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Environmental characteristics shape macroinvertebrate community structure across spatiotemporal scales in a subtropical African river system

Linton F. Munyai[1]✉, Busisiwe P. Gumede[1], Farai Dondofema[2] & Tatenda Dalu[1]

Understanding the impact of human activities and environmental drivers on macroinvertebrate communities is critical to adequately manage river ecosystems under multiple stressors. In this study, we assessed macroinvertebrate community structure in relation to water and sediment chemistry. Samples (i.e., water, sediment and macroinvertebrates) were collected from 16 sites along the subtropical Luvuvhu River (South Africa) mainstem and its tributaries across two seasons (i.e., cool–dry (June), hot–wet (November)). The analysed data was assessed using multivariate analyses and diversity matrices. Significant differences were observed across seasons and river sections for most water (i.e., pH, temperature, resistivity, ammonium, phosphates) and sediment (i.e., potassium, sodium, copper, zinc, boron, sediment organic carbon) variables. Macroinvertebrates exhibited high diversity during hot–wet season compared to the cool–dry season, with a six distinct macroinvertebrates families (i.e., Odonata, Diptera, Coleoptera, Hemiptera, Trichoptera, Ephemeroptera) having a high taxon abundances. Based on CCA analysis, seasons were positively associated with CCA axis 2, and were characterised by high Mg, Na, pH, sediment organic carbon, ammonium and phosphates, with all highlighted variables having a significant effect on macroinvertebrate community composition. The results obtained from this study highlighted that water and sediment chemistry had significant associations with changes in macroinvertebrate communities and composition. Therefore, understanding the relationship between water and sediment chemistry, and macroinvertebrates diversity matrices in rivers that are impacted by human activities is essential for comprehending the integrity of river ecosystem and for providing guidance to conservation managers. This knowledge will assist on how to effectively manage and safeguard these systems against further deterioration from anthropogenic activities.

Keywords Macroinvertebrate community structure, Sediment, Water, Multivariate analysis, Diversity matrices

Macroinvertebrate communities are widely used to assess the ecological integrity of river systems across spatial and temporal scales[1,2]. They are reliable bio–indicators and play a vital role in supporting the overall aquatic ecosystem health, with their life cycles being lengthy enough to respond to changes brought on by anthropogenic activities and perturbations[3]. According to Ogidi et al.[4], bioindicators are species or a community of organisms that represent an environment's abiotic and biotic status and demonstrate how environmental change affects the habitat, community, and/or ecosystem. To effectively evaluate water quality and formulate strategies for reducing pollution in streams and rivers, it is essential to obtain comprehensive data on the status of the aquatic ecosystem, particularly its biodiversity[5]. As anthropogenic activities such as water abstraction, sewage discharge, waste disposal and urban run–off continue to exert pressure on these fragile river ecosystems, it has become imperative to assess and monitor the impacts of these activities on aquatic biodiversity[6]. Several studies [e.g.,

[1]Aquatic Systems Research Group, School of Biology and Environmental Sciences, University of Mpumalanga, Nelspruit 1200, South Africa. [2]Department of Geography and Environmental Science, University of Venda, Thohoyandou 0950, South Africa. ✉email: munyailinton@gmail.com

7–9] have indicated that the ecological status of many rivers is being threatened by anthropogenic activities such as urbanisation, deforestation, poor agricultural practices, pollution, overexploitation and water abstraction.

Macroinvertebrates are one of the most widely used organisms compared to other groups of organisms (i.e., fish, macrophytes) when assessing the environmental quality of river systems[10–13]. They serve as a food source for fish and other aquatic organisms, contribute to nutrient cycling, and are involved in various ecological processes[14,15]. Macroinvertebrate monitoring can help determine population dynamics and river changes, revealing information about the ecosystem's general health and functioning and thereby aiding in enabling focused biodiversity conservation efforts and assessing environmental change[16,17]. Macroinvertebrates can be considered as (i) pollution–sensitive (e.g., Leptophlebiidae, Siphlonuridae, Arctopsychidae and Perlidae) that need good water quality to survive, and may require clear or non–turbid waters and/or high dissolved oxygen levels[18], and (ii) pollution–tolerant taxa (e.g., Chironomidae, Lymnaeidae, Planorbidae and Glossiphoniidae) which can survive in poor water quality, with unique adaptations that allows them to survive in turbid and nutrient–enriched waters containing low dissolved oxygen[19,20]. Therefore, any changes in water quality plays have a significant role in structuring macroinvertebrate communities in aquatic ecosystems.

