−0.15	−0.31	−0.11	0.03	**0.77**	-	-
IHF1	0.21	**0.50**	-	-	-	-	-	-
IHF	0.32	0.22	0.10	**−0.50**	0.33	−0.28	**−0.54**	0.06
QBR4	−0.41	−0.24	0.28	−0.03	**0.49**	−0.00	-	-
QBR	−0.41	0.25	0.30	−0.43	**0.45**	0.23	0.14	**0.89**

Notes: BOD5 = biochemical oxygen demand; N = nitrates; P = total phosphorus; FC = fecal coliforms; IHF1 = substrate inclusion and limitation; IHF = final value of the River Habitat Quality Index; QBR4 = degree of naturalness of the river channel; QBR = final value of the Riparian Vegetation Index. "-" indicates that the variables for a specific river type were not used in the PCA after a correlation analysis.

In the urban rivers (URB), the first axis of the PCA explained 49.8% and the second axis 20.4% of the variability. Conductivity (r = 0.59), turbidity (r = 0.55), and IHF (r = −0.54) correlated with the first axis, while QBR (r = 0.89) had a strong correlation with the second axis (Table 5). Most stations in this river type had higher conductivity and turbidity and low IHF and QBR values. Conductivity had a significant Pearson correlation with FC (r = 0.84); hence, most URB stations also had high FC concentrations. The few stations that reached a QBR value of 20 were related positively to this variable.

3.5. Taxa Present in the Different River Types and Identification of the Important Environmental Variables for Benthic Communities

In total, 89 different taxa were identified in the four river types (Table 6). The páramo river type had all 89 taxa. The MOF-HS river type had 60 taxa, the TAR river type 32, the URB river type 45, and the PC river type 63 taxa. Twelve taxa were found in all river types, 2 taxa appeared only in the PAR rivers, and 10 taxa were present only in PAR and PC rivers (Table 6).

In the páramo river dataset, the first and second axis of the RDA explained 53.2% and 18.6%, respectively, of the variability in the macroinvertebrate community. Riverbed heterogeneity (IHF6) (14.8% contribution), QBR (9.4% contribution), BOD5 (7.4% contribution), and shade of the riverbed (IHF5) (6.7% contribution) were the most influential variables on the distribution of macroinvertebrates in the páramo rivers (Figure 3a).

Table 6. List of taxa found in the different river types. PAR = páramo; MOF-HS = mountain forest and humid shrub; TAR = Tarqui; URB = urban; PC= Pacific coast.

Class	Family	Genus	PAR	MOF-HS	TAR	URB	PC	Class	Family	Genus	PAR	MOF-HS	TAR	URB	PC
Arachnida	Clade: Hydracarina		x	x		x	x	Insecta	Hydraenidae	*Hydraena*	x		x		
Bivalvia	Sphaeriidae	*Pisidium*	x	x	x	x				*Ochthebius*	x		x		x
Clitellata	Glossophonidae	*Helobdella*	x	x	x	x	x		Hydrobiosidae	*Atopsyche*	x	x		x	x
	Subclass: Oligochaeta		x	x	x	x	x			*Cailloma*	x	x			
Gastropoda	Ancylidae		x		x				Hydrophilidae	*Enochrus*	x				
	Lymnaeidae	*Fossaria*	x			x	x			*Tropisternus*	x				
		Pseudosuccinea	x	x		x			Hydropsychidae	*Leptonema*	x				
	Physidae	*Haitia*	x	x	x	x	x			*Smicridea*	x	x			x
	Planorbidae		x		x	x			Hydroptilidae	*Leucotrichia*	x	x		x	x
Insecta	Aeshnidae	*Rhinoaeshna*	x		x		x			*Metrichia*	x	x	x	x	
	Anomalopsychidae	*Contulma*	x	x			x			*Neotrichia*	x	x			x
	Baetidae	*Andesiops*	x	x	x	x	x			*Ochotrichia*	x	x	x	x	
	Blephariceridae	*Baetodes*	x	x	x	x	x			*Oxyethira*	x				x
		Camelobaetidius	x	x	x	x	x		Leptoceridae	*Grumichella*	x				
		Limonicola	x	x		x				*Nectopsyche*	x	x			x
		Mayobaetis	x				x		Leptohyphidae	*Ecuaphlebia*	x	x			x
		Nanomis	x				x			*Farrodes*	x	x		x	
		Paltostoma	x	x		x	x			*Leptohyphes*	x	x	x	x	x
		Prebaetodes	x				x			*Tricorythodes*	x	x			x
	Calamoceratidae	*Phylloicus*	x	x		x	x			*Thraulodes*	x	x			x
	Ceratopogonidae	*Bezzia*	x				x		Limnephilidae	*Anomalocosmoecus*	x	x			
		Palpomyia	x	x	x	x	x		Limoniidae	*Hexatoma*	x		x		x
	Chironomidae		x	x	x	x	x			Tribe: Hexatomini	x	x	x		x
	Coenagrionidae	*Ishnura*	x		x		x			*Limonia*	x	x		x	x
	Corydalidae	*Corydalus*	x				x			*Molophilus*	x	x		x	x
	Dixidae	*Dixa*	x							*Orimarga*	x	x		x	
	Dytiscidae	*Liodessus*	x				x			*Polymera*	x	x			x
		Rhantus	x		x	x			Muscidae	*Limnophora*	x	x		x	x
	Elmidae	*Austrelmis*	x	x		x	x		Perlidae	*Anacroneuria*	x	x			x
		Austrolimnius	x	x		x	x			*Claudioperla*	x	x			x
		Heterelmis	x	x	x	x	x		Polycentropodidae	*Polycentropus*	x	x			
		Hexanchorus	x	x	x		x		Psephenidae	*Pheneps*	x	x			x
		Huleechius	x	x	x	x	x		Psychodidae	*Maruina*	x	x		x	x
		Macrelmis	x	x			x			*Pericoma*	x			x	x
		Microcylloepus	x				x			*Psychoda*	x	x	x	x	
		Neoelmis	x	x		x	x		Scirtidae	*Cyphon*	x	x		x	x
		Notelmis	x				x		Simuliidae	*Gigantodax*	x	x	x	x	x
		Onychelmis	x	x						*Simulium*	x	x	x	x	x
		Pharceonus	x				x		Tabanidae	*Tabanus*	x	x			x
		Pseudodisersus	x				x		Tipulidae	*Tipula*	x	x		x	
	Empididae	*Neoplasta*	x	x	x	x	x		Xiphocentronidae		x	x		x	
	Ephydridae		x		x	x		Malacostraca	Hyalellidae	*Hyalella*	x	x	x	x	x
	Glossosomatidae	*Mortoniella*	x				x	Ostracoda			x		x	x	
	Grumichellinae	*Atanatolica*	x	x			x	Turbellaria	Dugesiidae	*Girardia*	x	x	x	x	x
	Helicopsychidae	*Helicopsyche*	x	x			x								

