

## Article

# Functional Diversity of Macroinvertebrate Communities in River Nature Reserves of Spain

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**Abstract:** In recent decades, aquatic ecosystems have suffered a series of impacts that have made them some of the most threatened ecosystems on a global scale. So, protection measures are needed to conserve the biodiversity we find in some of the less impacted ecosystems. In the Spanish legislation, a category arose for this purpose in lotic ecosystems, the River Natural Reserve (RNR). In this work, we analyse the taxonomic and functional diversity of 145 macroinvertebrate biocoenoses from 128 different RNRs belonging to 10 different basins and representing 18 typologies. Most of the analysed biocoenoses have an overall high taxonomic diversity, with some exceptions corresponding to particular reaches suffering occasional disturbances or with very special conditions. An intermediate functional homogeneity has been also detected, related to a relatively low average functional richness. We also found medium levels of functional evenness, a high functional divergence, and low functional dispersion and Rao index values, the latter supporting similarities among taxa in functional terms. In our studied systems, there is high taxa turnover, but functional turnover is very low. This means that most of the trait dissimilarity between taxa is found within a community, but not among communities, though there are relatively strong dissimilarities in community composition. Our results support the fact that the RNRs are protecting communities of great diversity, not only taxonomic, but also functional, which contributes to the proper functioning of the ecosystems found in these stream reaches. Thus, the analysis of the functional diversity of the communities, as in the present approach, should be implemented to identify and prioritize protection of reaches with higher functional diversity, where enhanced ecosystem functioning can be expected.

**Keywords:** traits; protected areas; streams; functional indices; diversity; Water Framework Directive



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## 1. Introduction

Despite the importance of freshwater ecosystems in maintaining life on Earth, these habitats are globally threatened by a wide array of pressures [1]. As pointed out by [2], several studies have demonstrated decreases in freshwater species populations, biodiversity, and ecosystem services, making these environments some of the most endangered ecosystems worldwide [3,4]. The degradation of freshwater ecosystems particularly impacts the ecosystem services they provide, many of which have diminished drastically or ceased existing over the last years [5]. Ecosystem services include the ecological processes that directly or indirectly benefit human beings, as well as the way in which functions are efficiently accomplished. Many organisms within the aquatic communities play a central role in these processes. As stated by [6], human impacts at different scales can cause declines in community diversity and functional shifts by driving species replacement, which can lead

to reverberating impacts on ecosystem functioning. The potential severity of these impacts is evident, since ecosystems are not primarily governed by the taxonomic composition but by the functional traits and distribution of individuals and the abundance of species and their biological activity [7]. In fact, the understanding that ecosystem functioning depends more on species functional traits and less on taxonomic diversity is becoming a dominant paradigm [8]. Many indices have been developed to assess functional diversity (see [9]), fueled by the rising interest in studies of functional ecology and diversity and functional species traits in general [10–12] and in aquatic ecosystems in particular [13]. Ref. [14] propose to quantify all three components of functional diversity separately (functional richness, evenness, and divergence) and avoid summarising all data within a single index [15]. Once quantified, more efficient decisions on biodiversity conservation may be made.

An essential strategy to conserve biodiversity is the establishment of protected areas such as natural reserves. Several studies have concluded that they have a positive effect on the number of individuals and species (e.g., [16–18]), particularly in high-impacted regions (e.g., [19]). In this sense, [20] underlined the importance of integrating different taxocenoses when designing protected areas, as some flagship taxa may not properly represent other groups of organisms. A weak concordance was shown to exist in the composition patterns of assemblages of macroinvertebrates and waterbirds, and an inverse or negligible relationship was found in their taxonomic richness patterns. Thus, protected areas should be designed and managed considering the widest possible range of organism groups. In many cases, the declaration of a protected area does not consider important aspects affecting the conservation of biodiversity within it, such as understanding the problems beyond the limits of the protected area in or the particularities of a lotic system when designing the area [21]. Furthermore, these protected areas should represent the total biodiversity of these ecosystems, a goal that requires alternatives to species-centred approaches that consider also phylogenetic or trait information [22], thus combining taxonomic and functional diversity.

In Spanish legislation, a specific category of nature protection arose in 2016 [23], the Hydrological Reserves, within which the category of River Natural Reserves (hereafter RNRs) appeared, to face the worrying declines in conservation status of freshwater ecosystems and, particularly, of streams and rivers. RNRs are specifically defined as watercourses whose natural flows have not been affected by human activities. This designation is based mainly on factors related to the physical environment, such as abiotic, hydromorphological, water quality, and landscape integration factors. Their designation as RNRs is aimed at protecting and conserving the distinctive biodiversity of representative stretches of different types of Spanish rivers. Additionally, they serve as reference stations for monitoring the effects of climate change under the Water Framework Directive (WFD; [24]), as links between Natura 2000 network areas, and as a means of raising the citizens' awareness of the respectful use of water and river spaces.

Under this protection category, particular reaches of rivers with scarce or null anthropogenic perturbances and pressures are catalogued, which have a good or high ecological status, even if they are used by humans (e.g., for recreation), within the standards of the WFD. Although there are now 208 RNRs declared in Spain, at the time of sampling for this study (2017), there were 135 legally declared RNRs spread over 10 of the 16 river basin districts (Cantábrico Occidental, Cantábrico Oriental, Duero, Ebro, Guadalquivir, Guadiana, Júcar, Miño-Sil, Segura, and Tajo). They comprised a total of 2686.23 km of protected reaches within the streams and rivers of Spain [25]. Apart from including reaches with relatively natural conditions and with no, or only occasional, impacts, there was a representation of most of the distinct typologies of rivers in the Iberian Peninsula. Thus, RNRs strive to preserve spots of favourable conditions where organisms can thrive and develop proper communities, with the aim to conserve and protect large amounts of biodiversity and natural resources.

The goal of this study is to analyse the functional diversity of macroinvertebrates from most of the then-existing RNRs in Spain and to compare it with the taxonomic diversity

calculated from data coming from the protocols of the WFD to determine if, at these sites, a proper representation of both taxonomic and functional macroinvertebrate diversity was being conserved. For this purpose, we have focused on the macroinvertebrate community collected during the analysis of such sites, as they form an important part of the lotic biocoenoses and are one of the most important indicators that must be studied within the WFD monitoring programs. Macroinvertebrates have the advantage of being relatively easy to identify at the family level and easy to collect and to study, they represent a wide range of sensitivity to environmental conditions, and they have a long enough life cycle to reflect long-lasting condition changes [26].

## 2. Materials and Methods

### 2.1. Study Sites

Peninsular Spain is geomorphologically characterised by the presence of several large West–East mountain systems, which divide the Iberian Peninsula into different basins. Moreover, a broad gradient of climatic conditions, ranging from Atlantic to Mediterranean climates, results in many river typologies.

In our analyses, we have included 145 macroinvertebrate biocoenoses from 128 different RNRs (in some RNRs, more than one reach was sampled; see Supplementary Data S1; Figure 1) belonging to 10 different basins and representing 18 typologies ([27]; Supplementary Data S2) from which we had data available on the macroinvertebrate community. The sampling site within each RNR was selected to represent the section with the lowest number of impacts or pressures. To select this site, a previous assessment was carried out prioritising locations as far upstream and accessible within the RNR as possible. Additionally, this process incorporated the official discharge shapefile provided by the General Directorate of Water of the Ministry of the Environment combined with a survey using aerial photographs to detect possible hydromorphological pressures. The coordinates were chosen to identify where water bodies were influenced by the discharges and hydromorphological pressures, ensuring that the sampling sites were both representative and minimally disturbed. Each site was visited once in spring–summer of 2017 (from the 22nd of May to the 6th of July).