Understanding the relationship between macroinvertebrates and environmental variables can provide insights into the complex interactions within the river systems[21,22]. Environmental variables such as water temperature, metals and nutrient concentrations can directly influence the distribution and abundance of macroinvertebrates[23]. For example, increased nutrient loads, metals and other toxic substances can negatively affect macroinvertebrate abundances and diversities, leading to shifts in community composition[24]. The present study aimed to investigate macroinvertebrate community structure in relation to water and sediment chemistry variables along a subtropical river system (i.e., the Luvuvhu River) in South Africa. Specifically, this study aimed to: (i) examine the macroinvertebrate's community structure and composition across seasons and sites within the Luvuvhu River, and (ii) assess the relation between water and sediment chemistry variables and macroinvertebrate community structure and composition. We hypothesized that macroinvertebrate community structure will differ across various river sections, mainstream lower reaches and tributaries in relation to major barriers such as dams and weirs which will alter river flows and disrupt habitats, and across seasons which would result in differences in water and sediment concentration levels along the river system.

Materials and methods
Study area
The Luvuvhu River originates from the Soutpansberg Mountains, together with its other tributaries. The river flows over a stretch of approximately 200 km from source to its confluence with the Limpopo River at Pafuri, within the Kruger National Park. The Luvuvhu River is situated within the savanna biome, characterised by a hot and wet summer climate, with mild and dry winters. According to Munyai et al.[25] most of rain (over 80%) is received between November and March. The mean annual precipitation varies between 610 mm and 800 mm. The local climate throughout the summer and winter months are characterised by high (i.e., 40 ^{0}C) and low (i.e., 9 ^{0}C) temperatures, respectively. The Luvuvhu River exhibits several hydrological features, including rapid flows, riffles, runs, and pools, particularly in its lowveld stretches making a good habitat for various macroinvertebrates within these biotopes. This study involved the collection of macroinvertebrates, water and sediment samples from 16 sites (in different river sections: *Upstream*, *downstream and tributaries*) located along the Luvuvhu River system across two seasons (i.e., hot–wet, cool–dry) (Fig. 1). All 16 sites were selected based on associated land use activities (*mainstream sites*: agriculture, water abstraction and solid waste disposal; *tributaries*: location relative to the wastewater sewage plant, sand mining and brick making) that could potentially negatively impact the river system.

Water variables
Conductivity (μS cm^{-1}), salinity (ppt), pH, water temperature (°C), and total dissolved solids (TDS) (mg L^{-1}) were measured in–situ at 16 sampling sites ($n = 2$ per site) along the Luvuvhu River and its tributaries across two seasons (i.e., cool–dry, hot–wet), using a portable handheld multiparameter Cyberscan Series portable meter (Eutech Instruments, Singapore). Furthermore, two integrated water samples ($n = 5$ replicates per sample) were randomly taken from each site using a bucket before being placed into a labelled bottle and stored on ice awaiting further analysing for nutrients (i.e., ammonium (NH_4^+), phosphates (PO_4^{3-})) in the laboratory. The water samples were analysed within 8 h of collection using a HANNA HI83300 multiparameter photometer (HANNA Instruments Inc., Rhode Island). A HANNA freshwater ammonia high range test kit (HI3824) with a range of 0–100 mg L^{-1} and a resolution of 0.1 mg L^{-1} was used based on the Nessler method from the American Society for Testing and Materials (ASTM) Manual of Water and Environmental Technology D1426. Phosphate concentration was measured using the HANNA phosphate high range (HI717) test kit, with a range of 0–30 mg L^{-1} and resolution of 0.1 mg L^{-1} based on the adaptation of the ammonia acid method for the Standards Methods for the Examination of Water and Wastewater, 18th edition.

