water

MDPI

Article

Exploring Macroinvertebrates Ecological Preferences and Trait-Based Indicators of Suspended Fine Sediment Effects in the Tsitsa River and Its Tributaries, Eastern Cape, South Africa

Phindiwe Ntloko *, Carolyn G. Palmer, Frank C. Akamagwuna and Oghenekaro N. Odume *

Unilever Centre for Environmental Water Quality, Institute for Water Research, Rhodes University, P.O. Box 94, Makhanda 6140, South Africa; tally.palmer@ru.ac.za (C.G.P.); akamagwunafrank@yahoo.com (F.C.A.)
* Correspondence: ntloko80@gmail.com (P.N.); n.odume@ru.ac.za (O.N.O.); Tel.: +27-83-479-0921 (P.N.)

Abstract: The taxonomy-based response pattern of macroinvertebrates to sediment stress is well established, with tolerant taxa increasing in impacted conditions, while sensitive taxa decrease along a deteriorating water quality gradient. However, the distribution patterns of traits in response to environmental stress gradient, including suspended sediments, remain unclear, particularly in Africa, where trait-based studies are under-explored. We examined the distribution patterns of macroinvertebrate traits along a suspended sediment stress gradient and identified tolerant and sensitive traits for suspended sediment stress. We sampled macroinvertebrates and environmental variables seasonally in winter, spring, summer and autumn of 2016 to 2018 in eight selected sites in the Tsitsa River and its tributaries. We selected 12 traits and ecological preferences, resolved them into 47 trait attributes, and analysed them using the RLQ and fourth-corner analyses. Our results revealed that macroinvertebrate traits and ecological preferences were differentially influenced by fine suspended sediments in the Tsitsa River and its tributaries. Traits such as a preference for CPOM, collector-filtering, and a high sensitivity to oxygen depletion, were deemed sensitive to suspended sediments stress, exhibiting positive associations with the control sites, and negatively associated with any of the environmental parameters (sediment grain sizes, turbidity, TSS and EC). Tolerant indicator traits included a high tolerance of oxygen depletion, skating and a preference for FPOM. The fourth-corner analysis results indicated that suspended fine sediment grain sizes, (including coarse sand, fine silt and clay) were the most important variables influencing macroinvertebrate trait distribution patterns during the dry season, while gravel, mud and medium sand were more important during the wet season. Overall, our study provided critical insights towards trait-based responses of macroinvertebrates communities to suspended sediment stress, key information that could stimulate the development of macroinvertebrate trait-based biomonitoring tools for the assessment of suspended sediment stress in the Afrotropical region.

Keywords: biomonitoring; freshwater; pollution; RLQ; sediments

Citation: Ntloko, P.; Palmer, C.G.; Akamagwuna, F.C.; Odume, O.N. Exploring Macroinvertebrates Ecological Preferences and Trait-Based Indicators of Suspended Fine Sediment Effects in the Tsitsa River and Its Tributaries, Eastern Cape, South Africa. *Water* **2021**, *13*, 798. https://doi.org/10.3390/w13060798

Academic Editor: Christophe Piscart

Received: 16 January 2021
Accepted: 6 March 2021
Published: 15 March 2021

1. Introduction

Sedimentation is among the most common freshwater ecosystem stressors impacting on macroinvertebrate communities [1–3]. Fine sediments' impacts on aquatic biota are wide-ranging and can be profound because their effects can be complex and are mediated by a range of factors including exposure duration, particle size distribution, sediments load, sources, geomorphological setting, and the vulnerability of resident biota [4–6]. Sediment effects on macroinvertebrates can be direct, e.g., clogging of fragile and exposed gills and filter-feeding structures, burial, abrasion; or indirect effects, e.g., alteration of the physical and chemical condition of streams such as reduction of dissolved oxygen and increases in turbidity [1,7–9].

Macroinvertebrates assemblages have been reported to change in response to human-induced stressors in river and stream ecosystems. Globally, the taxonomy-based approaches to analysing the effects of water quality impacts on macroinvertebrates are widely used [10–12]. The taxonomic approach compares macroinvertebrate communities across a stress gradient, and the degree of impact is inferred by assessing the deviation of the assemblages at the impacted sites from those at the control or reference sites [5,13].

Metrics, tools, and methods developed based on the taxonomy-based approaches have found routine application in many countries such as European WFD [14], South Africa, e.g., the South African Scoring System version 5 [15], the Biological Monitoring Working Party System in the United Kingdom [16], Hilsenhoff's Biotic Index (HBI) in the United States of America [17], and the Australian River Assessment System, AUSRIVAS in Australia [18,19].

The taxonomy-based approach is useful because apart from its use in inferring water quality impact, it provides important biological information needed for biodiversity conservation and protection. Such important information about biodiversity may include the occurrence and distribution of rare, endangered, dominant, keystone, or vulnerable species, which may be impacted by stressors [10,20]. Further, because of the widespread application of the taxonomy-based approach, key water quality indicators have been established. For example, the richness and diversity of macroinvertebrate taxa such as Ephemeroptera, Plecoptera, and Trichoptera (EPT) have been known to decrease in response to water quality stressors like organic and acid mine drainage pollution as well as sedimentation [21,22]. On the other hand, the compositions and abundances of taxa such as chironomids, and many other dipterans are usually reported to increase in relation to stressors such as organic pollution and sedimentation. Similar to the taxonomic indicators, it is asked in this paper whether trait-based indicators can also be identified for monitoring effects of sedimentations in impacted river and stream ecosystems. The approach followed in this paper offers an opportunity to identify indicator traits based on the specific environmental stressor.

The Tsitsa River and its tributaries are subject to elevated fine sediments inputs (i.e., animal grazing and crop production) from the surrounding landscape. The river is situated in the rural part of the Eastern Cape Province of South Africa, where duplex, dispersive and easily erodible soils have caused the influx of fine sediments into the Tsitsa river, impacting both structure and function of biological communities [21]. In an earlier study, elevated fine sediments have been found to impact on EPT community structures in the Tsitsa River and its tributaries [5]. Because these rivers are situated in rural catchments, water quality is relatively good, but elevated sediments remain a critical challenge, particularly during the wet seasons. Grazing as a form of agriculture contributes to fine sediments delivery into the streams. Given that these rivers are mainly impacted by elevated fine sediments, they provide an opportunity for exploring macroinvertebrate traits responses and identifying trait-based indicators of suspended fine sediments effects, without the confounding effects from other water quality stressors such as urban pollution. Identifying trait-based indicators of elevated suspended fine sediments effects is useful because traits mediate organism–environmental interaction, potentially providing mechanistic insights for predicting assemblage response to a given environmental filter [23,24]. Environmental filters such as fine sediments favour particular suits of traits [1,5,7].

The trait-based approach (TBA) is informed by the habitat templet theory [25,26], which is based on an autecology, which predicts that a correspondence is expected between prevailing habitat conditions and traits [27]. The TBA has been used to explore the impact of fine sediment stress on macroinvertebrates, e.g., [1,8,21,28]. Further, it has been argued that unlike the taxonomy-based indicators, trait-based indicators can link macroinvertebrate response to ecosystem function, e.g., material fluxes are directly related to feeding behaviour and body sizes [29–32]. However, only a few studies, e.g., [21,33,34] have explored the TBA in Africa, especially in sediment-impacted rivers, so it remains unclear how trait-based studies from other regions apply to highly-sedimented rivers in Afrotropical region.

In the Afrotropical region where taxonomic expertise is sparse, identifying useful trait-based indicators of suspended fine sediments stress can help contribute to and accelerate the science and practice of freshwater biomonitoring without the necessity of species identification as not all traits are constrained by taxonomy, for example, body shape, body size, and many ecological preferences of macroinvertebrates at family level [35]. Thus, in the present study, we examined the effects of suspended fine sediments on macroinvertebrate communities by means of multivariate RLQ and fourth corner analysis. Eight sites that represent an increasing fine sediments loads were selected for this study. The objectives of this paper, therefore are to (i) explore the distribution of macroinvertebrate ecological preferences and biological traits in relation to elevated suspended fine sediments and (ii) identify trait-based indicators potentially useful for monitoring sedimentation effects.

2. Materials and Methods

2.1. Study Area and Selected Sampling Sites

This study was conducted in the Tsitsa River catchment in five streams, which include the Tsitsa, Qurana, Pot, Little Pot Rivers, and the Millstream (Figure 1). The Tsitsa River catchment is situated in the Eastern Cape Province of South Africa, with a catchment area of about 4924 km^2. The Tsitsa River and its tributaries form part of the broader Mzimvubu River catchment. The Tsitsa rises in the Drakensberg 15 km to the south-east of Rhodes, a small town close to Maclear and about 80 km west of Mount Frere, and flows eastwards. The Tsitsa catchment is subject to widespread gully erosion, having more than 10,000 gullies [36]. Gullies, together with the duplex and dispersive soils, are the contributing factors of fine sediments influx into the river system.