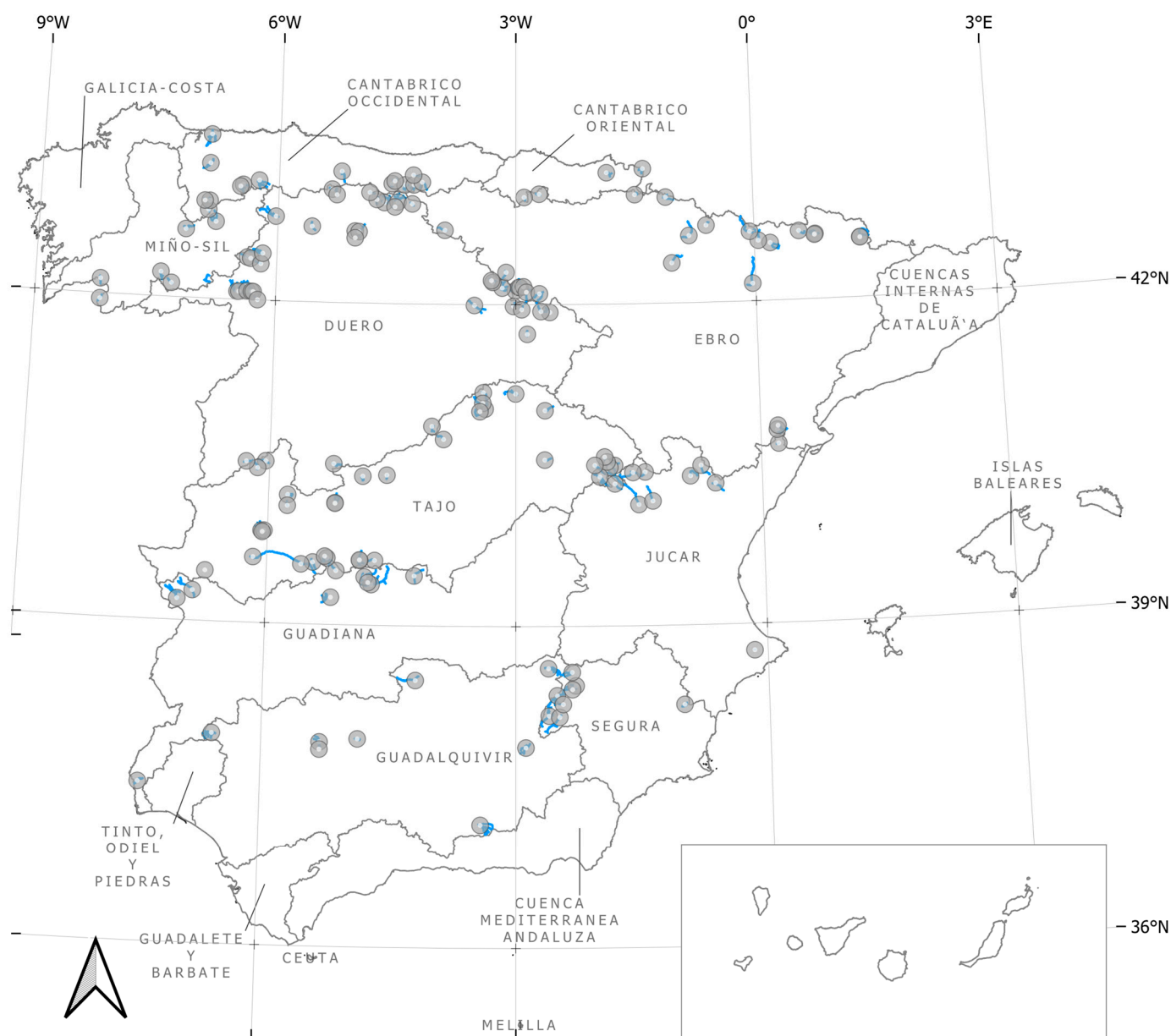
### 2.2. Physicochemical Characterisation of the Sampled Sites

Several physicochemical parameters (pH, conductivity, dissolved oxygen, and temperature) were recorded in situ with a multi-parametric probe (Hach HQ4d, with IntelliCAL™ PHC101, Hach, Düsseldorf, Germany). Water velocity, together with depth, was measured in different points of a transversal section of the reach using a propeller-meter with a scale in it (Global Water Digital FP-211, Global Water Instrumentation, College station, TX, USA), and mean values of both variables were obtained to calculate discharge after measuring the width of the channel. A bottle of water (1 L) was collected to analyse ammonium, nitrates, and orthophosphates (Supplementary Data S1). Also, some environmental indexes such as QBR, IHF, IBMWP, and IPS were calculated in each reach (Supplementary Data S1), although they are not considered for this study.

### 2.3. Macroinvertebrate Sampling, Identification, and Trait Assignment

Benthic macroinvertebrates were collected using a kick net 0.25 m wide and of 500 µm mesh following a semiquantitative, stratified approach in which 20 subsamples were taken, each from an area covering approximately 0.125 m<sup>2</sup> and from different microhabitats, which were then integrated. Microhabitats were visually identified and selected from the shore within a 100 m long reach before the macroinvertebrate sampling. They were classified within one of the following categories: hard substrates (rocks, stones, and gravel, especially in areas of rapids), plant debris (leaf litter, logs of different sizes, plant debris, etc.), vegetated banks, submerged macrophytes, and sand and other fine sediments. Freshly fallen leaves were never included as a microhabitat. Samples were distributed across microhabitats proportionally to the amount of them, i.e., more samples were taken from

more abundant microhabitats. This procedure follows the Spanish national normative [28] to meet the WFD requirements to evaluate the ecological status of the water masses.



**Figure 1.** Map of Spain showing names and administrative limits of the hydrological demarcations and the analysed River Natural Reserves (as dots) within them.

Macroinvertebrates were identified to the family level (except Oligochaeta), following the recommendations of the WFD protocol. As pointed out by several authors (e.g., [29]), this level is a useful approximation to account for the structure and function of macroinvertebrate communities, despite the intrinsic differences in particular ecological roles of different genera and species within the same family. This has been also supported for other animal groups in terrestrial ecosystems, where it has been established that family-level diversity can be a meaningful proxy for determining species-level diversity patterns in biodiversity studies [30].

We used biological and ecological traits from the database of [31]. No other, more recent, data were included because they provided little new information on biological traits (and none on ecological traits) for the taxa present in the RNRs analysed here. For

instance, [32] compiled new trait data on genera from the Mediterranean region, but from them, only a reduced number of genera were present in our database from the Spanish RNRs. In addition, some of the trait data came from family mean trait values; hence, their inclusion in our analysis would have changed the result. No traits were listed in [32] for Melanopsidae, but this family was only present at two sites in relatively low abundances. Thus, to have comparable databases, this family was removed from both taxonomic and functional analyses. A selection of 11 biological traits and 4 ecological traits was used for the analyses (Table 1), considering the most relevant to assess functional diversity, ecosystem services, and/or capacity of resistance/resilience to perturbations. This trait database uses a fuzzy coding system in which a score (from 0 to 3 or from 0 to 5, depending on the information available for each trait and the categories within each trait) is assigned to each trait category for each taxon depending on its affinity (see [33]). The category “hydrostatic vesicle” from trait “respiration” was removed from the database, as none of the taxa present in the RNRs had this respiration mechanism.

**Table 1.** Biological and ecological traits, and corresponding categories, selected for the analyses from [31], together with the short code assigned to each category in the analysed database.

Biological Trait	Category	Short Code
Maximal potential size	≤0.25 cm	V1.1
	>0.25–0.5 cm	V1.2
	>0.5–1 cm	V1.3
	>1–2 cm	V1.4
	>2–4 cm	V1.5
	>4–8 cm	V1.6
	>8 cm	V1.7
Life cycle duration	≤1 year	V2.1
	>1 year	V2.2
Potential number of cycles per year	<1	V3.1
	1	V3.2
	>1	V3.3
Aquatic stages	egg	V4.1
	larva	V4.2
	nymph	V4.3
	adult	V4.4
Reproduction	ovoviviparity	V5.1
	isolated eggs, free	V5.2
	isolated eggs, cemented	V5.3
	clutches, cemented or fixed	V5.4
	clutches, free	V5.5
	clutches, in vegetation	V5.6
	clutches, terrestrial	V5.7
	asexual reproduction	V5.8
Dispersal	aquatic passive	V6.1
	aquatic active	V6.2
	aerial passive	V6.3
	aerial active	V6.4
Resistance forms	eggs, statoblasts	V7.1
	cocoons	V7.2
	housings against desiccation	V7.3
	diapause or dormancy	V7.4
	none	V7.5
Respiration	tegument	V8.1
	gill	V8.2
	plastron	V8.3
	spiracle	V8.4

Table 1. Cont.