Sediment variables
Integrated sediment samples ($n = 2$ per each site, with a sample consisting of 5 replicates) were collected using a small plastic shovel, and each sample was placed in new polyethylene Ziplock bag to avoid cross–contamination. Samples were quickly placed in an ice–filled cooler box and transported to the University of Mpumalanga laboratory for further processing. In the laboratory, the sediment samples were oven–dried at 60 °C for 72 h to a constant weight before being disaggregated in a porcelain mortar. The dried sediment samples were then homogenised using a riffle splitter, and thereafter, a sediment subsample of 0.5 kg was separated and sent to BEMLAB, Cape Town, for further analysis. The following elements were analysed on the sediment samples:

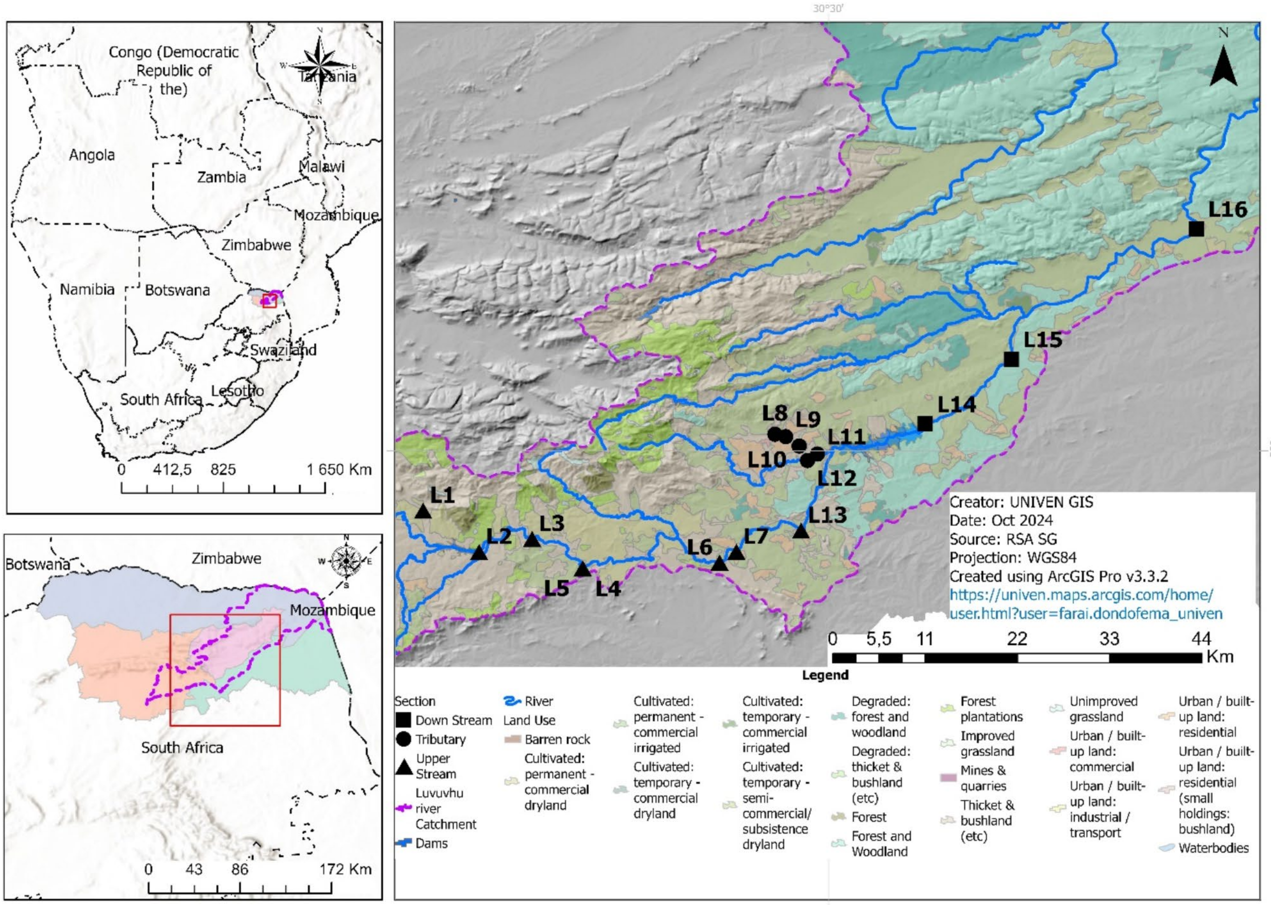

Fig. 1. Map highligting the study sites along the subtropical Luvuvhu River system, South Africa.