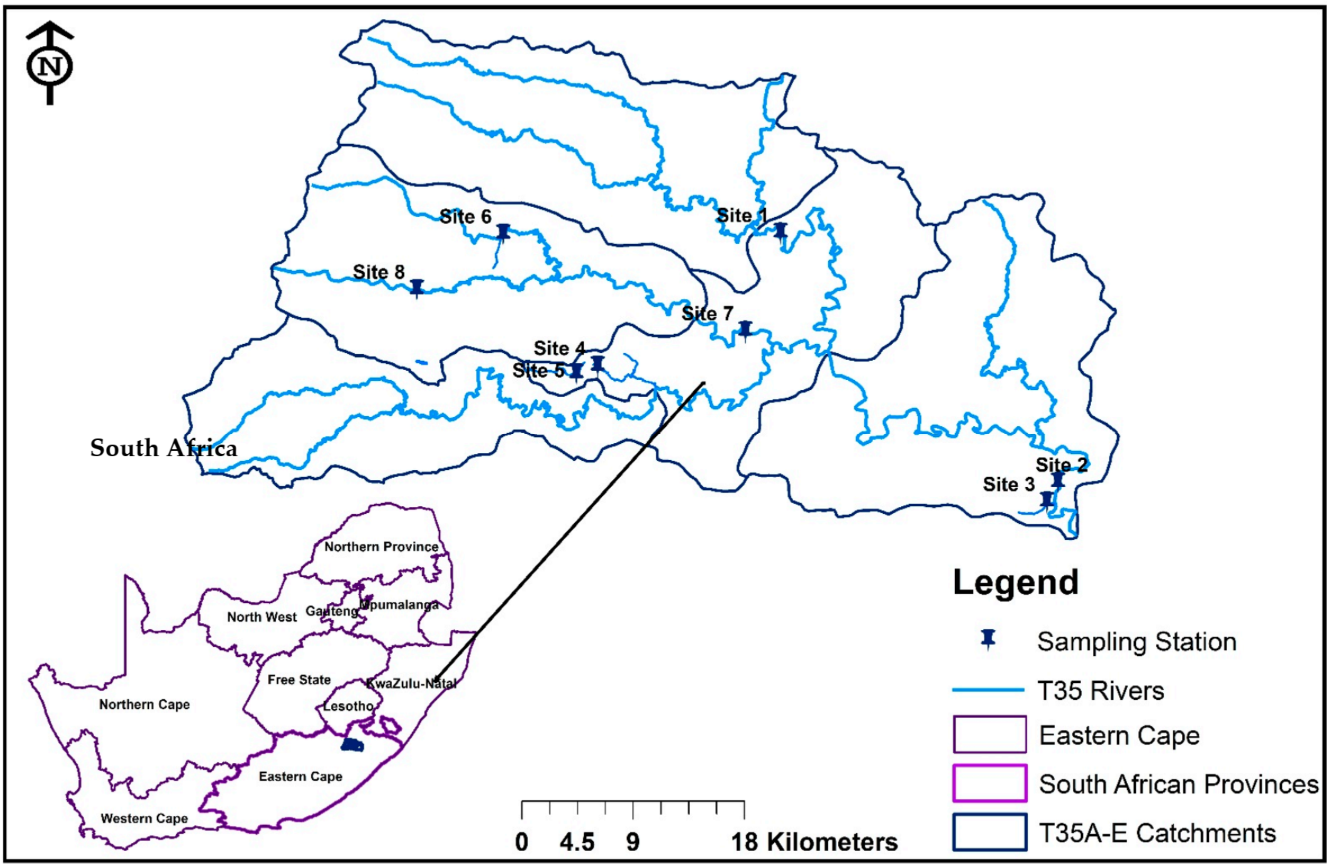

Figure 1. Map of the study area showing the locations of the eight selected sampling sites in the Tsitsa River and its tributaries. The study area location in South Africa is shaded in dark blue in the Eastern Cape Province on the map of South Africa.

The Tsitsa River joins the Mzimvubu River after a flow length of approximately 200 km northwest to southeast at Port St. Johns, where it finally empties into the Indian ocean. The Tsitsa River serves as an ecological asset in providing basic ecosystem services such as water and aquatic resources that are beneficial to rural and peri-urban communities on the catchment. Additionally, the river is one of the few remaining rivers in South Africa in their near-natural state. The river is a source of water supply for subsistence agricultural activities such as livestock farming, and rural settlements that rely on it for drinking and cooking.

The study was conducted seasonally at eight selected sampling sites over a period of two years, beginning in late winter (August 2016), spring (October 2016), summer (December 2016), autumn (March 2017), winter (July 2017), spring (September 2017), summer (December 2017) and ending in autumn (March 2018). The selected sites included two sites in the Tsitsa River (Sites 1 and 2), one site in the Qurana River (Site 3), two sites in the Millstream (Sites 4 and 5), two sites in the Pot River (Sites 6 and 7) and one site in the Little Pot River, i.e., Site 8 (Figure 1). The sites were selected to indicate a gradient of sediment impact based on turbidity, total dissolved solids (TSS) and land use practices (privately owned, well-maintained catchment versus communally owned, poorly maintained landscape). The site classification was based on the extent of erosion, land-use practices and a previous study [5]

Other factors that were considered when selecting the sites were the availability of macroinvertebrate biotopes: stones, vegetation and gravel, sand and mud. Sites 1, 2 and 3) were considered as the highly sedimented sites. The Sites in the Millstream (i.e., Site 4 and Site 5) were considered as moderately sedimented. Sites 6, 7 (i.e., Pot upstream and Pot downstream) and Site 6 (i.e., Little Pot) were situated in the well-maintained area of the catchment and were collectively referred to as the control sites (CLS).

2.2. Sampling of Selected Water Quality Variables and Fine Sediment Grain Sizes

Physico-chemical variables were measured seasonally at all the sampling sites. For each sampling event, the selected physico-chemical variables measured on-site include dissolved oxygen (DO), electrical conductivity (EC), temperature and pH using a multi-parameter probe; model H198, Hanna instrument. Turbidity was measured on site using the portable turbidity Orbeco-Helliage 966 Metre. Water samples were collected and transported to the laboratory for analysis of total suspended solids (TSS). TSS was measured through the filtration and oven-dried method according to the protocol described by [37].

Water samples for sediments grain size characterisation were collected using the disturbance technique [38]. To sample suspended sediments, an open-ended, cylindrical polyethylene bucket (height 75 cm; diameter 48.5 cm) was carefully inserted into the water column. The water column within the cylindrically shaped container was then agitated using a wooden pole of about 15 cm long. The agitation of the water column was done so that the stream bed is not disturbed, in order not to collect settled fine sediments. While the water was still vigorously in motion, suspended fine sediments samples were collected and then through filtered 2000 µm pore size sieve into 250 mL acid washed sampling bottles. Filtration remove particles larger than 2000 µm such as debris. Filtered samples were then transported to the laboratory and refrigerated until analysed. Suspended fine sediments grain sizes were characterised using the Mastersizer 3000 laser diffraction particle size analyser as fully described in [5]. The fine sediment grain size output from the Mastersizer 3000 was further analysed for grain size classes using the GRADISTAT version 8.0 [38]. Suspended fine sediments were separated into gravel, mud, sand, silt and clay. Sand was composed of very coarse sand (>1000–2000 µm), coarse sand (<2000–1000 µm), medium sand (<1000–500 µm), fine sand (<500–250 µm) and very fine sand (<250–125 µm), silt in µm was composed of very coarse silt (<125–63 µm), coarse silt (<63–31 µm), medium silt (<31–16 µm), fine silt (<16–8 µm), very fine silt (<8–4 µm) and clay (<4 µm).

2.3. Macroinvertebrates Sampling

Concurrent with physico-chemical sampling, macroinvertebrates were collected using a 30 × 30 cm, 1000 µm mesh net in accordance with the South African Scoring System version 5 (SASS5) protocol [15]. The SASS5 protocol is a standardised kick sampling technique that requires the collection of macroinvertebrates from three distinct biotopes: stones (in-current and out-of-current), sediments (gravel, sand and mud; GSM), and aquatic and marginal vegetation. The samples were pooled and analysed as composite samples per site, per sampling event. Three replicate samples were collected per site per sampling event over the study period. Samples were preserved in jars containing 75% ethanol and transported to the laboratory for sorting and identification [39]. In the laboratory, the samples were identified to family levels according to the identification guide by [40].

2.4. Selected Macroinvertebrates Traits and Ecological Preferences

A total of 12 traits and ecological preferences were selected for the study and further resolved into 47 attributes (Table 1). The selection of trait and ecological preferences was informed by the literature and mechanistic link between fine sediment modes of stress and the particular traits. Overall, the selection of traits and ecological preferences were informed by (i) mechanistic relationships between the trait and fine sediment modes of stress, (ii) availability of trait and ecological preference data, and (iii) ease of measurement and observation. For example, respiration is selected for analysis because fine sediments have been hypothesised to clog respiratory trait attributes such as gills [11]. Feeding was also selected because, like respiration, organisms which feed by filtering particulate organic matter have been shown to have their feeding apparatus clogged by elevated fine sediments. Accumulation of fine sediments has also been shown to alter food quality, cover stable surfaces on which many macroinvertebrates feed [38]. Concerning velocity preference, for example, Odume et al [6] and Jones et al [7] [argued that velocity mediates fine sediment impact on macroinvertebrates because, at a higher velocity, the frictional force between organisms' body surfaces and the moving fine sediments is likely to be aggravated causing increased abrasion of soft and exposed body surfaces. Information on trait and ecological preferences was retrieved from the newly compiled trait database for South African macroinvertebrates at family level [24,41] and supplemented by other sources [5,42,43]

Table 1. Selected macroinvertebrates ecological preferences and traits, as well as their respective attributes. Abbreviations; FPOM (fine particulate organic matter), CPOM (coarse particulate organic matter) and GSM (gravel, sand, and mud) (Table was adapted from [8]).