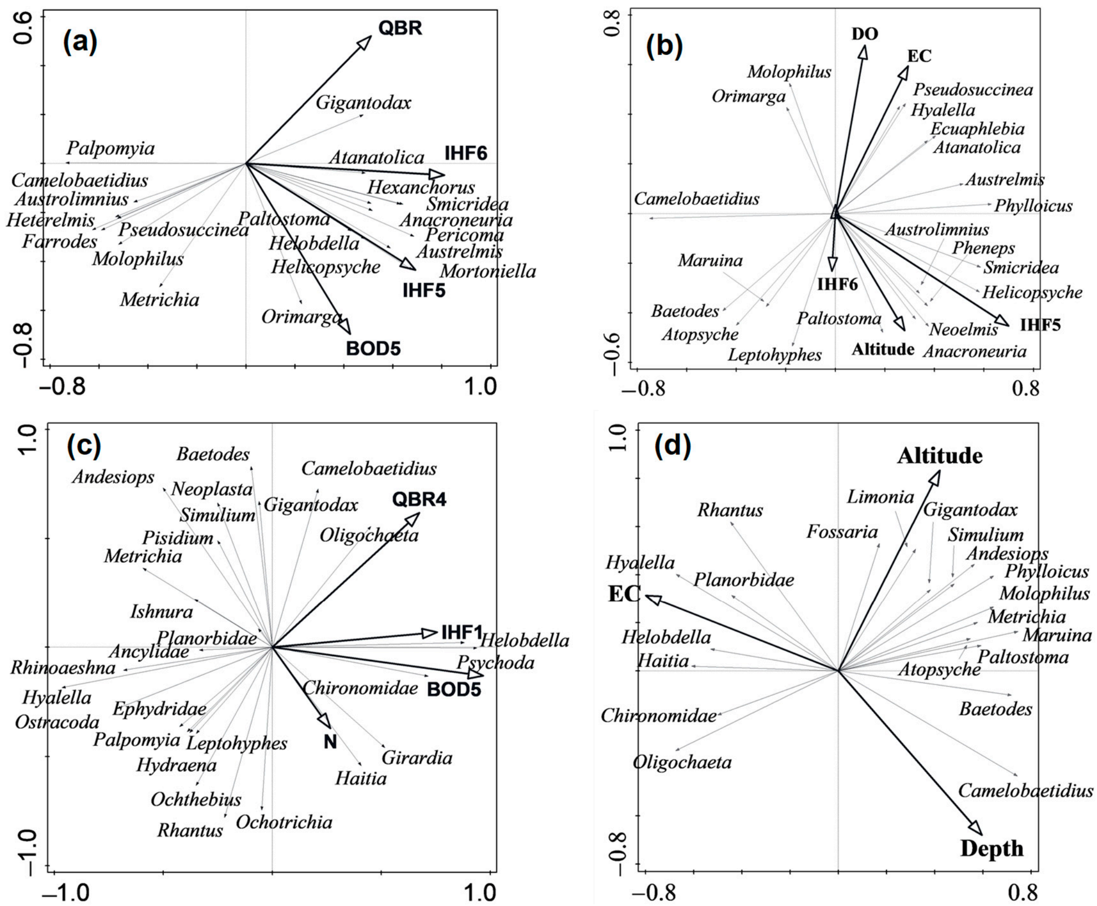

Figure 3. Result of the redundancy analyses (RDA) for the river types: (**a**) páramo; (**b**) mountain forest and humid shrub; (**c**) Tarqui; and (**d**) urban. BOD5 = biochemical oxygen demand; IHF1 = substrate inclusion and limitation; IHF5 = shade of the riverbed; IHF6 = riverbed heterogeneity; QBR = final value of the Riparian Vegetation Index; QBR4 = degree of naturalness of the river channel; DO = dissolved oxygen; EC = electric conductivity; N = nitrates; depth = water depth; altitude = elevation.