sediment pH (sedpH), resistivity (sedRes), stone, phosphorus (sedP), potassium (K), calcium (Ca), magnesium (Mg), sodium (Na), copper (Cu), zinc (Zn), manganese (Mn), boron (B), iron (Fe), sediment organic carbon (SOC), and sulphur (S) were quantified for each site and season using an inductively coupled plasma atomic emission spectroscopy (ICPMS) instrument (see Rice[26] for detailed methodology).

Macroinvertebrate sampling

Macroinvertebrate samples ($n = 2$ per site) were collected from the 16 river sites across the 2 seasons using a standardised handheld kick net (frame 30 cm × 30 cm, mesh size 500 µm, handle 1.5 m). The following habitats were sampled along a 100 m transect for each sample: pools, bedrock, littoral/riparian vegetation/macrophytes, stones in current, stones out of current and rapids/riffles, gravel, sand and mud for a total time of 8 min. The net was fully immersed in the aquatic environment, and the collection of macroinvertebrates was conducted by systematically sweeping a designated area. The methodology encompassed the act of traversing the water while utilising a sampling net, and employing forceful movements to displace aquatic macrophytes, sand, and boulders. This action was undertaken with the purpose of dislodging macroinvertebrates that might be attached to the mentioned substrates. To avoid the potential escape of active macroinvertebrates, the net was expeditiously retrieved from the water column. The macroinvertebrates were afterwards transferred into a 500 mL plastic container and preserved in 70% ethanol solution. Macroinvertebrates were then taken to the laboratory for identification up to genus and/or species level in most instances following Fry[27] identification guides.

Data analysis

Sediment metal, nutrient concentrations and water environmental variables (with the exception of pH) were log–transformed to test for homogeneity of variances and normality. The collinearity of environmental variables was tested using correlation analysis, with all highly significantly ($r > 0.8$) correlated variables being removed. The differences in sediment metal, nutrient concentrations, and environmental variables between the 16 sampling sites and seasons (i.e., cool–dry, hot–wet) were assessed using a two–way ANOVA in SPSS version 25.0.

Macroinvertebrate diversity metrices (i.e., taxa richness, evenness, Margalef's, Shannon–Wiener and Simpson's diversity indices) were calculated using macroinvertebrate community dataset in PAST version 4.03. The effects of different sites (16 sites), and seasons (cool–dry, hot–wet), and their interaction on environmental variables (i.e., water and sediments) and macroinvertebrates diversity metrics (i.e., taxa richness, evenness, Margalef's, Shannon–Wiener, Simpson's) and total abundances were examined using a two–way ANOVA coupled with Tukey's posthoc –using SPSS version 25.0. In all analyses, significance was inferred at $p < 0.05$. A distance–based permutational analysis of variance (PERMANOVA[28]) was conducted using Bray–Curtis and Euclidean distance dissimilarities based on 9 999 permutations with Monte Carlo tests to examine differences in macroinvertebrate community structure across sites and seasons.

To determine the relative importance of water and sediment chemistry variables responsible for structuring macroinvertebrate taxa among sites and seasons, an multivariate ordination approach was used. First, to determine whether to use unimodal or linear ordination method for the macroinvertebrate community sturucture and environmental characteristics, a Detrended Correspondence Analysis (DCA) was used. Since the gradient length were > 4, a unimodal Canonical Correspondence Analysis (CCA) was used based on significant ($p < 0.05$) forward selected environmental variables using 9999 Monte Carlo Permutations in CANOCO version 5.1[29].