Traits and Ecological Preferences	Code
Maximum body size (mm)	
Very small (≤5)	A1
Small (>5 to 10)	A2
Medium (>10 to 20)	A3
Large (>20 to 40)	A4
Very large (>40)	A5
Respiration	
Gills	B1
Tegument	B2
Aerial; spiracles	B3
Aerial vegetation: breathing tube, straps/other apparatuses, e.g., elytra	B4
Aerial: lung	B5
Mobility	
Climbing	C1
Crawling	C2
Sprawling	C3
Swimming	C4
Skating	C5
Burrowing	C6

Table 1. *Cont.*

Traits and Ecological Preferences	Code
Body shape	
Streamlined	D1
Flattened	D2
Spherical	D3
Cylindrical	D4
Preferred food	
FPOM (fine particulate organic matter)	E1
CPOM (coarse particulate organic matter)	E2
Feeding habit	
Shredding	F1
Collector-gathering	F2
Collector-filtering	F3
Scraping (grazing, brushing)	F4
Predation	F5
Preferred biotope	
Sediment (gravel, sand, and mud)	G1
Stones	G2
Vegetation	G3
Attachment mechanism	
Free-living	HI
Temporarily attached	H2
Permanently attached	H3
Respiratory type	
Aerial	I1
Aquatic	I2
Sensitivity/tolerance to dissolved oxygen depletion	
Highly sensitive	J1
Moderately sensitive	J2
Tolerant	J3
Highly tolerant	J4
Body protection	
Exposed and soft	K1
Cased/tubed	K2
Exposed but sclerotised	K3
Completely sclerotised	K4
Velocity preference (m/s)	
Very fast-flowing (>0.6)	L1
Moderately flowing (0.3–0.6)	L2
Slow flowing (0.1–3)	L3
Very slow-flowing (<0.1)	L4

2.5. Data Analysis

Exploring the Distribution Pattern of Ecological Preferences and Traits and Identifying Trait-Based Indicators of Elevated Suspended Fine Sediments

A two-way multivariate analysis of variance (MANOVA) was employed as a statistical test method to test for differences in suspended sediment grain sizes and physico-chemical variables between the sites and seasons. MANOVA simultaneously used multiple independent variables to compare the sites in terms of the sediment particle size distribution and the physico-chemical variables. While using MANOVA, the basic assumptions of normality and homogeneity of variance need to be examined using the Shapiro–Wilk test and the Levene's test, respectively. If assumptions are not met, then data were transformed logarithmically, but normalized if assumptions were still not met. One-way ANOVA followed by a Tukey's post hoc test was undertaken to indicate where the significant differences lay.

The macroinvertebrate data were consisted in two matrices, a taxon-site matrix (L) and a trait-taxon matrix (Q), with the trait data fuzzy-coded and abundance data log (x + 1) transformed. Fuzzy coding (Supplementary Data, Table S1) was used to describe the association of each taxon to each trait attribute. Affinity scores ranging from 0 to 5 were used, considering potential functional variation between species within a family and also in between life stages within a taxon [33]. Each taxon per trait attribute was assigned a score of 0 indicating no affinity, 5 high affinity to a trait attribute [33]. The fuzzy coding was particularly useful because working at the family level, it allows for potential plasticity, variability, and functional diversity that exist within a given family. The approach has been widely used in other studies [42,44,45].

RLQ analysis for each season was used to relate fine sediment grain sizes, TSS, and turbidity, DO, EC (R), macroinvertebrates taxa (L), and the traits and ecological preferences (Q) along a gradient of increasing sedimentation. RLQ was developed by Doledec [5] for three-dimensional data analysis: environmental data, taxa and trait data. In RLQ ordination, the first ordination (correspondence analysis, CA) is performed on the taxa data set L-table, second ordination (principal component analysis, PCA) on the environmental data sets, in this case, the sediment grain sizes, turbidity, EC, TSS and DO, R-table, which links the taxa data set to the physico-chemical variable data set by using the sample scores result of the CA as row weights. A third ordination was performed to links the L data set to the trait data set by using the taxon score results of the CA as row weights. A final ordination (combined RLQ) analysis was performed that simultaneously conducts ordination on the three separate ordinations (CA, PCA, and HS) by searching for a linear combination of traits-taxon scores in the traits-taxon scores in Q-HS ordination and physico-chemical variables sample scores in R-PCA ordination, by maximising the covariance between Q and R through L ordination. The significance of the RLQ analysis was tested using the Monte Carlo permutation test with 999 permutations at *alpha* = 0.05. The RLQ ordination thus allows the spatial visualisation of the distribution of traits and ecological preferences in relation to the sediment grain sizes and physico-chemical variables. RLQ were undertaken using the ADE-4 statistical package for R version 3.4.1 in R-programming environment [46].

To identify potential trait-based indicators of suspended fine sediment stress, ecological preferences and trait attribute associated with sites in the Tsitsa River and Qurana River were designated as tolerant traits, whereas trait attributes associated with the control sites were designated as potential fine sediment sensitive traits. The Fourth-corner analysis [46] was then further conducted to confirm designated trait-based indicators of suspended fine sediment effects. The Fourth-corner analysis is a multivariate permutational test that searches for significant association between traits and environmental variables. In this study, it was used to test the association between suspended sediment grain sizes, turbidity, TSS, and DO with the selected traits/ecological preferences. A trait was confirmed to be a fine sediment tolerant if it was positively associated with highly impacted sites and positively correlated with at least one environmental variable (TSS, EC, turbidity) or any of the suspended grain sizes associated with the highly sedimented sites. A trait was confirmed as a sensitive indicator trait if the correlation was significantly negative with TSS, turbidity, or any of the suspended grain sizes associated with the highly sedimented sites [47].

3. Results

3.1. Physico-Chemical Variables and Grain Size Distribution

The means, standard deviations, and ranges of the basic physico-chemical variables including dissolved oxygen (DO), total suspended solids (TSS), Electrical conductivity (EC) and turbidity, recorded in the Tsitsa River and its tributaries for the current study are presented in Table 2 and only Turbidity and TSS were statically significantly different between sites. Sites (i.e., TSU, TSD, and QHR) were mainly dominated by very fine sand, very fine silt, medium silt, very coarse silt, and clay (Table 2). Whereas, the two sites situated in the Millstream (i.e., MLU and MLD) were primarily dominated by clay, constituting more than 60% of the volumetric grain sizes at the two sites. Grain sizes within the control sites were evenly distributed, with the dominant grain size (i.e., coarse silt) constituting only 15% of the overall difference in (Table 2). Overall differences in grain sizes were statistically significant ($p < 0.05$) across the sites, but not across the two seasons as shown in (Table 3). The interactions between the sites and seasons in terms of the suspended sediment grain sizes were statistically not significant ($p > 0.05$). The one-way ANOVA results indicated that very fine sand, very coarse silt, medium silt, and fine silt were significantly higher at TSU, TSD, QHR compared with the MLD, MLU, and CLS. The rest of the grain sizes did not differ statistically between sites.

Table 2. Means, ± standard deviations, and ranges (in parentheses) for the volumetric distribution of suspended fine sediment grain sizes and physico-chemical variables across sites in the Tsitsa River and its tributaries. Different superscript letters for very fine sand, very coarse silt, medium silt, fine silt, and total suspended solids across sites that indicate significant differences ($p < 0.05$) revealed by Tukey HSD post-hoc test. The same superscript letter between sites per variable indicates no significant differences ($p > 0.05$). The sediment grain sizes are reported a volumetric fraction of 1.