In the high montane forest–humid shrub river type dataset, the first axis of the RDA explained 27.2% and the second axis explained 22.1% of the variability. Although IHF5 was still essential in the distribution of the taxa (10.5% contribution), other variables also became important, such as dissolved oxygen (DO) (8.6% contribution), altitude (8.3% contribution), IHF6 (7.0% contribution), conductivity (EC) (6.4% contribution), and nitrate concentration (7.1% contribution) (Figure 3b). In the Tarqui rivers, the first two axes of the RDA explained, respectively, 41.2% and 30.9% of the variability in the macroinvertebrate community. The following parameters significantly explained the variability in the macroinvertebrate community: BOD5 (33.9% contribution), QBR4 (22.1% contribution), nitrate (17.4% contribution), and substrate inclusion and limitation (IHF1) (11.9% contribution) (Figure 3c). In the URB river types, the first and second axes of the RDA explained 72.2% and 16.8%, respectively, of the variability in the macroinvertebrate community. The following variables determined the distribution of the benthic community: EC (21.6% contribution), altitude (8.2% contribution), and water depth (12.9% contribution) (Figure 3d).

4. Discussion

In the high mountains, millions of people depend on the use of surface water for human consumption [42]. Hence, ensuring river ecosystem health is a fundamental requirement to provide the vital liquid [43]. Establishing an extensive biomonitoring network and a regular ecosystem health assessment is an important tool of integrated watershed management. To evaluate ecosystem health, it is important to consider the anthropogenic pressure gradient but also the natural variability of the ecosystems; hence, classification of rivers is necessary. River classification is a long-established practice developed for a range of applications [44]. In Europe, the classification of rivers was elaborated at different spatial scale, such as national [45] or European [46]. In most cases, the development of these typologies needs extensive datasets of hydrological regimes [47] or geomorphology [48]. In Ecuador, Villamarín et al. [49] identified two different elevation bioregions, but a more detailed classification does not exist, mainly due to the complex topography and climatology [14]. Owing to the lack of detailed hydrological datasets, a condition that importantly differs from what is observed, for instance, in northern countries, simple morphological/climatological/geological characteristics were used, which proved to be a successful approach for identifying river types; an information that may be useful for optimizing local biomonitoring costs and efforts.

Benthic macroinvertebrates are demonstrated to be the most useful biological assessment methods for freshwater ecosystem health monitoring (i.e., Álvarez-Cabria et al. [50], Liu et al. [51]). Thereby, many indices have been developed using macroinvertebrates for evaluating the ecological status of lotic systems [52]. However, macroinvertebrate communities generally are not used to define river typology. Nevertheless, this approach was successful in the present study to refine the previously developed river typology that was based exclusively on morphological/climatological/geological characteristics. This approach could be also replicated in similar studies.

In the defined river types, inorganic, organic, and microbial pollution and the state of the riparian vegetation seem to be the most important anthropogenic pressures. Turbidity and electric conductivity strongly correlate with the presence of human activities related to industrial discharges [53]. Turbidity increases with mining activities [54] or cattle raising [55]. Average turbidity increased by more than 90% due to the activity of cattle in a British lowland river [55]. In the current study, the two less impacted river types, namely, PAR and MOF-HS, had lower values of conductivity and turbidity: 70.5 µS cm^{-1} and 1.5 NTU in PAR rivers and 81.1 µS cm^{-1} and 3.2 NTU in the MOF-HS rivers, respectively. The catchment of the Tarqui river type is strongly dominated by cattle raising, having importantly higher values of conductivity and turbidity (average 131.4 µS cm^{-1} and 8.8 NTU, respectively). Urban rivers (URB) receive important industrial discharge (average electric conductivity 127.5 µS cm^{-1}), and upstream deforestation resulted in the highest turbidity value (in average, 12.8 NTU) among the studied river types.

Fecal coliforms is one of the most important microbial contaminants derived, among others, from urban sewage and/or animal husbandry [56]. Wilson and Everard [55] reported that cattle raising doubled the level of fecal coliforms in the British lowland river. The páramo and mountain forest–humid shrub rivers had very low fecal coliforms concentrations (on average, 49 and 1318 MPN $(100\ mL)^{-1}$, respectively). However, a few sampling stations, which had slightly higher concentrations compared to the rest of the stations, caused fecal coliforms to become important variables in these two river types. These stations were affected by the presence of cattle, which shows the advancement of agricultural/cattle raising frontiers toward higher altitudes. In the TAR rivers, the concentration of fecal coliforms was the most important anthropogenic pressure variable, reflecting the very active cattle raising activity occurring in this catchment. Similarly, Jayakod et al. [57] reported that livestock operations and failing septic systems are the two main sources of fecal coliforms in the Pelahatchie watershed in Mississippi. The present study confirmed elevated values of fecal coliforms in the urban river type, which most probably originate from urban septic systems. The presence of fecal coliforms result in the increase of BOD5 as reported already several decades ago [58]. In the present study, relatively low BOD5 values were recorded with highly elevated fecal coliforms concentrations, which is in line with the results of local studies [59,60]. BOD5 levels at a sampling location with slower, deeper water might be higher than the respective BOD5 levels for a similar site in aerated waters [34]. This might be the case in the sampled rivers, which are high mountain water courses with more pronounced slopes, faster flow and more turbulence, features that facilitate stream water aeration.