Results

Water variables

During the study, water mean pH was generally neutral ranging from 7.0 – 7.1 during hot–wet season and slightly acidic during the cool–dry season with a range of 5.8–6.1. The water temperature at all the sites also showed seasonal variation with low temperatures recorded during hot–wet (mean range 15.8–19.6 °C) (Table 1). Significant differences (ANOVA, $p < 0.001$) among seasons and river section were observed for temperature, pH and resistivity. Water variables such as total dissolved solids and salinity showed significant differences among river section (Table 2). Ammonium concentration range was 1.2–2.4 mg L^{-1} (hot–wet) and 0.1–0.3 mg L^{-1} (cool–dry), whereas, phosphate concentration range was 2.4 – 6.4 mg L^{-1} and 0.2 – 0.8 mg L^{-1} for the hot–wet and cool–dry season, respectively. The high concentration of ammonium were recorded downstream during hot–wet season (Table 1). Ammonium and phosphate concentrations were found to be significantly different (ANOVA, $p < 0.001$) across seasons (Table 2).

Sediment variables

Sediment metal concentrations were high in the tributaries than in the mainstem river sites across seasons (Table 1). Metal concentration were generally high in the downstream river sections with no clear seasonal

Variable	Hot–wet			Cool–dry		
	Upstream	Downstream	Tributary	Upstream	Downstream	Tributary
Water						
Temperature (°C)	15.8 ± 0.9	16.4 ± 1.3	16 ± 0.9	17.8 ± 0.5	19.6 ± 1.5	17.6 ± 0.7
pH	7.0 ± 0.3	7.0 ± 0.2	7.1 ± 0.1	5.8 ± 0.2	5.9 ± 0.5	6.1 ± 1.1
Total dissolved solids (mg L^{-1})	73.2 ± 9	93.3 ± 7.9	90.5 ± 22.9	82.9 ± 6.6	101.2 ± 13.7	98.3 ± 28.5
Conductivity (μS cm^{-1})	225 ± 22.1	276.7 ± 36.7	273.5 ± 82.1	228.5 ± 23.8	271.2 ± 71.6	267.7 ± 95
Salinity (ppm)	108.1 ± 12.2	132.7 ± 17.6	141 ± 46.1	110.1 ± 10.6	124.6 ± 21.7	129.7 ± 44.4
Resistivity (Ω)	6989.7 ± 774.1	5352 ± 527	5556 ± 1338.5	6091.5 ± 431.2	4220.9 ± 2046.8	5407.1 ± 1331.1
Ammonium (mg L^{-1})	1.5 ± 1.2	2.4 ± 2	1.9 ± 1.3	0.2 ± 0.3	0.1 ± 0.2	0.2 ± 0.3
Phosphates (mg L^{-1})	5.0 ± 2.4	3.2 ± 2.9	6.4 ± 4.6	0.6 ± 0.2	0.6 ± 0.7	0.8 ± 0.8
Sediment						
pH	5.4 ± 0.8	6.2 ± 1.0	6.2 ± 0.9	6.0 ± 0.6	6.3 ± 1.1	6.2 ± 1.1
Resistivity (Ω)	2850.8 ± 1231.7	2409.2 ± 1173.6	2845.0 ± 1173.6	2043.3 ± 975.7	1618.3 ± 623.4	1977.5 ± 623.4
Stone (%)	23.2 ± 22.7	9.8 ± 5.3	12.8 ± 5.3	24.4 ± 20.3	25.2 ± 16.9	20.7 ± 16.9
P (mg kg^{-1})	6.2 ± 2.2	9.7 ± 9.2	5.5 ± 9.2	7.5 ± 3.4	11.4 ± 11.3	6.7 ± 11.3
K (mg kg^{-1})	23.0 ± 8.6	20.6 ± 3.7	18.2 ± 3.7	30.2 ± 16.4	30.0 ± 15.6	23.0 ± 15.6
Ca (mg kg^{-1})	2.2 ± 1.4	3.6 ± 1.7	2.5 ± 1.7	4.5 ± 3.7	4.2 ± 2.9	2.8 ± 2.9
Mg (mg kg^{-1})	1.4 ± 1.0	1.4 ± 0.6	1.1 ± 0.6	2.1 ± 1.5	1.8 ± 0.9	1.2 ± 0.9
Na (mg kg^{-1})	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
Cu (mg kg^{-1})	3.0 ± 2.4	7.9 ± 5.8	3.2 ± 5.8	5.3 ± 6.0	8.6 ± 11.0	6.1 ± 11.0
Zn (mg kg^{-1})	0.8 ± 0.4	3.2 ± 2.3	1.6 ± 2.3	1.4 ± 1.2	2.3 ± 1.6	1.8 ± 1.6
Mn (mg kg^{-1})	135.5 ± 74.2	269.5 ± 174.3	328.4 ± 174.3	370.7 ± 690.0	305.1 ± 249.8	348.0 ± 249.9
B (mg kg^{-1})	0.1 ± 0.05	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0.1
Fe (mg kg^{-1})	166.5 ± 74.7	3341 ± 171.8	431.9 ± 171.9	359.3 ± 383.6	224.6 ± 140.3	286.9 ± 140.4
C (%)	0.3 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.8 ± 0.6	0.6 ± 0.1	0.7 ± 0.2
S (mg kg^{-1})	6.8 ± 2.8	17.1 ± 15.1	8.1 ± 15.1	16.1 ± 10.5	13.3 ± 8.1	9.8 ± 8.1
T Value (cmol kg^{-1})	4.2 ± 2.7	5.5 ± 1.7	3.9 ± 1.8	7.1 ± 5.4	6.5 ± 3.7	4.4 ± 3.7
Acid Saturation (%)	9.0 ± 6.4	5.1 ± 6.4	5.1 ± 6.4	4.0 ± 4.7	3.1 ± 4.9	3.9 ± 4.9