Suspended Sediment Grain Size (μm) Measured as a Fraction of 1 and Physico-Chemical Variable	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	*p*-Value
Coarse sand	0.15 ± 0.19 (0.02–0.49)	0.13 ± 0.18 (0.01–0.47)	0.02 ± 0.05 (0.04–0.14)	0.13 ± 0.17 (0.003–0.38)	0.01 ± 0.002 (0.004–0.04)	0.004 ± 0.001 (0.002–0.005)	
Medium sand	0.07 ± 0.09 (0.03–0.15)	0.06 ± 0.08 (0.01–0.18)	0.17± 0.033 (0.067– 0.19)	0.05 ± 0.07 (0.003–0.15)	0.02 ± 0.04 (0.003– 0.13)	0.01 ± 0.002 (0.003–0.07)	
Fine sand	0.13 ± 0.19 (0.03–0.39)	0.14 ± 0.10 (0.07–0.37)	0.02 ± 0.008 (0.073–018)	0.13 ± 0.03 (0.07–0.15)	0.14 ± 0.07 (0.09–0.36)	0.09 ± 0.03 (0.069–0.13)	
Very fine sand	0.77 ± 0.19 (0.44–0.96) ^{ab}	0.41 ± 0.23 (0.14–0.67) ^{a}	0.06 ± 0.02 (0.08–0.57) ^{a}	0.09 ± 0.30 (0.23–0.92) ^{a}	0.26 ± 0.15 (0.44–0.96) ^{ad}	0.25 ± 0.21 (0.39–0.86) ^{ac}	0.038
Very coarse silt	0.06 ± 0.04 (0.04–0.11) ^{b}	0.16 ± 0.09 (0.01–0.26) ^{a}	0.14 ± 0.23 (0.067–0.19) ^{a}	0.06 ± 0.11 (0.08–0.36) ^{bd}	0.07 ± 0.06 (0.17– 0.37) ^{ad}	0.03 ± 0.07 (0.17–0.33) ^{a}	0.032
Coarse silt	0.05 ± 0.11 (0.08–0.9)	0.08 ± 0.05 (0.001–0.17)	0.08 ± 0.06 (0.070–018)	0.10 ± 0.04 (0.03–0.14)	0.11 ± 0.023 (0.067– 0.14)	0.15 ± 0.11 (0.068–0.29)	
Medium silt	0.02 ± 0.01 (0.01– 0.04) ^{ac}	0.02 ± 0.04 (0.03–0.08) ^{a}	0.4 ± 0.08 (0.04–0.15) ^{a}	0.18 ± 0.19 (0.04–0.51) ^{ac}	0.04 ± 0.009 (0.01– 0.056) ^{ac}	0.06 ± 0.04 (0.034–0.11) ^{b}	0.027
Fine silt	0.27 ± 0.15 (0.48–0.59) ^{a}	0.04 ± 0.06 (0.02– 0.10) ^{a}	0.2 ± 0.11 (0.09–0.36) ^{bc}	0.02 ± 0.03 (0.01–0.08) ^{bc}	0.02 ± 0.04 (0.01– 0.22) ^{b}	0.02 ± 0.016 (0.01–0.04) ^{bc}	0.046
Very fine Silt	0.13 ± 0.03 (0.07–0.15) ^{b}	0.23 ± 0.26 (0.01–0.69) ^{bd}	0.69± 0.26 (0.25–0.99) ^{b}	0.15 ± 0.20 (0.01–0.47) ^{b}	0.04 ± 0.11 (0.005–0.38) ^{b}	0.002 ± 0.006 (0.007–0.017) ^{bc}	0.043
Clay	0.15 ± 0.20 (0.01–0.47) ^{ab}	0.18 ± 0.17 (0.15–0.25) ^{a}	0.01 ± 0.10 (0.04–0.47) ^{a}	0.26 ± 0.23 (0.15–0.31) ^{bd}	0.28 ± 0.24 (0.25–0.59) ^{a}	0.10 ± 0.21 (0.18–0.17) ^{a}	0.004
Turbidity (NTU)	4.10 ± 8.04 (2.40 -10.03) ^{a}	20.04 ± 20.11 (1.38–8.05) ^{ab}	17.8 ± 6.0 (5.8–24.0) ^{ab}	0.8 ± 0.24 (0.25–0.99) ^{b}	0.4 ± 1.0 0.0–2.9 ^{ab}	0.1 ± 0.2 (0.0–0.6) ^{ab}	0.001
DO (mg/L)	8.8 ± 6.9 (3.03–11.09)	9.0 ± 18.0 (4.6–21.0)	6.7 ± 5.8 (2.7–10.0)	6.4 ± 4.6 (2.5 -14.2)	6.8 ± 5.2 (8.7–15.0)	6.9 ± 5.8 (4.3–17)	
TSS (mg/L)	10883 ± 11220 (1333–34567) ^{ab}	2095 ± 2353 (198.3–9268) ^{b}	9120 ± 10656 (2310–34567) ^{b}	5404 ± 9883 (198–34618) ^{b}	5727 ± 8286 (231.0–19604) ^{b}	2265 ± 3456 (1988–16946) ^{ad}	0.011
EC (mS/m)	66.3 ± 20.4 (43.0–93.0) ^{a}	108.8 ± 64.2 (38.0–246.0) ^{ac}	88.9 ± 41.8 (49–175) ^{ab}	64.9 ± 35.3 (39.0–146.0) ^{a}	66.3 ± 21.8 (35.0–105.5) ^{a}	23.1 ± 14.2 (39.0–74.0) ^{bc}	0.003

Table 3. Multivariate analysis of variance MANOVA results for suspended sediment grain size distribution between sites and seasons, indicating significant difference ($p < 0.05$) between sites during the study period (August 2016–March 2018).

Effect	Test	Value	F–Value	Effect df	Error df	*p*-Value
Intercept	Wilks	0.016936	126.9761	16	3.0000	0.000000
Sites	Wilks	0.185105	1.6679	48	104.8927	0.015544
Season	Wilks	0.261618	1.2447	48	104.8927	0.176893
Sites*season	Wilks	0.028669	1.1528	144	292.5042	0.156192

3.2. *Spatial Distribution of Traits and Ecological Preferences*

The results of the RLQ analysis during the dry season revealed that the first two axes explained 99.1% cumulative variance of the dataset. The first axis accounted for 81.94% and the second axis 17.16% of the total variance. The ordination plot revealed that medium silt, coarse sand, and medium sand were clustered together and were positively associated with QHR, MLU, MLD, and CLS. During the dry season, except on few occasions, the control site (Site 6–8) and the moderately sedimented sites, i.e., 4 and 5 (MLU and MLD) were closely clustered together and were mainly influenced by increasing DO and clay. The traits that were mainly associated with the control sites were a preference for climbing, aerial respiration, temporary attachment and skating. These traits were associated with taxa such as Dyticidae, Oligonuridae, Baetidae, Syphidae and Muscidae (Figure 2). The highly sedimented Sites 1 (TSU) and 2 (TSD) and Site 3, Qurana River (QHR) were clustered together. Ceratopogonidae, Baetidae, Caenidae, Leptophlabidae and Helodidae taxa were found to be associated with these sites. Traits associated with these taxa include a preference for very fast-flowing waters, predation and scraping. These sites clustering was mainly influenced by increasing fine sand, very fine silts and TSS (Figure 2). For the wet season, the first two axes of the RLQ explained 92.87% cumulative variance. The first axis accounted for 81.87% variance, and the second axis 11% total variance. The ordination plot revealed that MLU, QHR), TSD, and TSU) were clustered together and showed a positive association with fine sand, coarse silt, fine silt, turbidity, and TSS. Collector-filterers, shredders, CPOM, and a preference for slow-flowing waters were the traits attributes associated with these sites (Figure 2). These traits were associated with taxa such as Oligonuridae, Syphidae, Dyticidae and Muscidae.

3.3. *Identifying Trait-Based Indicators of Fine Sediment Stress*

The fourth-corner analysis was used to test the significance of individual trait-environment association, and to further explore the significant association between the individual trait attribute/ecological preference and suspended sediments grain sizes, turbidity, EC, DO and TSS, (Figure 3). During the dry season, only coarse sand, fine silt and clay correlated significantly with macroinvertebrate traits and ecological preferences (Figure 3). A positive correlation was detected between fine silt, and large (>20 to 40 mm) and very large (>40 mm) body sizes, possession of lungs and spherical body shape, whereas clay was negatively correlated with crawling, a high tolerance of DO depletion and all attributes of velocity preference (m/s), except slow-flowing (0.1–3 m/s). Coarse sand indicated significant positive correlations with gills, crawling, CPOM, scraping, a preference for stone biotope, aerial, and all attributes of sensitivity/tolerance to dissolved oxygen depletion and velocity preference, except very fast-flowing (>0.6 m/s) (Table 4).