Strongly reduced River Vegetation Index and lack of vegetation naturalness characterized the Tarqui and urban rivers. Cattle raising eliminated or reduced riverine vegetation in the Tarqui river, and city development had the same effect in the urban rivers. Despite several studies that argue about the upmost importance of riverine vegetation to enhance the ecological status [61] and its impact on important ecosystem processes [62,63], the currently existing national regulation, which prohibits the construction of civil infrastructures within a 50 m buffer zone from the riversides, is rarely fulfilled. Sotomayor et al. [37] stated for the whole Paute river basin, which the seven study catchments belong to, that the presence of native vegetation has the potential, along the river courses, to form buffer systems for the enhancement of riparian ecosystems and, finally, of improving the downstream water quality.

Macroinvertebrate communities responded to different environmental variables in the four river types. Species of the caddisfly (*Atanatolica*, *Mortoniella*, and *Helicopsyche*), stoneflies (*Anacroneuria*), and diptera (*Paltostoma*) are present in the neotropical high mountains that normally inhabit little-impacted sites, with a good water quality and heterogeneous fluvial habitat [64,65]. Similarly, in the present study, these were related to high River Habitat Index in the higher elevations, páramo sites. *Orimarga*, another genus from the páramo rivers, are dipterans that usually live associated with fine substrates, such as sand or silt [66], where oxygen concentrations are lower and there may be accumulations of decomposing debris, which may explain the association of this genus with the higher BOD5 in the present study. Further, genera such as *Anacroneuria*, *Helicopsyche*, *Paltostoma*, *Atopsyche*, *Pheneps*, and *Maruina* are associated with heterogeneous fluvial habitats and high altitude rivers [67–69]. In the current study, these genera were related to higher elevation sampling stations of mountain forest and humid shrub river types, where more shade and riverbed heterogeneity were present. Chironomidae and *Psychoda* (Psychodidae) dipteran, present in lower elevation rivers, are usually resistant to conditions with little oxygen and high organic load [70]. In the current study, these taxa were associated with higher BOD5 in the lower elevation Tarqui rivers. In the same rivers, the presence of snail *Haitia* was linked to elevated nitrate concentrations similarly to other studies, which found this snail in eutrophic environments with excesses of nutrients [71].

Genera of rheophilic mayflies (*Baetodes* and *Camelobaetidius*) that usually inhabit wide and deep river channels [72] were detected in urban rivers where river channels were much deeper and wider than the rivers of the other types. Some genera with a preference for highlands (*Paltostoma*, *Gigantodax*, *Andesiops*, and *Atopsyche*) [73–75] were still observed in some upper reaches of urban rivers (such as the Machángara), although with low abundances. Some of them may appear in these rivers as a consequence of dragging from the headwaters immediately after rainy periods. High conductivity, which in turn is highly correlated with other variables such as lower oxygen concentration, high fecal coliforms, and total dissolved solids, was associated with taxa such as Chironomidae, Oligochaeta, and *Haitia*. This result could be due to the fact that Chironomidae and Oligochaeta were not identified further to genus or species levels, which was the case of the study of Rosa et al. [76], who reported that the presence/absence of Chironomidae and Oligochaeta was able to detect the river's pollution gradient. Further, Scheibler et al. [77] reported that elevated conductivity levels and increased river discharge produced low chironomid density values in the Andes region of western–central Argentina.

5. Conclusions

Evaluations of ecological status are especially important in rivers where water is taken from surface ecosystems for drinking purposes. This study revealed that evaluations should include, in addition to physical–chemical variables, the river habitat and riparian vegetation quality, as these characteristics were important to identify anthropogenic pressures and seemed to strongly influence macroinvertebrate communities. The 90 sampling stations were grouped into five different river types, in which anthropogenic pressure gradients were inorganic (i.e., conductivity, turbidity), organic (i.e., fecal coliform), river habitat, and riparian vegetation quality. The macroinvertebrate communities were strongly influenced by different aspects of river habitat and riparian vegetation quality such as the presence of shade, riverbed heterogeneity, substrate inclusion, degree of vegetation naturalness, and Riparian Vegetation Quality Index. BOD5, representing organic pollution, was also an important variable for macroinvertebrates in two river types. Electric conductivity indicated inorganic pollution and was also correlated with biological pollution such as fecal coliforms and influenced macroinvertebrates in the mountain forest and humid shrub river types. The novelty of the study included the development of river typology on a strongly understudied region using an extensive sampling network for evaluation of river ecosystems health. Further, in addition to the physical and hydro-geomorphological characteristics, which are usually used to define river types, in the current study, analyses of macroinvertebrate communities helped to refine the river typology, which may lead to a more optimal local biomonitoring.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/w15091742/s1, Table S1: Sampling stations grouped into six classes (river types) defined by the K-means cluster analysis.

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Data Availability Statement: Restrictions apply to the availability of data used in this research. Data were collected by the researchers of the University of Cuenca and the Municipal Public Enterprise of Telecommunications, Drinking Water, Sewerage, and Sanitation of Cuenca (ETAPA EP); as such, data requests need to be addressed to both institutions and to the authors of those institutions.