Table 1. Mean (± standard deviation) of water and sediment chemistry variables measured from Luvuvhu River system, South Africa.

Variable	Season		Sections		Season × Sections	
Water	**F**	***p***	**F**	***p***	**F**	***p***
Temperature	**63.816**	**< 0.001**	**8.875**	**< 0.001**	2.823	0.068
pH	**66.125**	**< 0.001**	0.706	0.498	0.549	0.58
Total dissolved solids	3.473	0.067	**6.143**	**0.004**	0.018	0.982
Conductivity	0.024	0.879	2.874	0.065	0.029	0.971
Salinity	0.54	0.466	**3.561**	**0.035**	0.228	0.797
Resistivity	**5.027**	**0.029**	**9.129**	**< 0.001**	0.956	0.390
Ammonium	**33.803**	**< 0.001**	0.614	0.545	0.846	0.434
Phosphates	**40.571**	**< 0.001**	2.663	0.078	2.109	0.131
Sediment						
pH	1.202	0.278	2.748	0.072	0.693	0.504
Resistivity	**6.994**	**0.011**	1.089	0.343	0.002	0.998
Stone	**5.194**	**0.026**	0.588	0.559	0.307	0.737
P	0.480	0.491	1.933	0.154	0.002	0.998
K	**4.694**	**0.034**	1.127	0.331	0.048	0.953
Ca	2.750	0.103	1.754	0.182	1.043	0.359
Mg	1.697	0.198	1.228	0.300	0.319	0.728
Na	**6.264**	**0.015**	1.480	0.236	0.546	0.582
Cu	1.217	0.275	**5.087**	**0.009**	0.743	0.480
Zn	0.024	0.877	**8.596**	**0.001**	1.238	0.298
Mn	0.845	0.362	2.528	0.089	0.515	0.600
B	**8.032**	**0.006**	**3.482**	**0.037**	0.696	0.503
Fe	0.097	0.756	1.754	0.182	2.634	0.080
C	**24.603**	**< 0.001**	0.676	0.513	0.896	0.414
S Am.acet	3.481	0.067	2.552	0.087	2.678	0.077
T Value	2.031	0.159	1.776	0.178	0.669	0.516
Acid Sat.	2.316	0.133	1.318	0.275	0.483	0.619

Table 2. Two–way ANOVA analysis of environmental variables in Luvuvhu River system, South Africa. Values in bold indicate significant differences at $p < 0.05$, season df = 1, site df = 2.