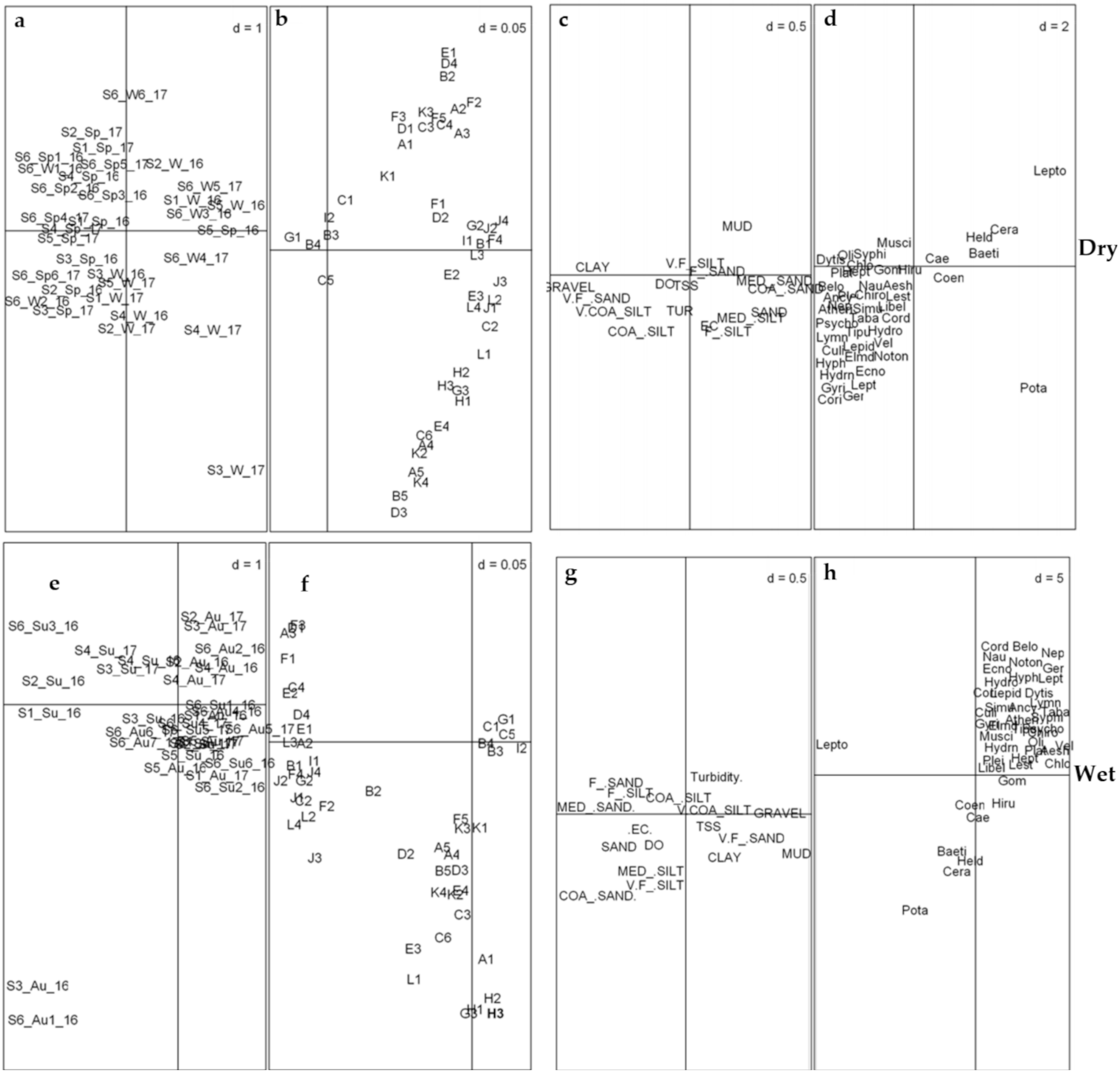

Figure 2. RLQ plot showing the site clustering (**a**,**e**) during the sampling seasons based on environmental variables (**c**,**g**), trait (**b**,**f**) and macroinvertebrates (**d**,**h**) during the dry and wet seasons over the study period (August 2016–March 2018) in the Tsitsa River and its tributaries. Abbreviations: traits are as in Table 1, for traits and ecological preferences, physico-chemical variables and suspended grain sizes in Table 2; taxa: Oli: Oligonuridae, Baeti: Baetidae, Coen: Coenogranidae, Gom: Gomphidae, Lepto: Leptophlabidae, Held: Helodidae, Dyti: Dytiscidae, Pota: Potamonautidae, Cera: Ceratopogonidae, Syph: Syrphidae and Caen: Caenidae sites: S6_W4_17; Control site_winter_year 2017, Control site_winter_year 2017, S2_W_16;Tsitsa downstream_winter_year 2016, S4_W_16; Millstream upstream_winter_year 2016, S5_W_17; Millstream downstream_winter_year 2017 and S3_W_16; Qurhana River_winter_year 2016, S3_Au_17: Qurhana River_autumn_year 2017; S1_Su_16: Tsitsa upstream_summer_year 2016 and S2_Su_16: Tsitsa downstream_summer_year 2016.

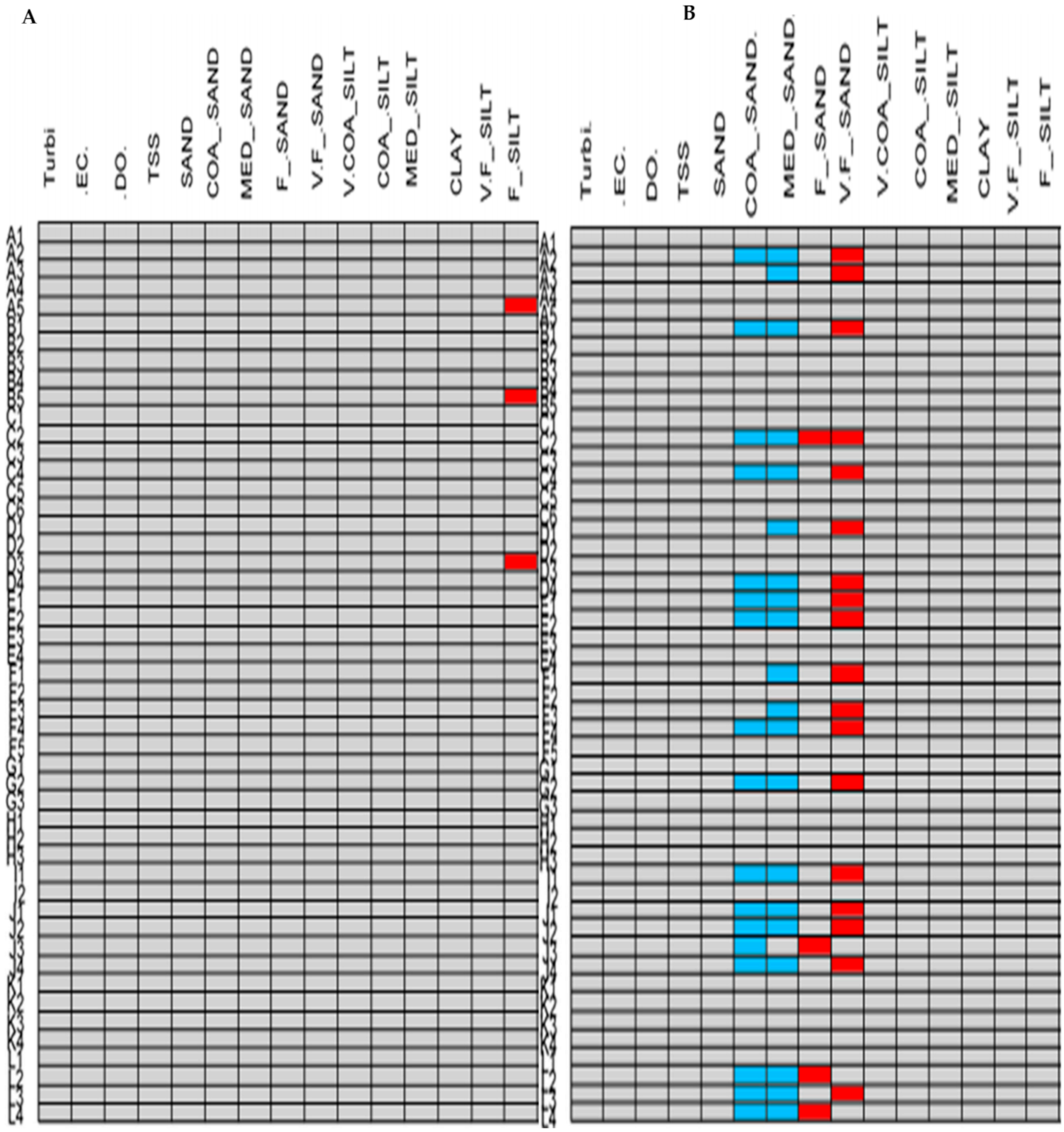

Figure 3. Results of the fourth corner analysis showing the correlations between macroinvertebrates traits/ecological preferences, and the fine suspended sediments grain sizes as well as selected physico-chemical variables in the Tsitsa River and its tributaries during the dry season (**A**) and wet season (**B**). Red indicates a significant positive correlation ($p \leq 0.05$) and blue indicates significant negative correlation ($p \leq 0.05$); grain sizes and physico-chemical variables: Turb: turbidity, EC: electrical conductivity, TSS: total suspended solids, F_SAND: fine sand, COA_SAND: coarse sand, MED_SAND: medium sand, VF_SAND: very fine sand, V.COA_SILT: very coarse silt, COA_SILT: coarse silt, MED_SILT: medium silt, F_SILT: fine silt and VF_SILT: very fine silt.

Table 4. Fourth-corner statistics after 4999 permutations showing correlation coefficient and level of probability of statistical significance for correlation between traits and environmental variables during the dry and wet seasons. * ($p \leq 0.05$). Only significant correlations are shown.