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References

1. Viviroli, D.; Archer, D.R.; Buytaert, W.; Fowler, H.J.; Greenwood, G.B.; Hamlet, A.F.; Huang, Y.; Koboltschnig, G.; Litaor, M.I.; López-Moreno, J.I.; et al. Climate change and mountain water resources: Overview and recommendations for research, management and policy. *Hydrol. Earth Syst. Sci.* **2011**, *15*, 471–504. [CrossRef]
2. Sotomayor, G.; Hampel, H.; Vázquez, R.F. Water quality assessment with emphasis in parameter optimisation using pattern recognition methods and genetic algorithm. *Water Res.* **2018**, *130*, 353–362. [CrossRef] [PubMed]
3. Arumí, J.L.; Muñoz, E.; Oyarzún, R. Andean Mountain Groundwater, Drinking Water Sources, and Vulnerability: A Case Study in Central Chile. In *Groundwater*; Modreck, G., Ed.; IntechOpen: Rijeka, Croatia, 2019.
4. Grayson, R.; Blöschl, G. *Spatial Patterns in Catchment Hydrology Observations and Modelling*; Cambridge University Press: Cambridge, UK, 2000; p. 404.
5. Mosquera, P.V.; Hampel, H.; Vázquez, R.F.; Alonso, M.; Catalan, J. Abundance and morphometry changes across the high-mountain lake-size gradient in the tropical Andes of Southern Ecuador. *Water Resour. Res.* **2017**, *53*, 7269–7280. [CrossRef]
6. Minaya, V.; Camacho Suarez, V.; Wenninger, J.; Mynett, A. Runoff generation from a combined glacier and páramo catchment within the Antisana Reserve in Ecuador. *J. Ecohydraul.* **2021**. [CrossRef]