Biological Trait	Category	Short Code
Food	microorganisms	V9.1
	detritus < 1 mm	V9.2
	dead plant $\geq$ 1 mm	V9.3
	living microphytes	V9.4
	living macrophytes	V9.5
	dead animal $\geq$ 1 mm	V9.6
	living microinvertebrates	V9.7
	living macroinvertebrates	V9.8
	vertebrates	V9.9
Feeding habits	absorber	V10.1
	deposit feeder	V10.2
	shredder	V10.3
	scraper	V10.4
	filter-feeder	V10.5
	piercer	V10.6
	predator	V10.7
	parasite	V10.8
Temperature	psychrophilic	V11.1
	thermophilic	V11.2
	eurythermic	V11.3
Locomotion and substrate relation	flier	V12.1
	surface swimmer	V12.2
	full water swimmer	V12.3
	crawler	V12.4
	burrower	V12.5
	interstitial	V12.6
	temporarily attached	V12.7
	permanently attached	V12.8
Longitudinal distribution	crenon	V13.1
	epirithron	V13.2
	metarithron	V13.3
	hyporithron	V13.4
	epipotamon	V13.5
	metapotamon	V13.6
	estuary	V13.7
	outside river system	V13.8
Substrate (preferendum)	flags/boulders/cobbles/pebbles	V14.1
	gravel	V14.2
	sand	V14.3
	silt	V14.4
	macrophytes	V14.5
	microphytes	V14.6
	twigs/roots	V14.7
	organic detritus/litter	V14.8
	mud	V14.9
Current velocity (preferendum)	null	V15.1
	slow	V15.2
	medium	V15.3
	fast	V15.4

### 3. Data Analysis

All the analyses were performed in R software v. 4.4.1 [34] using packages vegan [35], FD [36], psych [37], picante [38], and ade4 [39].



From a taxonomic point of view, each macroinvertebrate community was characterised using the Shannon–Wiener diversity index (S-W) and the complement of Simpson’s index (1-D) with the diversity function in the vegan package.

To ordinate RNRs in accordance with their macroinvertebrate communities, a non-metric multidimensional scaling (NMDS) analysis was performed using the metaMDS function in the vegan package. For this, a Wisconsin square root standardisation was applied to the community data (abundance), and dissimilarities among communities were calculated using the Bray–Curtis distance. The number of dimensions (“k” in function metaMDS) was adjusted to reach a stress below 0.2. Function envfit in the vegan package was used to test statistical significance of the physicochemical parameters measured in each site, and those that were statistically significant were represented as vectors and isoclines in the NMDS bidimensional plots.

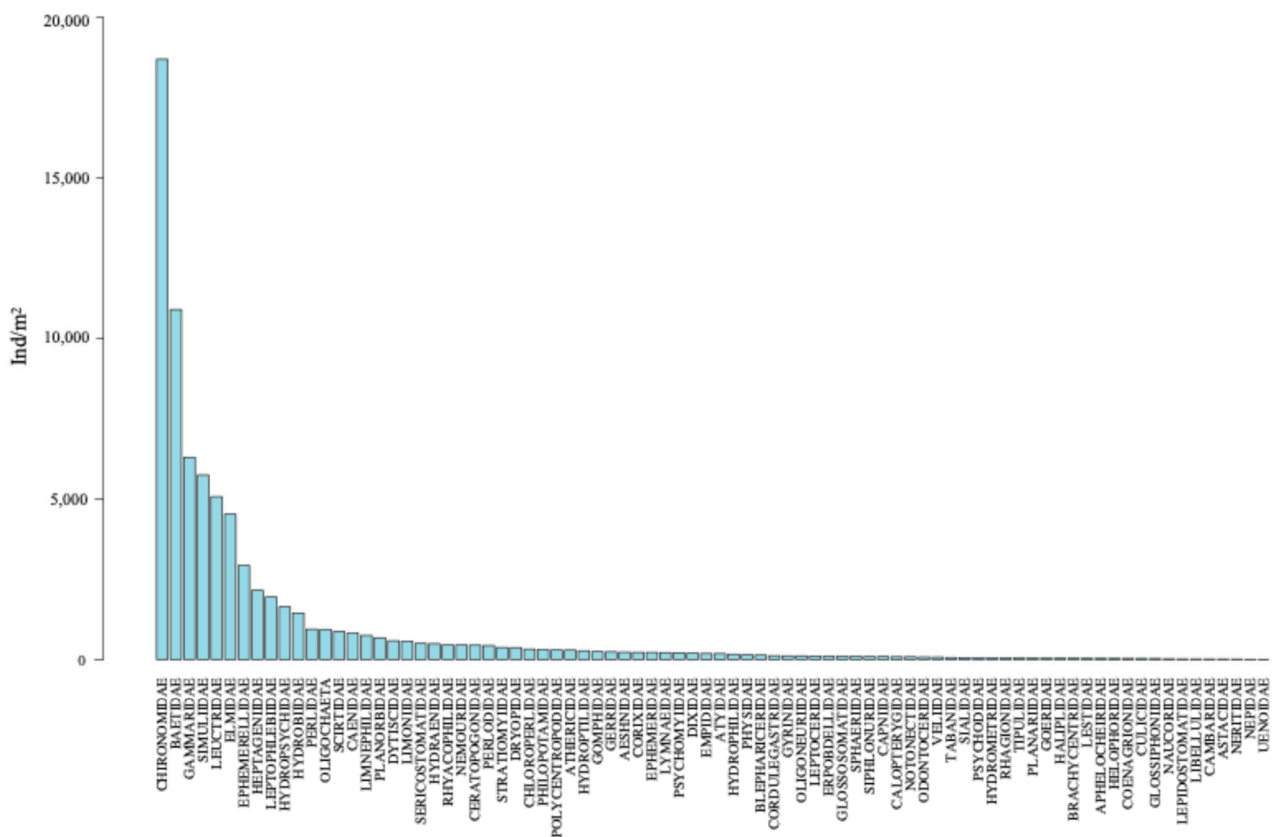
For the statistical analyses of functional diversity, we transformed each trait variable into a dummy variable (sensu [40]) by changing each score to proportions within each trait variable of the [31] original database so that the score of each category within each trait ranged from 0 to 1. Following calculations in [40], we calculated the community weighted mean trait value (CWM), a measure of community trait structure, for each category within each trait and performed an NMDS analysis (as described before) with this new variable. For all the RNRs, we estimated functional richness (FRic), functional evenness (FEve), functional divergence (FDiv), functional dispersion (FDis), and Rao’s quadratic entropy (Rao), together with the number of taxa (S) and the quality of the resulting reduced-space representation (computed as described by [41] and interpreted as a  $R^2$ -like ratio). To compute them, we calculated the Gower’s distance for each trait separately and then averaged the dissimilarity across traits. We obtained all these indexes with the dbFD function, which uses principal coordinates analysis (PCoA) to return PCoA axes, which are then used as “traits” to compute functional diversity [36]. We weighted the indexes for which it was possible by taxa abundances (see [11]). After obtaining the functional diversity indexes, we applied a Spearman’s test with the function cor.test in R to test for correlations between them.

Finally, we analysed diversity partitioning by applying the Rao function from [42] to both taxonomic and functional data, transforming the values of the index into equivalent numbers, as recommended by the authors. The Rao index has the unique property of providing one of the few direct measures of species redundancy within and among biological communities, as well as being the only existing estimator of diversity that combines different measures of species dissimilarity with relative species abundances, standardising the comparison of  $\alpha$ ,  $\beta$ , and  $\gamma$  components between taxonomic, phylogenetic, and functional diversity [42]. In our analysis, we obtained  $\alpha$ ,  $\beta$ , and  $\gamma$  components of functional diversity by applying the hierarchical apportionment of quadratic entropy defined by [43] with the apqe function of the ade4 package [39].

## 4. Results

### 4.1. Taxonomic Diversity

In our study, a total of 145 different macroinvertebrate biocoenoses (communities) were analysed. Merging all, 98 families of macroinvertebrates were recorded, represented by more than 192,000 individuals. When combining the data from all the RNRs, Chironomidae was, by far, the most abundant family, followed by Baetidae, and a group of four families also quite abundantly represented (Gammaridae, Simuliidae, Leuctridae, and Elmidae; Figure 2). Most of the other families were more scarce or even quite rare in the whole RNRs. Both diversity indices, S-W and 1-D, showed overall high median values, with some exceptions corresponding to particular reaches (Figure 3). Eight sampling points had an S-W index below 1.5, and four had a value of 1-D lower than 0.5 (Figure 3).