Traits	DO	TSS	SAND	GRAVEL	MUD	COA_ SAND	MED_ SAND	F_ SAND	VF_ SAND	VC_ SILT	COA_ SILT	MED_ SILT	F_ SILT	VF_ SILT	CLAY
Dry															
A4													0.042 *		
B1						0.034 *							0.040 *		
B5															−0.045 *
C2						0.041 *							0.039 *		
D3															
E2						0.049 *									
E3						0.045 *									
F4						0.035 *									
G2						0.042 *									
I1						0.034 *									
J1						0.031 *									
J2						0.040 *									
J3						0.029 *									
J4						0.044 *							0.040 *		
L1															−0.049 *
L2						0.039 *									
L3						0.043 *									
L4						0.035 *									−0.048 *
Wet															
A2				−0.035 *	−0.032 *							0.034 *			
A3					−0.023 *		0.022 *								
B1				−0.027 *	−0.024 *		0.035 *								
C2				−0.041 *	−0.040 *	0.037 *	0.046 *								
C4				−0.037 *	−0.017 *		0.020 *								
D1							0.024 *					0.020 *			
D4				−0.037 *	−0.023 *		0.026 *								
E1				−0.036 *	−0.031 *		0.038 *								
E2				−0.041 *	−0.020 *		0.015 *								
F1					−0.018 *		0.019 *								
F3					−0.023 *		0.027 *								
F4				−0.024 *	−0.030 *		0.040 *								
G2				−0.044 *	−0.034 *		0.040 *								
I1				−0.027 *	−0.024 *		0.035 *								
J1				−0.027 *	−0.035 *	0.05 *	0.044 *								
J2				−0.023 *	−0.025 *	0.074	0.033 *								
J3				−0.044 *		0.0436 *									
J4				−0.028 *	−0.024 *	0.079	0.029 *								
L2				−0.039 *	0.052	0.032 *									
L3				−0.020 *	−0.017 *		0.019 *								
L4				−0.034 *	−0.052	0.034 *									

During the wet season, a total of 36 traits attributes and ecological preferences were significantly correlated with suspended fine sediments grain sizes. Out of the 36 traits/ecological preferences that were significantly correlated, 18 traits attributes and ecological preferences such as small body size, medium body size, gills, crawling, swimming, a preference for FPOM, CPOM as food sources, collector-filtering, scraping, a preference for the stone biotope, high sensitivity to DO depletion, a moderate sensitivity to DO depletion were negatively significantly correlated with gravel and mud. Conversely, 18 traits attributes and ecological preferences were significantly positively correlated with coarse sand and medium sand. These traits include small, medium body sizes, gills, crawling, swimming, streamlined body shape, cylindrical body, a preference for FPOM, CPOM, predation, permanently attached, and aquatic respiration. Of the traits that negatively correlated with coarse sand and fine sand, CPOM, collector-filterer, high sensitivity to DO depletion were associated with the control sites. These traits were therefore deemed sensitive traits to elevated suspended fine sediments (Table 4). Of the traits that showed a positive correlation with medium sand and very fine sand, a high tolerance to DO depletion, skating, and a preference for FPOM were associated with the highly sedimented sites. These traits were thus deemed tolerant traits (Table 4).

4. Discussion

In this study, we explored the influence of elevated sediments on the distribution patterns of macroinvertebrate traits and ecological preferences in selected streams in the

Tsitsa River Catchment, Eastern Cape Province of South Africa. Our results showed that elevated suspended sediments differentially affected macroinvertebrate traits and ecological preferences, consistent with other freshwater studies, e.g., [8,47–49], marine [29] and wetlands ecosystems [29] who have demonstrated significant effects of fine sediments on macroinvertebrate traits. Traits such as collector-filterer, CPOM, and a high sensitivity to DO depletion were positively associated with the control sites; they were identified as potentially sensitive traits of suspended fine sediments. These traits and preferences exhibited significant positive correlations with any of TSS, EC, turbidity and suspended sediments grain sizes (Figure 3). Conversely, a high tolerance to DO depletion, skating and a preference for FPOM were associated with the highly sedimented sites and were deemed tolerant indicator traits of suspended fine sediments.

Macroinvertebrate feeding activities and preferences for food are commonly reported to be affected by elevated fine sediment loads as they are linked to nutritional quality or impaired access to food resources [8,50]. In the present study, CPOM was associated with less sedimented sites, indicating that elevated sediments may have affected macroinvertebrate that feeds on detritus, CPOM, through the reduction in food quality. When suspended sediments are deposited on stream bottom or substrates, including CPOM; the fine sediments can cover the food items, thereby reducing the quality/palatability and access to food for macroinvertebrates shredders, feeding on CPOM [50]. For example, [51] who investigated the effects of fine sedimentation on CPOM availability and shredder abundance in Alpine streams in the Pellice River, Italy found elevated fine sediments to significantly reduced the amount of (CPOM), affecting the abundance of invertebrate shredders. Similarly, [8] found invertebrate shredders to be the only feeding groups that demonstrated reduced abundance with increased fine sediments in two lowland UK streams. Further, the palatability of CPOM for shredders depend on the initial actions of microbes on leaf litter [52]. However, suspended sediments can inhibit the rate of microbial breakdown of litter, through reduced light energy and temperature [52], thereby affecting the nutritional quality of CPOM for macroinvertebrate shredders. With regards to collector-filters, it is likely that suspended sediment in water column negatively affected filterers through clogging of respiratory and feeding apparatus, and thus their predominant association with the less sedimented sites. The sensitivity of CPOM and collector-filterers are consistent with other studies [52,53]. Rabeni et al. [50] studied changes in functional feeding groups associated with sedimentation and found a greater proportion of gatherers and a lower proportion of filterers and scrapers in four Missouri USA streams. Buendia et al. [54] who assessed the ecological effects of fine sediment in Isábena River, NE Iberian Peninsula Spain also found filterers to be the most sensitive macroinvertebrate feeding groups.

DO plays a critical role in the distribution of macroinvertebrates, particularly with regards to those species that have high sensitivities to DO depletion. Taxa sensitive to DO depletion were associated with the control sites, whereas those tolerant of DO depletion were associated with the sediment-impacted sites, providing support for the importance of DO in structuring stream communities [55]. Sediment delivery from catchment areas that are rich in organic materials is likely to stimulate microbial activities that can cause DO depletion. Moreover, increased sediment loads can also impact on the vertical distribution of oxygen, thereby influencing the depth to which organisms may burrow [7]. Thus, taxa (e.g., the EPTs) that have high sensitivity to DO, can be affected severely, and tolerant taxa of Chironomidae and Oligochaeta are favoured, and thus, the distribution pattern of sensitivity/tolerance to dissolved oxygen depletion observed in this study. Further, infiltration and deposition of fine sediment into the riverbed has been reported to modify macroinvertebrate community structure and functioning [53]. Taxa with low dissolved oxygen requirements frequently dominate substrates characterised by a high proportion of fine sediment (see [50], with absence of taxa vulnerable to fine sediment through damage of gills [47,56].

Macroinvertebrate filter-feeding structures are usually prone to clogging, particularly when levels of suspended sediments are elevated [7]. Collector-filterers that filter suspended FPOM from water column have been considered the most intolerant macroinvertebrate functional feeding group, [56,57]. Although fine sediments can lead to a reduction in macroinvertebrates that feed on FPOM [50,53], they can also serve as an important source of organic food particles [58]. In the present study, macroinvertebrate preferring FPOM proved tolerant of suspended sediments; thus, it is likely that suspended sediments increased the food availability for macroinvertebrates feeding on FPOM, thereby increasing their occurrence in the highly sedimented sites [58]. However, traits are unlikely to respond to environmental stress in isolation, but rather a combination of traits will determine the response of an individual species to a stressor [59]. Since collector-filters that feed on FPOM were earlier identified as sensitive indicators of suspended sediments in this study, it is possible that the observed response of FPOM was mediated by the interactive and correlative effects of other traits [59,60]. The mediating effects of traits on other traits to stressors have been observed in other studies, e.g., [61–63]. Nevertheless, previous studies have found macroinvertebrates feeding on FPOM to increase along suspended fine sediment gradient [64,65].

Concerning the skaters, their tolerance to suspended sediments was expected as they are active surface swimmers that live mainly on the water surface and thus can escape the effects of suspended sediments in the water [24]. Most actively swimming taxa are able to escape from danger and seek refuge [51]. Thus, the significant association of skaters with the highly sedimented sites suggests that skaters can actively move out of highly sedimented areas and return when conditions are normalised, and thus their tolerance in this study. The tolerance of actively mobile taxa such skaters observed in the Tsitsa River have been demonstrated in other studies investigating the effects of fine sediments on macroinvertebrate traits [21,28]. For example, Buendia et al. [54] who investigated the ecological effects of sediments on macroinvertebrates structure and function in the Isábena River, Spain observed active swimmers to be the most tolerant locomotion trait, increasing with increased sedimentation. The study attributed the positive correlation of swimmers with fine sediment to the ability of these actively swimming animals to move out of the most impaired areas. Overall, the results were in line with previous studies of sedimentation effects in sediment-impacted river catchments, with macroinvertebrates responding differentially to sediment stress. Thus, the identification of indicator traits of fine sediments is an important step towards the development of trait-based tool specific for monitoring sediment effects for Afrotropical river systems.

5. Conclusions

The results of this study showed that macroinvertebrates traits seem to be affected differentially by suspended fine sediments. Traits such as collector-filters, aquatic respiration, predators, a preference for very fast-flowing waters, a preference for low DO, skating and a preference for FPOM were associated with the highly sedimented sites. These sites were mainly influenced by increasing turbidity, TSS and sediments grain sizes such as fine sand and very fine silt. Traits such as a preference for low DO, skating and a preference for FPOM were regarded as tolerant traits, which can potentially increase resilience on taxa possessing them. On the other hand, traits such as CPOM, collector-filterers a high sensitivity to DO were deemed sensitive traits, and taxa possessing these traits are likely to be vulnerable to elevated suspended sediments. Overall, the present study provided information that could facilitate the development of trait-based biomonitoring tools for assessing the effects of elevated suspended sediments on riverine ecosystems, particularly in Africa, where trait-based studies remain scarce.

Supplementary Materials: Supplementary Materials are available online at https://www.mdpi.com/2073-4441/13/6/798/s1.

Author Contributions: Conceptualisation, O.N.O.; data collection, P.N. and F.C.A.; data analysis, P.N. and F.C.A.; methodology, P.N., O.N.O. and C.G.P.; funding acquisition, O.N.O.; writing of manuscript, P.N., O.N.O., F.C.A. and C.G.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Water Research Commission of South Africa under project No. K1/7157. The Rhodes University Council is also acknowledged for partial funding for this work.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data is contained within the article or Supplementary Material.