7. Mena-Vásconez, P.; Hofstede, R. Los Páramos Ecuatorianos. In *Botánica Económica de los Andes Centrales*; Moraes, M.R., Øllgaard, B., Kvist, L.P., Borchsenius, F., Balslev, H., Eds.; Universidad Mayor de San Andres: La Paz, Bolivia, 2006; pp. 91–109.
8. Peters, M.K.; Hemp, A.; Appelhans, T.; Becker, J.N.; Behler, C.; Classen, A.; Detsch, F.; Ensslin, A.; Ferger, S.W.; Frederiksen, S.B.; et al. Climate–land-use interactions shape tropical mountain biodiversity and ecosystem functions. *Nature* **2019**, *568*, 88–92. [CrossRef]
9. Baudino, O.M.; Suero, E.A.; Augusto, M.; Gimenez, M.E.; Flores, N. Monitoring Organochlorine Pesticides in Surface and Ground Water in San Juan Argentina. *J. Chil. Chem. Soc.* **2003**, *48*, 7–12. [CrossRef]
10. Kim, M.; Boithias, L.; Cho, K.H.; Silvera, N.; Thammahacksa, C.; Latsachack, K.; Rochelle-Newall, E.; Sengtaheuanghoung, O.; Pierret, A.; Pachepsky, Y.A.; et al. Hydrological modeling of Fecal Indicator Bacteria in a tropical mountain catchment. *Water Res.* **2017**, *119*, 102–113. [CrossRef]
11. Sotomayor, G.; Hampel, H.; Vázquez, R.F.; Forio, M.A.E.; Goethals, P.L.M. Implications of macroinvertebrate taxonomic resolution for freshwater assessments using functional traits: The Paute River Basin (Ecuador) case. *Divers. Distrib.* **2022**, *28*, 1735–1747. [CrossRef]
12. Zhao, C.; Pan, T.; Dou, T.; Liu, J.; Liu, C.; Ge, Y.; Zhang, Y.; Yu, X.; Mitrovic, S.; Lim, R. Making global river ecosystem health assessments objective, quantitative and comparable. *Sci. Total Environ.* **2019**, *667*, 500–510. [CrossRef]
13. Munné, A.; Prat, N.; Solà, C.; Bonada, N.; Rieradevall, M. A simple field method for assessing the ecological quality of riparian habitat in rivers and streams: QBR index. *Aquat. Conserv.* **2003**, *13*, 147–163. [CrossRef]
14. Acosta, R.; Ríos-Touma, B.; Rieradevall, M.; Prat, N. Propuesta de un protocolo de evaluación de la calidad ecológica de ríos Andinos (C.E.R.A) y su aplicación en dos cuencas en Ecuador y Perú. *Limnetica* **2009**, *28*, 35–64. [CrossRef]
15. Pardo, I.; Álvarez, M.; Moreno, J.L.; Vivas, S.; Bonada, N.; Alba-Tercedor, J.; Jaimez-Cuellar, P.; Moya, G.; Prat, N.; Robles, N.; et al. El hábitat de los ríos mediterráneos. Diseño de un índice de diversidad de hábitat. *Limnetica* **2002**, *21*, 115–134. [CrossRef]
16. Arias, P.A.; Bellouin, N.; Coppola, E.; Jones, R.G.; Krinner, G.; Marotzke, J.; Naik, V.; Palmer, M.D.; Plattner, G.K.; Rogelj, J.; et al. Technical Summary. In *Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*; Masson-Delmotte, V., Zhai, P., Pirani, A., Connors, S.L., Péan, C., Berger, S., Caud, N., Chen, Y., Goldfarb, L., Gomis, M.I., et al., Eds.; Cambridge University Press: Cambridge, UK; New York, NY, USA, 2021; pp. 33–144.
17. Mosquera, P.V.; Hampel, H.; Vázquez, R.F.; Catalan, J. Water chemistry variation in tropical high-mountain lakes on old volcanic bedrocks. *Limnol. Oceanogr.* **2022**, *67*, 1522–1536. [CrossRef]
18. Van Colen, W.; Mosquera, P.V.; Hampel, H.; Muylaert, K. Link between cattle and the trophic status of tropical high mountain lakes in páramo grasslands in Ecuador. *Lakes Reserv.* **2018**, *23*, 303–311. [CrossRef]
19. ETAPA-EP. *Diseños Definitivos de la Planta de Tratamiento de Aguas Residuales de Guangarcucho, Cantón Cuenca, Provincia del Azuay, Ecuador, Anexo 1: Informe de Condiciones de Tratabilidad*; Empresa Pública Municipal de Telecomunicaciones, Agua Potable, Alcantarillado y Saneamiento ETAPA-EP: Cuenca, Ecuador, 2017; p. 40.
20. G&H; ACSAN. *Determinación del Caudal de Aguas no Residuales que Ingresa al Sistema de Saneamiento y Propuestas Para la Aplicación de Medidas Correctivas. Greeley and Hansen (G&H), ACSAN Consultores*; Empresa Pública Municipal de Telecomunicaciones, Agua Potable, Alcantarillado y Saneamiento ETAPA-EP: Cuenca, Ecuador, 2023; p. 59.
21. Ríos-Touma, B.; Acosta, R.; Prat, N. The Andean Biotic Index (ABI): Revised tolerance to pollution values for macroinvertebrate families and index performance evaluation. *Rev. Biol. Trop.* **2014**, *62*, 249–273. [CrossRef]
22. Prat, N.; Ríos-Touma, B.; Acosta, R.; Rieradevall, M. Los macroinvertebrados como indicadores de la calidad del agua. In *Macroinvertebrados Bentónicos Sudamericanos: Sistemática y Biología*; Domínguez, E., Fernández, H.R., Eds.; Fundación Miguel Lillo: Tucumán, Argentine, 2009; pp. 631–654.
23. EU-Parliament. *Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 Establishing a Framework for Community Action in the Field of Water Policy*; European Parliament: Brussels, Belgium, 2000.
24. Segnini, S. El uso de los macroinvertebrados bentónicos como indicadores de la condición ecológica de los cuerpos de agua corriente. *Ecotropicos* **2003**, *16*, 45–63.
25. Alvites, C.; Battipaglia, G.; Santopuoli, G.; Hampel, H.; Vázquez, R.F.; Matteucci, G.; Tognetti, R.; De Micco, V. Dendrochronological analysis and growth patterns of *Polylepis reticulata* (Rosaceae) in the Ecuadorian Andes. *IAWA J.* **2019**, *40*, S331–S335. [CrossRef]
26. Boiten, W. *Hydrometry*, 2nd ed.; CRC Press: Boca Raton, FL, USA, 2008; p. 244.
27. Strahler, A.N. Quantitative analysis of watershed geomorphology. *Eos Trans. Am. Geophys. Union* **1957**, *38*, 913–920. [CrossRef]
28. Corral, L.R.; Montiel Olea, C.E. What Drives Take-up in Land Regularization: Ecuador's Rural Land Regularization and Administration Program, Sigtierras. *J. Econ. Race Policy* **2020**, *3*, 60–75. [CrossRef]
29. Sotomayor, G.; Romero, J.; Ballari, D.; Vázquez, R.F.; Ramírez-Morales, I.; Hampel, H.; Galarza, X.; Montesinos, B.; Forio, M.A.E.; Goethals, P.L.M. Occurrence Prediction of Riffle Beetles (Coleoptera: Elmidae) in a Tropical Andean Basin of Ecuador Using Species Distribution Models. *Biology* **2023**, *12*, 473. [CrossRef]
30. Manzo, V. Key to the South America genera of Elmidae (Insecta: Coleoptera) with distributional data. *Stud. Neotrop. Fauna Environ.* **2005**, *40*, 201–208. [CrossRef]
31. Domínguez, E.; Molineri, C.; Pescador, M.L.; Hubbard, M.D.; Nieto, M.C. *Ephemeroptera of South America*; Pensoft Publishers: Moscow, Russia, 2006; p. 646.