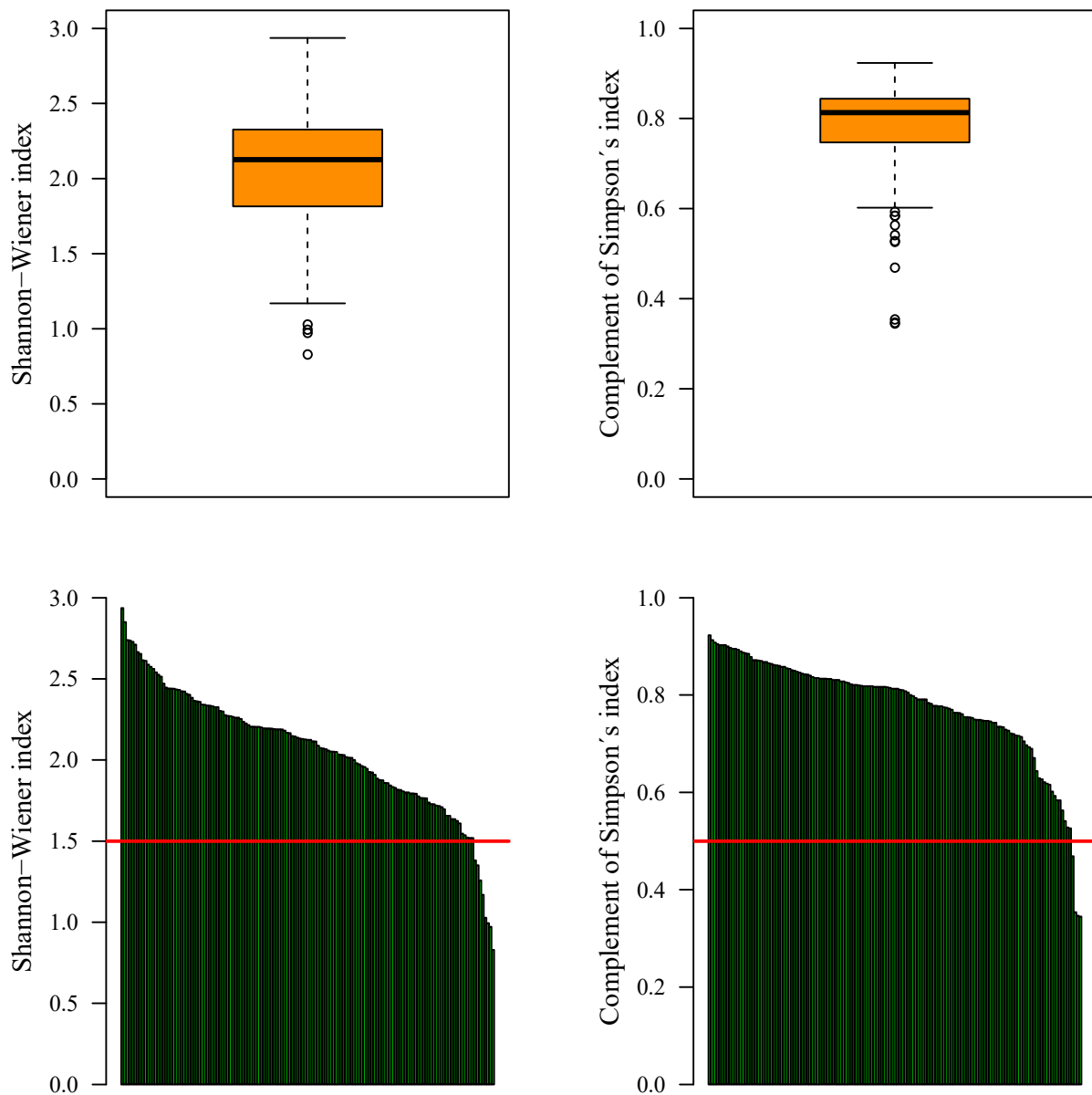
**Figure 2.** Rank-abundance diagram representing the recorded families in all the analysed River Natural Reserves combined.

The NMDS analysis showed (with a stress = 0.18) that most communities were quite similar, showing a great aggregation of points within the ecological space represented but a slight, statistically significant, gradient underlined in relation to four abiotic parameters: temperature, conductivity, discharge, and nitrates (Figure 4).

#### 4.2. Trait Characterisation of Macroinvertebrate Communities

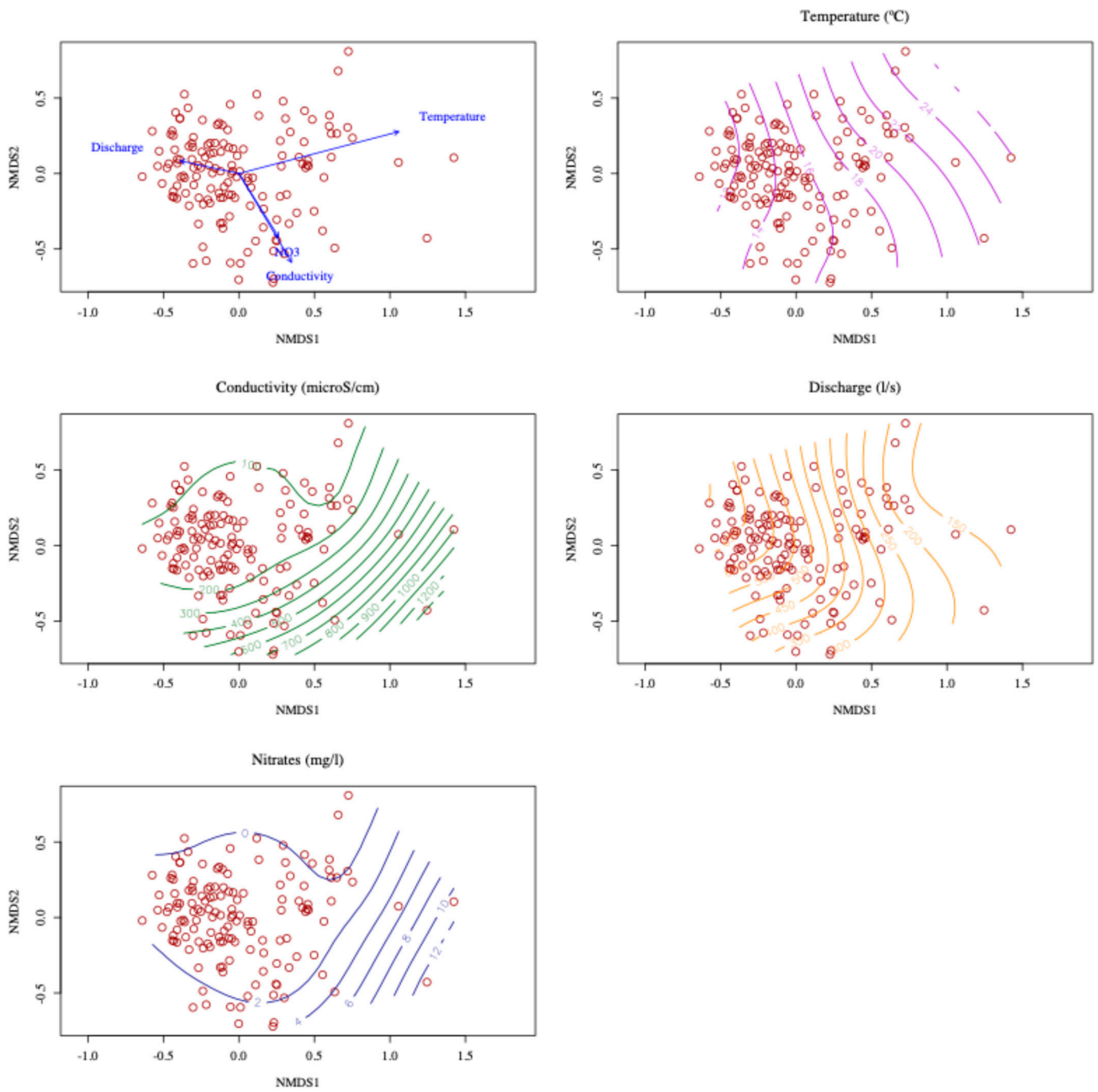
The CMW trait value was calculated for each category of each biological and ecological trait considered (Figure 5, Table 1). In most cases, there was one or a few categories within a trait that were more frequent in the macroinvertebrate community, and many of those better-represented categories provided a certain degree of sensitivity to perturbations to the communities. Macroinvertebrate communities from the studied RNRs were dominated by organisms of medium to relatively large maximal potential size, with a short duration of their life cycle and thus one or more generations per year. Immature stages and eggs were the most common aquatic stages, while adults were considerably scarce. They mainly laid isolated, cemented eggs and clutches, either cemented or free. Regarding dispersal, the aerial passive strategy was less common among these taxa. Most had no resistance forms, utilised the tegument or gills for respiration, and are classified as crawlers. Scrapers and shredders (feeding on biofilm and living microphytes, and dead, coarse particulate organic matter, respectively) were the most abundant in these communities, but also deposit feeders (feeding on detritus or fine particulate organic matter) were well represented. Most taxa had preference for reaches above the potamon and habitats associated with macrophytes, as well as those with substratum composed by boulders, cobbles, and pebbles. Taxa preferring slow to medium flow were more abundant, and they usually were eurythermic.