Acknowledgments: Water Research Commission of South Africa is acknowledged for funding this project under project No. K1/7157. The Rhodes University Council is also acknowledged for partial funding for this work.

Conflicts of Interest: The authors declared no conflict of interest.

References

1. Larsen, S.; Pace, G.; Ormerod, S.J. Experimental effects of sediment deposition on the structure and function of macroinvertebrate assemblages in temperate streams. *River Res. Appl.* **2011**, *27*, 257–267. [CrossRef]
2. Doretto, A.; Piano, E.; Fenoglio, S.; Bona, F.; Crosa, G.; Espa, P.; Quadroni, S. Beta-diversity and stressor specific index reveal patterns of macroinvertebrate community response to sediment flushing. *Ecol. Indic.* **2021**, *122*, 107256. [CrossRef]
3. Hubler, S.; Huff, D.D.; Edwards, P.; Pan, Y. The biological sediment tolerance index: Assessing fine sediment conditions in Oregon streams using macroinvertebrates. *Ecol. Indic.* **2016**, *67*, 132–145. [CrossRef]
4. Kaller, M.D.; Hartman, K.J. Evidence of a threshold level of fine sediment accumulation for altering benthic ma-croinvertebrate communities. *Hydrobiologia* **2004**, *518*, 95–104. [CrossRef]
5. Akamagwuna, F.C. Taxonomic and Trait-Based Responses of the Orders Ephemeroptera, Plecoptera, Odonata, and Tri-choptera (EPOT) to Sediment Stress in the Tsitsa River and Its Tributaries, Eastern Cape, South Africa. Master's Thesis, Rhodes University, Grahamstown, South Africa, 2018.
6. Odume, O.N.; Ntloko, P.; Akamagwuna, F.C.; Dallas, H.M.; Barber James, H. *A Trait Database for South African Macroin-Verte-Brates. Grant Report Submitted to Water Research Commission of South Africa*; WRC Report No. K1/7157; 2018; Unpublished Work.
7. Jones, J.I.; Murphy, J.F.; Collins, A.L.; Sear, D.A.; Naden, P.S.; Armitage, P.D. The Impact of Fine Sediment on Mac-ro-Invertebrates. *River Res. Appl.* **2017**, *28*, 1055–1071. [CrossRef]
8. Mathers, K.L.; Rice, S.P.; Wood, P.J. Temporal effects of enhanced fine sediment loading on macroinvertebrate community structure and functional traits. *Sci. Total Environ.* **2017**, *599–600*, 513–522. [CrossRef] [PubMed]
9. McKenzie, M.; Mathers, K.L.; Wood, P.J.; England, J.; Foster, I.; Lawler, D.; Wilkes, M. Potential physical effects of suspended fine sediment on lotic macroinvertebrates. *Hydrobiologia* **2019**. [CrossRef]
10. Gieswein, A.; Hering, D.; Lorenz, A.W. Development and validation of a macroinvertebrate-based biomonitoring tool to assess fine sediment impact in small mountain streams. *Sci. Total. Environ.* **2019**, *652*, 1290–1301. [CrossRef]
11. Naden, P.S.; Murphy, J.F.; Old, G.H.; Newman, J.; Scarlett, P.; Harman, M.; Jones, J.I. Understanding the controls on deposited fine sediment in the streams of agricultural catchments. *Sci. Total Environ.* **2016**, *547*, 366–381. [CrossRef]
12. Odume, O.; Muller, W.; Arimoro, F.; Palmer, C. The impact of water quality deterioration on macroinvertebrate communities in the Swartkops River, South Africa: A multimetric approach. *Afr. J. Aquat. Sci.* **2012**, *37*, 191–200. [CrossRef]
13. Doretto, A.; Piano, E.; Bona, F.; Fenoglio, S. How to assess the impact of fine sediments on the ma-croinvertebrate communities of alpine streams? A selection of the best metrics. *Ecol. Indic.* **2018**, *84*, 60–69. [CrossRef]
14. Filipe, A.F.; Feio, M.J.; Garcia-Raventós, A.; Ramião, J.P.; Pace, G.; Martins, F.M.; Magalhães, M.F. The European Water Framework Directive facing current challenges: Recommendations for a more efficient biological assessment of inland surface waters. *Inland Waters* **2018**, *9*, 95–103. [CrossRef]
15. Dickens, C.W.; Graham, P.M. The South African Scoring System (SASS) Version 5 Rapid Bioassessment Method for Rivers. *Afr. J. Aquat. Sci.* **2002**, *27*, 1–10. [CrossRef]
16. Biological Monitoring Working Party. *Final Report: Assessment and Presentation of the Biological Quality of Rivers in Great Britain*; Unpublished Report; Dept. of Envir., Water Data Unit, December 1978; p. 37.
17. Hilsenhoff, W.L. Rapid Field Assessment of Organic Pollution with a Family-Level Biotic Index. *J. N. Am. Benthol. Soc.* **1988**, *7*, 65–68. [CrossRef]
18. Jorgenson, D.W.; Mun, S.H.; Kevin, J.S. *Information Technology and the American Growth Resurgence*; The MIT Press: Cambridge, MA, USA, 2005.

19. Turak, E.; Waddell, N. *Australia-Wide Assessment of River Health: New South Wales AusRivAS Sampling and Pro-Cessing Manual. Monitoring River Heath Initiative Technical Report Number 13*; Commonwealth of Australia and New South Wales Environment Protection Authority: Canberra, Australia; Sydney, Australia, 2004.
20. IUCN 2015 IUCN Red List of Threatened Species. Version 2015.2. IUCN 2014. IUCN Red List of Threatened Species. Available online: http://www.iucnredlist.org (accessed on 6 March 2021).
21. Akamagwuna, F.C.; Mensah, P.K.; Nnadozie, C.F.; Odume, O.N. Trait-based responses of Ephemeroptera, Plecoptera, and Trichoptera to sediment stress in the Tsitsa River and its tributaries, Eastern Cape, South Africa. *River Res. Appl.* **2019**, *35*, 999–1012. [CrossRef]
22. Musonge, P.S.; Boets, P.; Lock, K.; Goethals, P.L. Drivers of Benthic Macroinvertebrate Assemblages in Equatorial Al-pine Rivers of the Rwenzoris (Uganda). *Water* **2020**, *12*, 1668. [CrossRef]
23. Statzner, B.; Bis, B.; Dole´dec, S.; Usseglio-Polatera, P. Perspectives for biomonitoring at large spatial scales: A unified meas-ure for the functional composition of invertebrate communities in European running waters. *Basic Appl. Ecol.* **2001**, *2*, 73–85. [CrossRef]
24. Odume, O.N.; Ntloko, P.; Akamagwuna, F.C.; Dallas, H.M.; Barber-James, H. *A Trait Database for South African Macroinvertebrates*; Unpublished WRC Report; Pretoria, South Africa, 2018.
25. Southwood, T.R.E. Habitat, the Templet for Ecological Strategies? *J. Anim. Ecol.* **1977**, *46*, 336. [CrossRef]
26. Townsend, C.R.; Hildrew, A.G. Species traits in relation to a habitat templet for river systems. *Freshwat. Biol.* **1994**, *31*, 265–275. [CrossRef]
27. Ter Braak, C.J.F.; Peres-Neto, P.R.; Dray, S. Simple parametric tests for trait–environment association. *J. Veg. Sci.* **2018**, *29*, 801–811. [CrossRef]
28. Herrera, L.S.; Brady, V.J.; Axler, K.; Peterson, C. Identifying the Impacts of Excess Fine Sediment on Benthic Macroinvertebrate Communities. 2016. Available online: https://conservancy.umn.edu/bitstream/hdle/11299/181796/Herrera_umn_0130M_16931.pdf?sequence=1&isAllowed=y (accessed on 6 March 2021).
29. Liu, G.; Verberk, J.Q.J.C.; Van Dijk, J.C. Bacteriology of drinking water distribution systems: An integral and multidimensional review. *Appl. Microbiol. Biotechnol.* **2013**, *97*, 9265–9276. [CrossRef]
30. Menezes, S.; Baird, D.J.; Soares, A.M.V.M. Beyond taxonomy: A review of macroinvertebrate trait-based community descriptors as tools for freshwater biomonitoring. *J. Appl. Ecol.* **2010**, *47*, 711–719. [CrossRef]
31. Dolédec, S.; Phillips, N.; Scarsbrook, M.; Riley, R.H.; Townsend, C.R. Comparison of structural and functional approach-es to determining land use effects on grassland stream invertebrate communities. *Basic Appl. Ecol.* **2006**, *25*, 44–60. [CrossRef]
32. Bolam, S.G.; Garcia, C.; Eggleton, J.; Kenny, A.J.; Buhl-Mortensen, L.; Gonzalez-Mirelis, G.; Rijnsdorp, A.D. Differ-ences in biological traits composition of benthic assemblages between unimpacted habitats. *Mar. Environ. Res.* **2017**, *126*, 1–13. [CrossRef]
33. Odume, O.N. Searching for urban pollution signature and sensitive macroinvertebrate traits and ecological preferences in a river in the Eastern Cape of South Africa. *Ecol. Indic.* **2020**, *108*, 105759. [CrossRef]
34. Edegbene, O.; Arimoro, O.; Odume, O. Exploring the distribution patterns of macroinvertebrate signature traits and ecological preferences and their responses to urban and agricultural pollution in selected rivers in the Niger Delta ecore-gion, Nigeria. *Aquat. Ecol.* **2020**, *9*, 1–18. [CrossRef]
35. Ochieng, H.; Okot-Okumu, J.; Odong, R. Taxonomic challenges associated with identification guides of benthic macroinvertebrates for biomonitoring freshwater bodies in East Africa: A review. *Afr. J. Aquat. Sci.* **2019**, *44*, 113–126. [CrossRef]
36. Parwada, C.; Van Tol, J. Soil properties influencing erodibility of soils in the Ntabelanga area, Eastern Cape Province, South Africa. *Acta Agric. Scand. Sect. B Plant Soil Sci.* **2016**, *67*, 67–76. [CrossRef]
37. Xie, Y.; Dang, X.; Zhou, Y.; Hou, Z.; Li, X.; Jiang, H.; Zhou, D.; Wang, J.; Hai, C.; Zhou, R. Using sediment grain size characteristics to assess effectiveness of mechanical sand barriers in reducing erosion. *Sci. Rep.* **2020**, *10*, 1–14. [CrossRef]
38. Blott, S.J.; Pye, K. Gradistat: A grain size distribution and statistics package for the analysis of uncon- solidated sed-iments. *Earth Surface Process. Landf.* **2012**, *26*, 1237–1248. [CrossRef]
39. Upton, M.S. *Methods for Collecting, Preserving, and Studying Insects and Allied Forms*, 4th ed.; The Australian Entomological Society: Brisbane, Australia, 1991; 86p.
40. Gerber, G.A.; Gabriel, M.J.M. *Aquatic Invertebrates of South African Rivers Field Guide*; Resource Quality Services, Department of Water Affairs and Forestry: Pretoria, South Africa, 2002.
41. Duerdoth, C.; Arnold, A.; Murphy, J.; Naden, P.; Scarlett, P.; Collins, A.; Sear, D.; Jones, J. Assessment of a rapid method for quantitative reach-scale estimates of deposited fine sediment in rivers. *Geomorphology* **2015**, *230*, 37–50. [CrossRef]
42. Archaimbault, V.; Usseglio-Polatera, P.; Garric, J.; Wasson, J.-G.; Babut, M. Assessing pollution of toxic sediment in streams using bio-ecological traits of benthic macroinvertebrates. *Freshw. Biol.* **2009**, *55*, 1430–1446. [CrossRef]
43. White, J.C.; Hill, M.J.; Bickerton, M.A.; Wood, P.J. Macroinvertebrate taxonomic and functional trait compositions within lotic habitats affected by river restoration practices. *Environ. Manag.* **2017**, *60*, 513–525. [CrossRef] [PubMed]
44. Charvet, S.; Statzner, B.; Usseglio-Polatera, P.; Dumont, B. Traits of benthic macroinvertebrates in semi-natural French streams: An initial application to biomonitoring in Europe. *Freshw. Biol.* **2000**, *43*, 277–296. [CrossRef]
45. Da Conceição, A.A.; Albertoni, E.F.; Milesi, S.V.; Hepp, L.U. Influence of Anthropic Impacts on the Functional Structure of Aquatic Invertebrates in Subtropical Wetlands. *Wetlands* **2020**, *40*, 2287–2296. [CrossRef]
46. R Core Team. *R: A Language and Environment for Statistical Computing*; R Statistical Computing Environment: Vienna, Austria, 2017; R Foundation for Statistical Computing; ISBN 3-900051-07-0.