patterns amongst river sites. The metal concentrations showed a slight variation across the cool–dry and hot–wet season, with high concentrations generally recorded in cool–dry season (Table 1). High mean resistivity was observed, with a range of 2 409.2–2 850.8 Ω during the hot–wet season and 1 618.3– 2 043.3 Ω during the cool–dry season. Potassium (K), Cu, Mn and Fe concentrations in sediments were generally high during cool–dry, while metals such as sedP, Ca, Mg, Na, Zn and B were low in hot–wet (Table 1). During the hot–wet season, the mean K concentration were 23.0 mg L^{-1} (upstream), 20.6 mg L^{-1} (downstream) and 18.2 mg L^{-1} (tributaries), while during cool–dry, the K concentration increased to 30.2 mg L^{-1}, 30.0 mg $L^{-1}$and 23.0 mg L^{-1} for upstream, downstream and tributaries, respectively. Manganese concentration were found to be low during hot–wet season (range 135.5– 269.5 mg L^{-1}), and increased during cool–dry season with a range of 305.1–370.7 mg L^{-1}. Boron and Na recorded low concentration ranging between 0.1 mg L^{-1} and 0.2 mg L^{-1} during both seasons and river sections (Table 1). Based on two–way–ANOVA, significant differences ($p < 0.001$) were observed for sedRes, stone, K, Na, B and C across seasons, while significant site differences ($p < 0.001$) were observed on Cu, Zn and B (Table 2). No significant differences were observed for the interaction between river sections and seasons for all sediment variables.

Macroinvertebrate community structure

A total of 1 973 individual macroinvertebrates belonging to 92 macroinvertebrates taxon and 11 orders were identified from Luvuvhu River system across the two seasons (i.e., hot–wet, cool–dry) (Table S1). Coleoptera, Odonata, Megaloptera and Diptera taxa comprised of 22.8%, 17.7%, 12.7% and 11.4%, respectively of total taxa. Hemiptera and Ephemeroptera comprised of 10.1% each of total percentage taxon found. Molluscs taxa have accounted for 6.3%, whereas Trichoptera had 5.1%, with Lepidoptera, Annelida and Crustacea accounting for 1.3% each. Based on PERMANOVA analysis, significant site (Pseudo–F = 1.692, d*f* = 2, *p* (Monte Carlo (MC)) = 0.007) and seasonal (Pseudo–F = 3.732, d*f* = 1, *p* (MC) < 0.001) differences were observed for macroinvertebrate community structure, with site pairwise comparisons indicating significant differences among downstream and tributary sites ($t = 1.493$, *p* (MC) = 0.005). Furthermore, ANOSIM analysis indicated significant seasonal differences ($p = 0.007$) with a low Global R value of 0.14. Sites were found to be not significantly different ($p = 0.321$) with a Global R value of 0.02. The SIMPER analysis showed low similarities for the hot–wet (21.1%) and cool–dry (11.9%) seasons, with a high dissimilarities across the two seasons (SIMPER value = 86.6%). Similarly, sites also had low similarity values, for example, upstream (16.8%), downstream (17.9%)