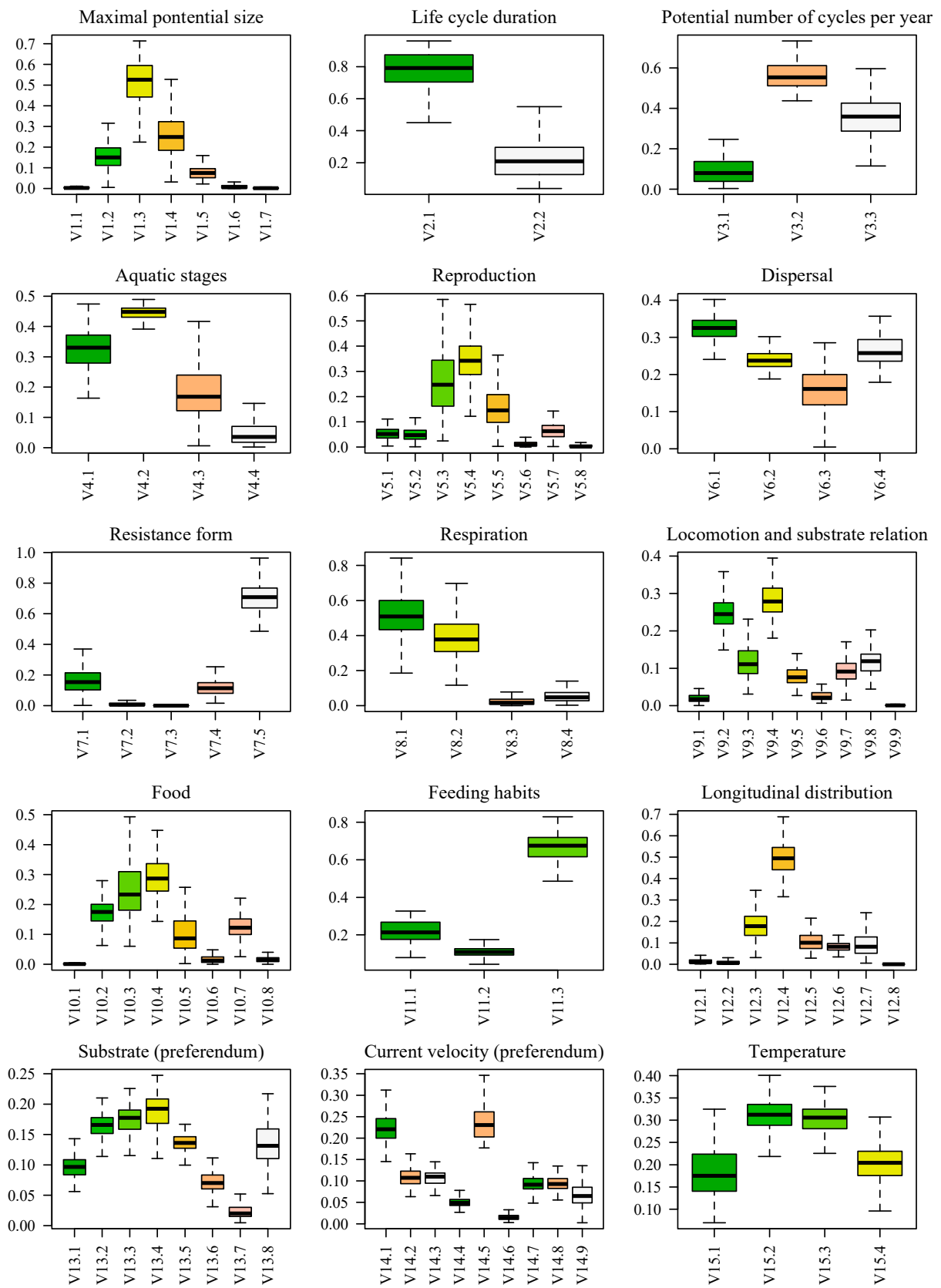


**Figure 3.** Boxplots (representing median, interquartile range, and maximum and minimum, with white points indicating outliers) showing the Shannon–Wiener diversity and complement of Simpson’s index of the studied RNRs (upper row) and the values of those indexes in each community ranked from the highest to the lowest diversity (lower row). Red lines indicate the limit established to detect low-diversity communities.

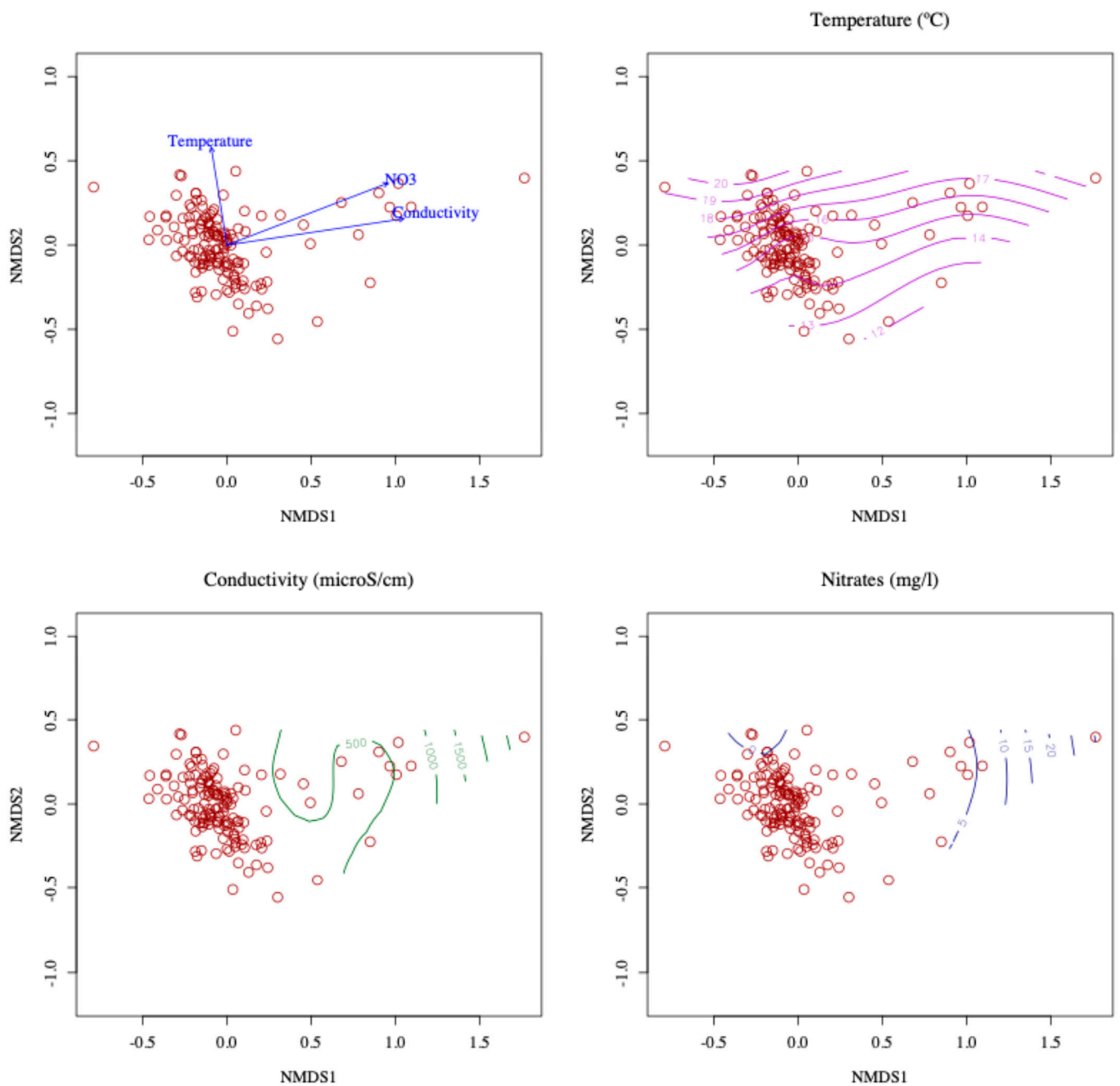
As when considering macroinvertebrate communities, the ordination using the CWM showed a great aggregation among communities in the ecological space represented (NMDS with three dimensions and stress = 0.1). In this case, only temperature, conductivity, and nitrates influenced the ordination and generated a slight, significant gradient (Figure 6).