32. Manzo, V.; Archangelsky, M. A key to the known larvae of South American Elmidae (Coleoptera: Byrrhoidea), with a description of the mature larva of *Macrelmis saltensis* Manzo. *Ann. Limnol. Int. J. Lim.* **2008**, *44*, 63–74. [CrossRef]
33. Domínguez, E.; Fernández, H.R. *Macroinvertebrados Bentónicos Sudamericanos: Sistemática y Biología*; Fundación Miguel Lillo: Tucumán, Argentina, 2009; p. 656.
34. APHA-AWWA-WEF. *Standard Methods for Examination of Water and Wastewater*, 23rd ed.; Baird, R.B., Eaton, A.D., Rice, E.W., Eds.; American Public Health Association (APHA), American Water Works Association (AWWA) and Water Environment Federation (WEF): Washington DC, USA, 2017.
35. Legendre, P.; Legendre, L. *Numerical Ecology*, 2nd ed.; Elsevier Science B.V.: Amsterdam, The Netherlands, 1998; Volume 24, p. 852.
36. Munné, A.; Prat, N. Defining river types in a Mediterranean area: A methodology for the implementation of the EU Water Framework Directive. *Environ. Manag.* **2004**, *34*, 711–729. [CrossRef] [PubMed]
37. Sotomayor, G.; Hampel, H.; Vázquez, R.F.; Goethals, P.L.M. Multivariate-statistics based selection of a benthic macroinvertebrate index for assessing water quality in the Paute River basin (Ecuador). *Ecol. Indic.* **2020**, *111*, 106037. [CrossRef]
38. Jain, A.K.; Dubes, R.C. *Algorithms for Clustering Data*; Prentice-Hall: Englewood Cliffs, NJ, USA, 1988; p. 320.
39. Clarke, K.R.; Gorley, R.N.; Somerfield, P.J.; Warwick, R.M. *Change in Marine Communities: An Approach to Statistical Analysis and Interpretation*, 3rd ed.; PRIMER-E: Plymouth, UK, 2014.
40. Quinn, G.P.; Keough, M.J. *Experimental Design and Data Analysis for Biologists*; Cambridge University Press: Cambridge, UK, 2002; p. 537.
41. Ter Braak, C.J.F.; Smilauer, P. *CANOCO Reference Manual and CanoDraw for Windows User's Guide: Software for Canonical Community Ordination*; Version 4.5; Microcomputer Power: Ithaca, NY, USA, 2002.
42. Bradley, R.S.; Vuille, M.; Diaz, H.F.; Vergara, W. Threats to Water Supplies in the Tropical Andes. *Science* **2006**, *312*, 1755–1756. [CrossRef]
43. Luo, Z.; Zuo, Q.; Shao, Q. A new framework for assessing river ecosystem health with consideration of human service demand. *Sci. Total Environ.* **2018**, *640–641*, 442–453. [CrossRef]
44. Tadaki, M.; Brierley, G.; Cullum, C. River classification: Theory, practice, politics. *WIREs Water* **2014**, *1*, 349–367. [CrossRef]
45. Rinaldi, M.; Surian, N.; Comiti, F.; Bussettini, M. A method for the assessment and analysis of the hydromorphological condition of Italian streams: The Morphological Quality Index (MQI). *Geomorphology* **2013**, *180–181*, 96–108. [CrossRef]
46. Rinaldi, M.; Gurnell, A.M.; del Tánago, M.G.; Bussettini, M.; Hendriks, D. Classification of river morphology and hydrology to support management and restoration. *Aquat. Sci.* **2016**, *78*, 17–33. [CrossRef]
47. Olden, J.D.; Kennard, M.J.; Pusey, B.J. A framework for hydrologic classification with a review of methodologies and applications in ecohydrology. *Ecohydrology* **2012**, *5*, 503–518. [CrossRef]
48. Orr, H.G.; Large, A.R.G.; Newson, M.D.; Walsh, C.L. A predictive typology for characterising hydromorphology. *Geomorphology* **2008**, *100*, 32–40. [CrossRef]
49. Villamarín, C.; Rieradevall, M.; Paul, M.J.; Barbour, M.T.; Prat, N. A tool to assess the ecological condition of tropical high Andean streams in Ecuador and Peru: The IMEERA index. *Ecol. Indic.* **2013**, *29*, 79–92. [CrossRef]
50. Álvarez-Cabria, M.; Barquín, J.; Antonio Juanes, J. Spatial and seasonal variability of macroinvertebrate metrics: Do macroinvertebrate communities track river health? *Ecol. Indic.* **2010**, *10*, 370–379. [CrossRef]
51. Liu, W.; Xu, M.; Zhao, N.; Zhou, X.; Pan, B.; Tian, S.; Lei, F. River health assessment of the Yellow River source region, Qinghai-Tibetan Plateau, China, based on tolerance values of macroinvertebrates. *Environ. Sci. Pollut. Res. Int.* **2019**, *26*, 10251–10262. [CrossRef]
52. Herman, M.R.; Nejadhashemi, A.P. A review of macroinvertebrate- and fish-based stream health indices. *Int. J. Ecohydrol. Hydrobiol.* **2015**, *15*, 53–67. [CrossRef]
53. Rahman, A.; Jahanara, I.; Jolly, Y.N. Assessment of physicochemical properties of water and their seasonal variation in an urban river in Bangladesh. *Water Sci. Eng.* **2021**, *14*, 139–148. [CrossRef]
54. Nasrabadi, T.; Ruegner, H.; Sirdari, Z.Z.; Schwientek, M.; Grathwohl, P. Using total suspended solids (TSS) and turbidity as proxies for evaluation of metal transport in river water. *Appl. Geochem.* **2016**, *68*, 1–9. [CrossRef]
55. Wilson, J.L.; Everard, M. Real-time consequences of riparian cattle trampling for mobilization of sediment, nutrients and bacteria in a British lowland river. *Int. J. River Basin Manag.* **2018**, *16*, 231–244. [CrossRef]
56. Reder, K.; Flörke, M.; Alcamo, J. Modeling historical fecal coliform loadings to large European rivers and resulting in-stream concentrations. *Environ. Model. Softw.* **2015**, *63*, 251–263. [CrossRef]
57. Jayakody, P.; Parajuli, P.B.; Brooks, J.P. Evaluating Spatial and Temporal Variability of Fecal Coliform Bacteria Loads at the Pelahatchie Watershed in Mississippi. *Hum. Ecol. Risk Assess.* **2014**, *20*, 1023–1041. [CrossRef]
58. Hiraishi, A.; Saheki, K.; Horie, S. Relationships of Total Coliform, Fecal Coliform, and Organic Pollution Levels in the Tamagawa River. *Nippon Suisan Gakk.* **1984**, *50*, 991–997. [CrossRef]
59. Fernández de Córdova, J.; González, H. *Evolución de la Calidad del Agua de los Tramos Bajos de los Ríos de la Ciudad de Cuenca*; ETAPA-EP: Cuenca, Ecuador, 2013; p. 99.
60. Mora-Campos, M.R.; Tamay-Heras, A.A. *Determinación Del íNdice de Calidad de Agua Mediante El Monitoreo de Macro Invertebrados, Parámetros Fisicoquímicos Y Microbiológicos en El Río Sinincay, Cuenca-Ecuador*; Universidad Politécnica Salesiana Cuenca: Cuenca, Ecuador, 2022.