47. Murphy, J.F.; Jones, J.I.; Arnold, A.; Duerdoth, C.P.; Pretty, J.L.; Naden, P.S.; Sear, D.A.; Collins, A.L. Can ma-croinvertebrate biological traits indicate fine-grained sediment conditions in streams? *River Res. Appl.* **2017**, *33*, 1606–1617. [CrossRef]
48. Bona, F.; Doretto, A.; Falasco, E.; La Morgia, V.; Piano, E.; Ajassa, R.; Fenoglio, S. Increased Sediment Loads in Alpine Streams: An Integrated Field Study. *River Res. Appl.* **2016**, *32*, 1316–1326. [CrossRef]
49. Meng, X.; Chen, J.; Li, Z.; Liu, Z.; Jiang, X.; Ge, Y.; Cooper, K.M.; Xie, Z. Degraded functional structure of macroinvertebrates caused by commercial sand dredging practices in a fl ood plain lake. *Environ. Pollut.* **2020**, *263*, 114415. [CrossRef] [PubMed]
50. Rabení, C.F.; Doisy, K.E.; Zweig, L.D. Stream invertebrate community functional responses to deposited sediment. *Aquat. Sci.* **2005**, *6705*, 395–402. [CrossRef]
51. Doretto, A.; Bona, F.; Falasco, E.; Piano, E.; Tizzani, P.; Fenoglio, S. Fine sedimentation affects CPOM availability and shredder abundance in Alpine streams. *J. Freshw. Ecol.* **2015**, *31*, 299–302. [CrossRef]
52. Bo, T.; Cammarata, M.; López-Rodríguez, M.J.; de Figueroa, J.M.T.; Baltieri, M.; Varese, P.; Fenoglio, S. The influence of water quality and macroinvertebrate colonization on the breakdown process of native and exotic leaf types in sub-alpine stream. *J. Freshw. Ecol.* **2014**, *29*, 159–169. [CrossRef]
53. Descloux, S.; Datry, T.; Usseglio-Polatera, P. Trait-based structure of invertebrates along a gradient of sediment colmation: Benthos versus hyporheos responses. *Sci. Total. Environ.* **2014**, *466-467*, 265–276. [CrossRef]
54. Buendia, C.; Gibbins, C.N.; Vericat, D.; Batalla, R.J.; Douglas, A. Detecting the structural and functional impacts of fine sediment on stream invertebrates. *Ecol. Indic.* **2013**, *25*, 184–196. [CrossRef]
55. Calapez, A.; Serra, S.; Santos, J.; Branco, P.; Ferreira, T.; Hein, T.; Brito, A.; Feio, M. The effect of hypoxia and flow decrease in macroinvertebrate functional responses: A trait-based approach to multiple-stressors in mesocosms. *Sci. Total. Environ.* **2018**, *637–638*, 647–656. [CrossRef] [PubMed]
56. Wood, P.J. Biological Effects of Fine Sediment in the Lotic Environment. *Environ. Manag.* **1997**, *21*, 203–217. [CrossRef] [PubMed]
57. Henley, W.F.; Patterson, M.A.; Neves, R.J.; Lemly, A.D. Effects of Sedimentation and Turbidity on Lotic Food Webs: A Concise Review for Natural Resource Managers. *Rev. Fish. Sci.* **2000**, *8*, 125–139. [CrossRef]
58. Feio, M.J.; Dolédec, S. Integration of invertebrate traits into predictive models for indirect assessment of stream functional integrity: A case study in Portugal. *Ecol. Indic.* **2012**, *15*, 236–247. [CrossRef]
59. Poff, N.L.; Olden, J.D.; Vieira NK, M.; Finn, D.S.; Simmons, M.P.; Kondratieff, B.C. Great insights into traits-based approaches in aq insects including which traits are more evolutionarily labile (and less correlated with each other) and an entire trait matrix for N. Functional trait niches of North American lotic insects: Traits-bas. *Bentholo. Soc.* **2006**, 35–93. [CrossRef]
60. Murphy, J.F.; Jones, J.I.; Pretty, J.L.; Duerdoth, C.P.; Hawczak, A.; Arnold, A.; Blackburn, J.H.; Naden, P.S.; Old, G.H.; Sear, D.A.; et al. Development of a biotic index using stream macroinvertebrates to assess stress from deposited fine sediment. *Freshw. Biol.* **2015**, *60*, 2019–2036. [CrossRef]
61. Kneitel, J.M.; Chase, J.M. Trade-offs in community ecology: Linking spatial scales and species coexistence. *Ecol. Lett.* **2004**, *7*, 69–80. [CrossRef]
62. Pilière, A.F.H.; Verberk, W.C.E.P.; Gräwe, M.; Breure, A.M.; Dyer, S.D.; Posthuma, L.; De Zwart, D.; Huijbregts, M.A.J.; Schipper, A.M. On the importance of trait interrelationships for understanding environmental responses of stream macroinvertebrates. *Freshw. Biol.* **2015**, *61*, 181–194. [CrossRef]
63. Usseglio-polatera, P.; Bournaud, M.; Richoux, P.; Tachet, H. Biomonitoring through biological traits of benthic ma-croinvertebrates: How to use species trait databases? *Hydrobiologia* **2000**, *423*, 153–162. [CrossRef]
64. Arruda, J.A.; Marzolf, G.R.; Faulk, R.T. The Role of Suspended Sediments in the Nutrition of Zooplankton in Turbid Reservoirs. *Ecology* **1983**, *64*, 1225–1235. [CrossRef]
65. Hart, R. Experimental studies of food and suspended sediment effects on growth and reproduction of six planktonic cladocerans. *J. Plankton Res.* **1992**, *14*, 1425–1448. [CrossRef]