**Figure 4.** NMDS ordination of the studied RNFs considering macroinvertebrate communities ( $k = 3$ , stress = 0.18), with linear vectors representing significant physicochemical variables (top left diagram), and with smooth surfaces representing each significant physicochemical variable (remaining diagrams).



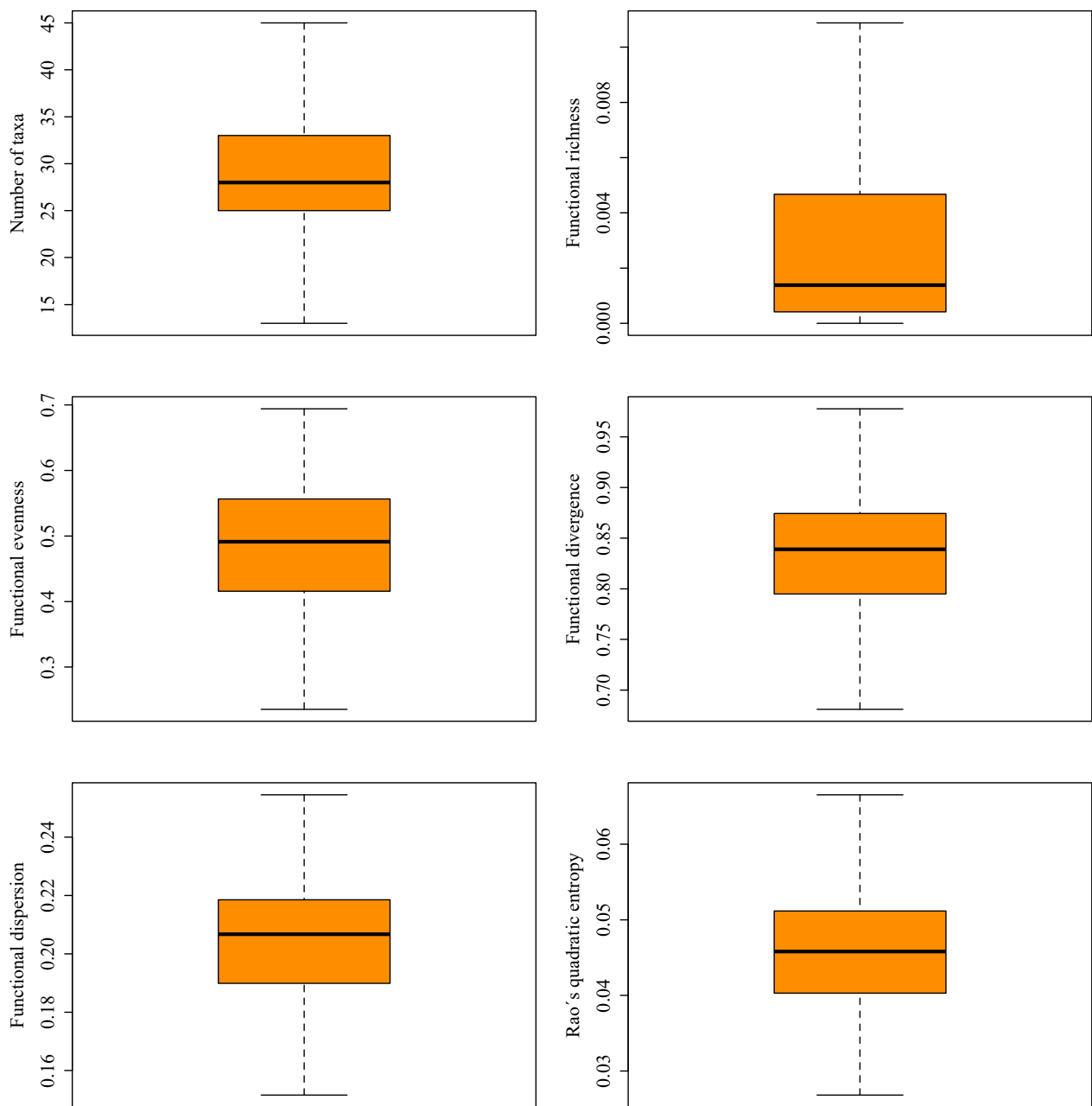
**Figure 5.** Boxplots (representing median, interquartile range, and maximum and minimum) showing the community weighted mean trait value (CWM) for each category within traits. Codes are as in Table 1.



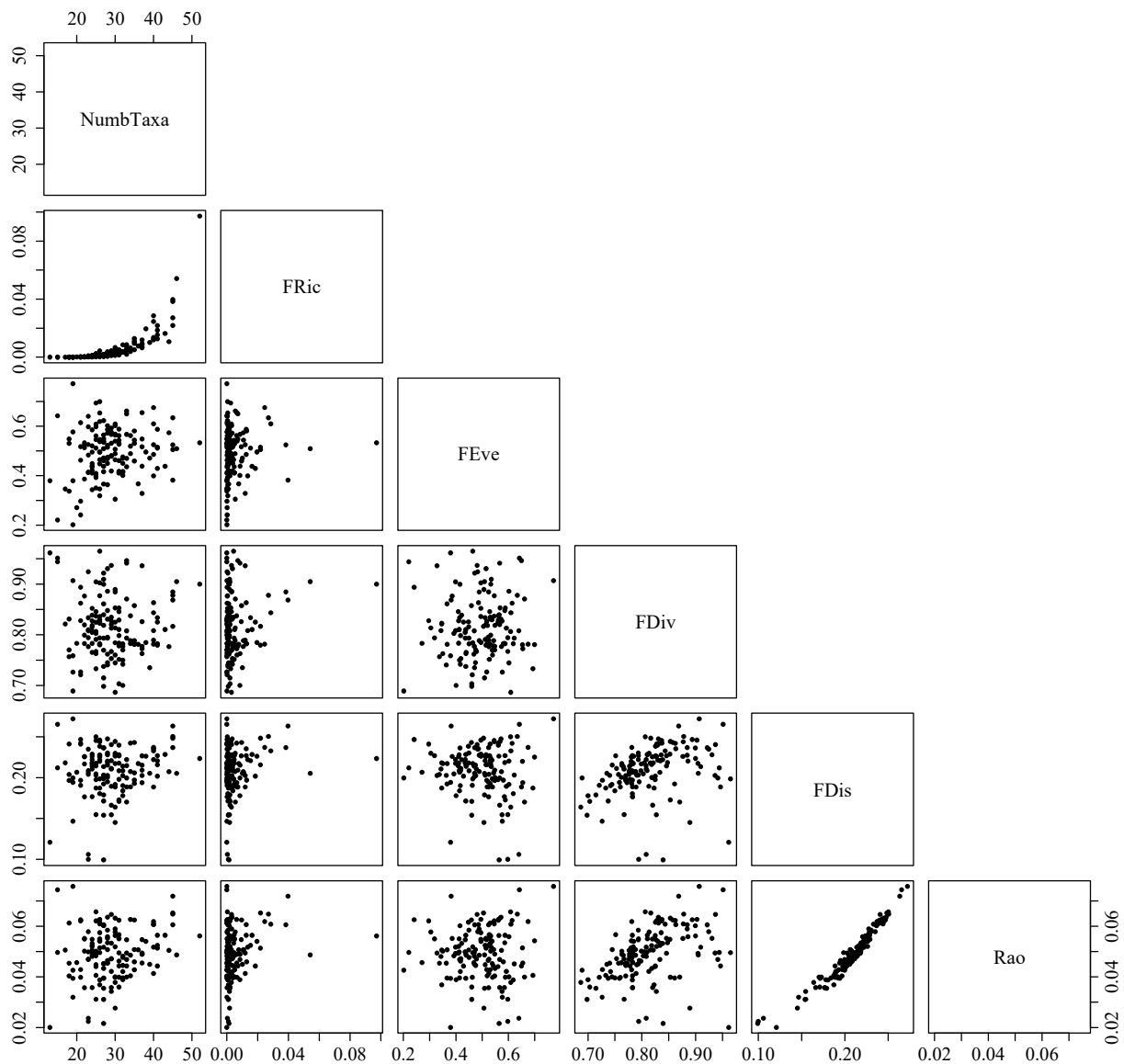
**Figure 6.** NMDS ordination of the studied RNFs considering CWM ( $k = 2$ , stress = 0.15), with linear vectors representing significant physicochemical variables (top diagram), and with smooth surfaces representing each significant physicochemical variable (remaining diagrams).