61. Rowiński, P.M.; Västilä, K.; Aberle, J.; Järvelä, J.; Kalinowska, M.B. How vegetation can aid in coping with river management challenges: A brief review. *Int. J. Ecohydrol. Hydrobiol.* **2018**, *18*, 345–354. [CrossRef]
62. Osterkamp, W.R.; Hupp, C.R.; Stoffel, M. The interactions between vegetation and erosion: New directions for research at the interface of ecology and geomorphology. *Earth Surf. Process. Landf.* **2012**, *37*, 23–36. [CrossRef]
63. Rutherford, J.C.; Marsh, N.A.; Davies, P.M.; Bunn, S.E. Effects of patchy shade on stream water temperature: How quickly do small streams heat and cool? *Mar. Freshw. Res.* **2004**, *55*, 737–748. [CrossRef]
64. Studholme, A.M.; Hampel, H.; Finn, D.S.; Vázquez, R.F. Secondary production of caddisflies reflects environmental heterogeneity among tropical Andean streams. *Hydrobiologia* **2017**, *797*, 231–246. [CrossRef]
65. Gutiérrez-Fonseca, P.E.; Springer, M. Description of the final instar nymphs of seven species from Anacroneuria Klapálek (Plecoptera: Perlidae) in Costa Rica, and first record for an additional genus in Central America. *Zootaxa* **2011**, *2965*, 16–38. [CrossRef]
66. Palatov, D.M.; Chertoprud, M.V.; Frolov, A.A. Fauna and types of soft-bottom macrozoobenthic assemblages in watercourses of mountainous regions on the eastern Black Sea coast. *Inland. Water. Biol.* **2016**, *9*, 150–159. [CrossRef]
67. Vázquez, R.F.; Vimos-Lojano, D.; Hampel, H. Habitat Suitability Curves for Freshwater Macroinvertebrates of Tropical Andean Rivers. *Water* **2020**, *12*, 2703. [CrossRef]
68. Ward, J.V. Ecology of alpine streams. *Freshw. Biol.* **1994**, *32*, 277–294. [CrossRef]
69. Sajamí-Reymundo, J.I.; Huamantinco-Araujo, A.A. Distribución espacial de Ephemeroptera, Plecoptera, Trichoptera y Coleoptera (Insecta) en una quebrada de primer orden, bosque montano, Junín, Perú. *Rev. Peru. Biol.* **2016**, *23*, 95–102. [CrossRef]
70. Braccia, A.; Voshell, J.R. Benthic Macroinvertebrate Fauna in Small Streams Used by Cattle in the Blue Ridge Mountains, Virginia. *Northeast. Nat.* **2006**, *13*, 269–286. [CrossRef]
71. Sokol, E.R.; Hoch, J.M.; Gaiser, E.; Trexler, J.C. Metacommunity Structure Along Resource and Disturbance Gradients in Everglades Wetlands. *Wetlands* **2014**, *34*, 135–146. [CrossRef]
72. Scheibler, E.E.; Claps, M.C.; Roig-Juñent, S.A. Temporal and altitudinal variations in benthic macroinvertebrate assemblages in an Andean river basin of Argentina. *J. Limnol.* **2014**, *73*. [CrossRef]
73. Finn, D.S.; Encalada, A.C.; Hampel, H. Genetic isolation among mountains but not between stream types in a tropical high-altitude mayfly. *Freshw. Biol.* **2016**, *61*, 702–714. [CrossRef]
74. Iñiguez-Armijos, C.; Hampel, H.; Breuer, L. Land-use effects on structural and functional composition of benthic and leaf-associated macroinvertebrates in four Andean streams. *Aquat. Ecol.* **2018**, *52*, 77–92. [CrossRef]
75. Ríos-Touma, B.; Holzenthal, R.W.; Rázuri-Gonzales, E.; Heckenhauer, J.; Pauls, S.U.; Storer, C.G.; Frandsen, P.B. De Novo Genome Assembly and Annotation of an Andean Caddisfly, *Atopsyche davidsoni* Sykora, 1991, a Model for Genome Research of High-Elevation Adaptations. *Genome Biol. Evol.* **2021**, *14*, evab286. [CrossRef]
76. Rosa, B.J.F.V.; Rodrigues, L.F.T.; de Oliveira, G.S.; da Gama Alves, R. Chironomidae and Oligochaeta for water quality evaluation in an urban river in southeastern Brazil. *Environ. Monit. Assess.* **2014**, *186*, 7771–7779. [CrossRef]
77. Scheibler, E.E.; Roig-Juñent, S.A.; Claps, M.C. Chironomid (Insecta: Diptera) assemblages along an Andean altitudinal gradient. *Aquat. Biol.* **2014**, *20*, 169–184. [CrossRef]