#### 4.3. Functional Diversity

Five functional diversity indexes were calculated for the whole RNR macroinvertebrate communities, in addition to the number of taxa (Figure 7). FRic was very low for all communities as a whole, but this index showed a high dispersion of values biased to higher than the median values. The quality of the resulting reduced-space representation was 0.54. FEve median was approximately 0.5, FDiv median was nearly 0.85, and FDis did not reach 0.22. Rao was also very low, with a median not reaching 0.05. Of them, FRic was highly correlated with S (Spearman  $R = 0.94$ ,  $p < 0.001$ ), as was FDis with Rao (Spearman  $R = 0.98$ ,  $p < 0.001$ ), and FDiv was moderately correlated both with FDis and Rao (Spearman  $R = 0.46$  and  $R = 0.50$ , respectively;  $p < 0.001$  in both cases) (Figure 8).



**Figure 7.** Boxplots (representing median, interquartile range, and maximum and minimum) showing the main functional diversity indexes for the studied RNRs combined, together with the number of taxa.

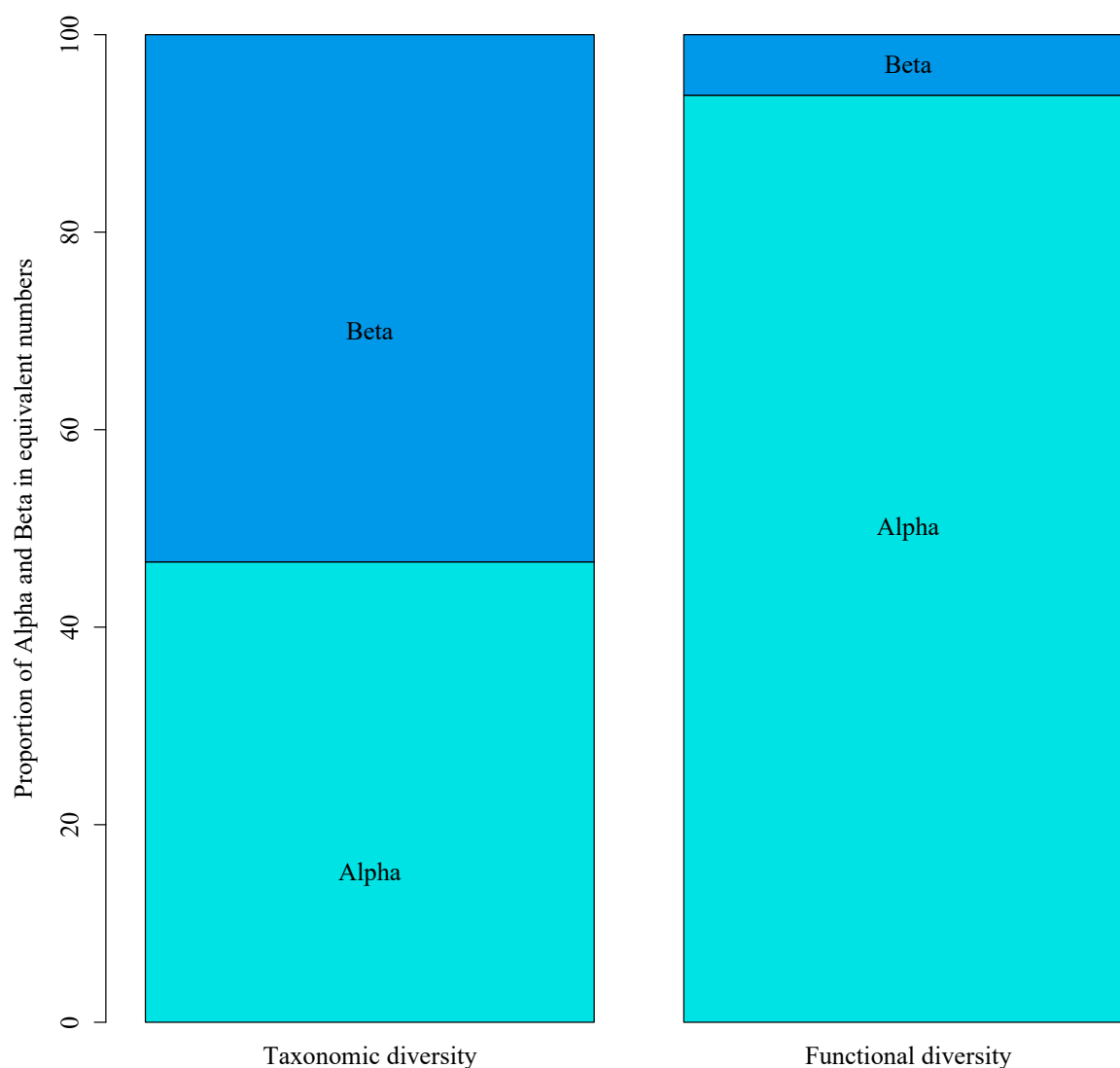


**Figure 8.** Scatterplots showing the relationships between each pair of functional diversity indexes.

#### 4.4. Taxonomic and Functional Diversity Partitioning

Diversity partitioning using Rao for both taxonomic and functional diversity showed a relatively high taxa turnover in the studied RNRs (Figure 9,  $\beta$  diversity in the left bar) but a very low functional turnover (Figure 8,  $\beta$  diversity in the right bar). The hierarchical apportionment of quadratic entropy analysis showed low  $\beta$  diversity (between samples diversity = 0.03), higher  $\alpha$  diversity (within samples diversity = 0.14), and a relatively low  $\gamma$  diversity (total diversity = 0.17).





**Figure 9.** Barplots comparing alpha and beta diversity, both taxonomic and functional.

## 5. Discussion

### 5.1. Taxonomic Diversity

Previous studies analysing taxonomic and functional diversity of particular taxocenoses of aquatic insects (beetles) found that reserve systems in the Iberian Peninsula perform relatively well for taxonomic diversity, though it remains uncertain if these results may apply to other taxa [17]. Our analyses of taxonomic diversity (considering family level) extended these conclusions to the macroinvertebrate biocoenoses, showing an overall high diversity in most of the RNR analysed. Nonetheless, some of the reaches had low or even very low values of S-W and 1-D. These sites were located in six different basins (Cantábrico Occidental, Ebro, Guadalquivir, Júcar, Segura, and Tajo) and belonged to seven different typologies (R-T01, R-T06, R-T08, R-T12, R-T13, R-T24, R-T26). Their location varied from plains and low-mountain reaches to high-mountain ones, and the geological influence ranged from calcareous to siliceous [44]. Despite being part of the RNR network, some anthropogenic impacts were detected in most of their catchments, such as cropping, livestock, water abstraction, an aggregate quarry, hydromorphological alterations, or the presence of garbage stemming from recreational areas. These alterations very likely affected the macroinvertebrate communities of these particular reaches and could explain those low taxonomic diversity values (e.g., [45–47]). Some of the sites with low taxonomic diversity

but without detected impacts corresponded to upper reaches, which could explain their relatively impoverished macroinvertebrate community [48].

Though most communities from RNRs were quite similar, among them, certain differences existed due to four of the seven main abiotic factors analysed by us: temperature, conductivity, discharge, and nitrates (Figure 4). The majority of RNRs had low water temperatures, relatively low quantities of dissolved ions, high discharges, and a very low concentration of nitrates. This corresponded to high-quality environments (a prerequisite to catalogue a stream reach with the RNR conservation category; [23]) in which a broad variety of macroinvertebrates may thrive, including those with restricted tolerance ranges such as most stoneflies (Plecoptera) and many caddisflies (Trichoptera; see Supplementary Data S1). A higher taxonomic diversity can be expected, which is also revealed by our community analysis on the family level, a taxonomic resolution which might homogenise communities and reduce diversity. Despite the gradients depicted by these four ecological factors, the range of variation of them was relatively narrow, with some exceptions corresponding to RNRs with very high conductivity or concentration of nitrates such as, for instance, the Chícamo river RNR in Murcia (southeastern Iberian Peninsula), which had conductivities around 2.500  $\mu\text{S}/\text{cm}$  and a concentration of nitrates over 25 mg/L. These parameters (mainly conductivity) may have important consequences for the survival of particular macroinvertebrate taxa and the entire community [49].

### 5.2. Trait Characterisation of Macroinvertebrate Communities

From the functional point of view, macroinvertebrate communities from RNRs may be considered relatively sensitive to disturbances, as they have a low representation of some trait categories important for resilience towards perturbations. Following the rationale of [50], derived from predictions by [51] regarding the relation between some traits and the resistance/resilience capacity of organisms to cope with disturbances, some trait categories should be more expected in streams such as the Mediterraneans, which suffer recurrent disturbances, both natural and anthropogenic. These disturbances include droughts, floods, wildfires, land-use changes, organic pollution, or biological invasions, among others [52]. Some of these resilience/resistance traits are certain sizes (very small and very large macroinvertebrates), short life-cycle duration, aerial active dispersal, the use of refuges against desiccation, the presence of a dormancy stage, or feeding on algae (scraping) (see Table 1 in [50] for an exhaustive list). In the macroinvertebrate communities from the studied RNRs, resistance forms were relatively scarce, as well as active dispersal, either in the aquatic or in the aerial phase (in the case of insects), and terrestrial clutches or clutches in vegetation were not the most frequent reproductive strategy (Figure 5). Thus, most of the organisms of the community would not have the ability to escape (spatially or temporarily) from their habitat if disturbed. This highlights the increased vulnerability of RNR communities under the current conditions of global change in the Mediterranean area [53–55]. In this scenario, changes of taxonomic and functional composition will very likely occur, with many sensitive taxa (mainly Plecoptera, Trichoptera, and Ephemeroptera) decreasing, tolerant ones and r-strategists (mainly Diptera) increasing, the spreading of alien taxa will be promoted and, consequently, secondary production and foodweb functioning will be modified in those reaches [55–57].

Functional homogeneity was supported by the NMDS analysis using CWM (Figure 6), after which we observed a great aggregation of communities in the two-dimensional space represented and only slight differences between a few communities following a gradient of temperature, conductivity, and nitrates concentration. This supports that under similar conditions, the most frequent trait categories are similar.

### 5.3. Functional Diversity

Functional homogeneity could be the reason why FRic was low on average (Figure 7). This parameter, as used here (see [58] for further discussion of the concept), is an adaptation of the trait range to multiple traits (the convex hull) and represents the minimum volume

(in a space defined by all ranges of the traits) within which we can find all the species in a community [40,59], i.e., the amount of functional space filled by the community [15]. Thus, usually, FRic is positively correlated with the taxonomic richness, as it was in our case (see results and Figure 8). As we mentioned before, these analyses have been carried out at the family level, thus the actual number of taxa at a lower level (genera or species) inhabiting the RNRs is surely higher, and functional richness is probably higher than shown here. Due to the time constraints of public agencies, with samples of a comparatively large spatial scale as in the presented study, subsequent identification of specimens to species level is hardly feasible, especially due to the absence of morphological characteristics in the immature stages of many species, which makes correct and rapid identification even more difficult or even impossible in many cases.

The aforementioned positive correlation between FRic and taxonomic richness would support the insurance hypothesis by [60]. This hypothesis states that more diverse communities ensure proper ecosystem functioning, even in intermittent systems. With a greater number of species and, thus, a greater abundance of traits in the community, it is more likely that traits and functions remain within the community, even if certain species disappear. So, this would be the case in most of the analysed RNRs.

On the other hand, functional richness (measured with FRic) is in some cases inversely related to functional evenness (estimated with FEve; [15]). As mentioned by [40], the bigger the trait range, the lower the chance that species will be unevenly distributed (though these authors pointed out this was counter-intuitive for them). In our case, we did not find a significant correlation (neither negative nor positive) between both indexes, and the FEve index showed an intermediate value. FEve should decrease if abundances differ among taxa and if functional distances among them are not uniform [15], so the macroinvertebrate communities of the studied RNRs showed medium levels of functional evenness. Otherwise, functional divergence (FDiv) was very high, indicating that the trait values of the most abundant taxa were relatively distant from the centre of the trait range, and functional dispersion (FDis), as well as the Rao index, was very low. Both FDis and Rao index are usually highly correlated [61], as corroborated by our results (Figure 8). Both represent how distant taxa are from the CWM in the functional space. Again, these low values indicate similarities among taxa in functional terms. Although the macroinvertebrate communities were functionally similar in the studied RNRs (low FDis and Rao), the most abundant taxa in them seem to differ in some traits from the rest of the community (high FDiv), even if the intermediate values of FEve suggest moderate importance. In this sense, we found a low correlation between FDiv and FEve (Figure 8), while simulations with artificial data sets previously supported the independence of these two parameters [15].

#### 5.4. Taxonomic and Functional Diversity Partitioning

In our studied systems, there was high taxa turnover, but functional turnover was very low (Figure 9), i.e., most of the trait dissimilarity between taxa was found within a community, but not among communities, though there were relatively strong dissimilarities in community composition. This reflects that each community was composed of taxa that, all together, contributed to the functioning of the ecosystem from different roles, covering a wide spectrum of features (even if we are working at the family level) and that this was common to most of the communities analysed in this study. Thus, functional complementarity likely enhances ecosystem functioning, and maintaining a high taxonomic and functional diversity probably increases the efficiency of natural processes in most of the studied RNRs. As stated by [62], under the complementarity effect concept, trait variation forms the basis for a permanent association of species that enhances collective performance.

## 6. Conclusions

Our results support the fact that most RNRs are protecting communities of great diversity, not only taxonomic (shown by the overall high values of the indexes calculated), but also functional, which contributes to the proper functioning of the ecosystems found

in these stream reaches. Nonetheless, for some animal groups (e.g., beetles), other studies show that particular protected areas seem to poorly represent their functional diversity at the regional scale, supporting the need to use an integrative approach, combining taxonomic and functional analyses, in aquatic ecosystems [17]. Trait-based approaches, together with taxonomic approaches, may be used to predict ecosystem functions and services at a given point in time [8], and recently even to predict the abundance of species considering a certain environmental scenario [63]. Thus, the approach used in this study, i.e., the incorporation of the functional analysis of the communities to the standard data obtained during the regular monitoring of these reaches, may provide a broader vision of the state of these environments and can be used in a complementary way in future planning, monitoring, and management strategies for lotic aquatic environments in general, and for these RNRs in particular. Furthermore, the establishment of new RNRs would enhance the functioning of several other stream ecosystems, as well as the services they provide, through the conservation of lotic taxonomic and functional diversity in general, and of macroinvertebrate communities in particular. The analysis of the functional diversity of the communities, as in the present approach, should be implemented to identify and prioritise protection of reaches with higher functional diversity, where enhanced ecosystem functioning can be expected.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/w16223290/s1>.

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