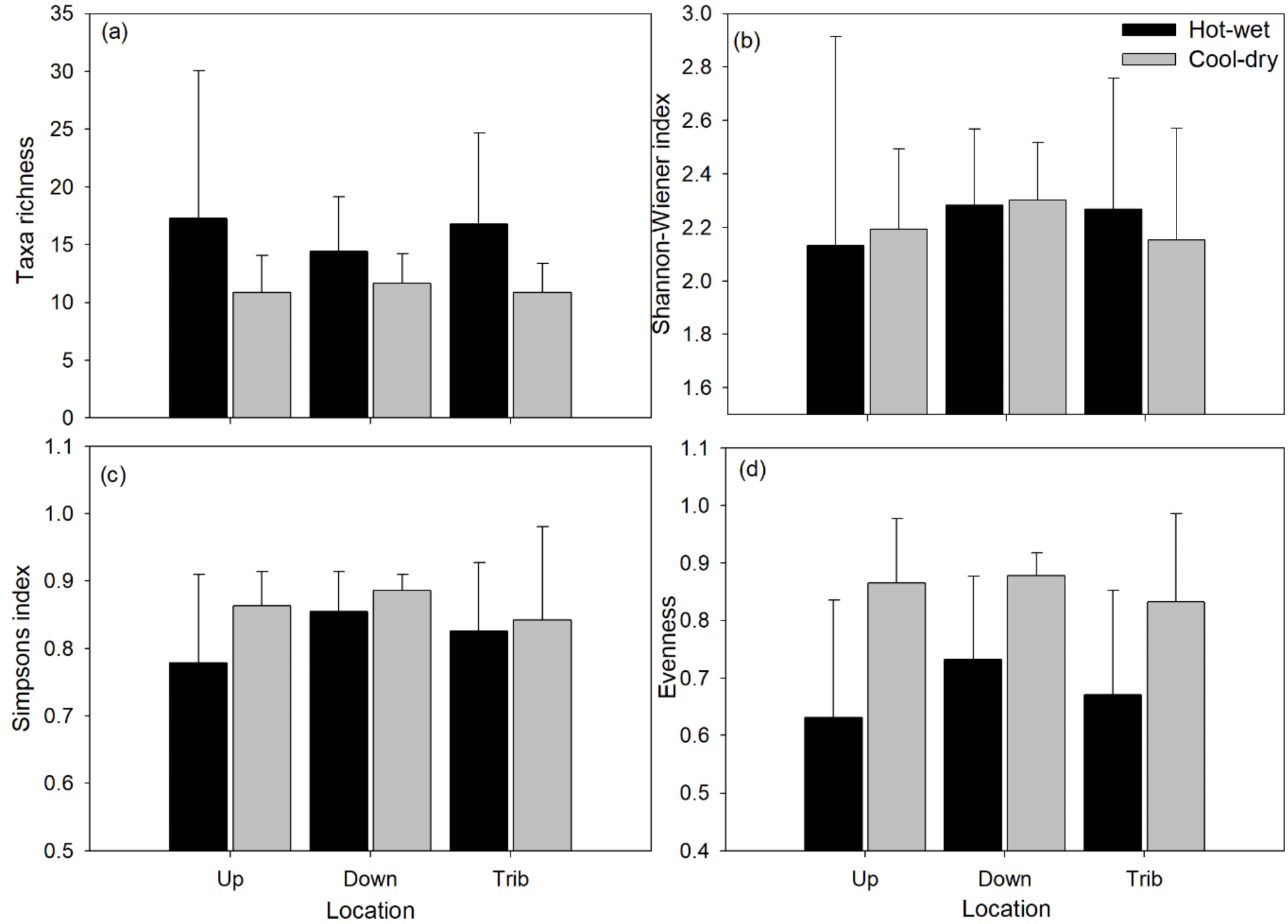

Fig. 2. Diversity indices matrices for macroinvertebrates: (a) taxa richness, (b) Shannon–Wiener, (c) Simpsons, and (d) evenness along the Luvuvhu River system. Abbreviation: Trib – tributary.

Index	Season		Section		Season × Section	
	$F_{2,58}$	*p*	$F_{1,58}$	*p*	$F_{2,58}$	*p*
Taxa richness	**9.833**	**0.003**	0.149	0.862	0.540	0.586
Abundances	**27.621**	**<0.001**	1.422	0.250	1.364	0.264
Shannon–Wiener	0.010	0.921	0.475	0.624	0.243	0.785
Simpson	3.362	0.072	1.573	0.216	0.681	0.510
Evenness	**23.375**	**<0.001**	1.060	0.353	0.457	0.635

Table 3. Two–way ANOVA analysis of macroinvertebrate diversity indices. Bold values indicate significant differences at $p < 0.05$.

and tributaries (15.0%). High dissimilarities were observed for upstream vs. downstream (83.0%), upstream vs. tributary (83.3%) and downstream vs. tributary (84.6%). The Corydalinae were the main contributors (>4%) for differences across river sections and seasons.

Taxa richness and Shannon–Wiener index were high during the hot–wet season, with Simpson index and evenness being low during the hot–wet season (Fig. 2a–c). Evenness (E) was relatively high during the cool–dry seasons, however not statistically significant (Fig. 2d). No significant differences ($p > 0.05$) were observed for all diversity matrices (i.e., taxa richness, Shannon–Wiener, Simpson's, evenness) among the study river sections and seasons (Table 3).

Relationship between macroinvertebrates and environmental variables

Canonical correspondence analysis (CCA) axes 1 and 2 variance was 21.8% and 20.9%, respectively, with both axes of the selected exploratory variables accounting for 42.7% of the total macroinvertebrate community data.

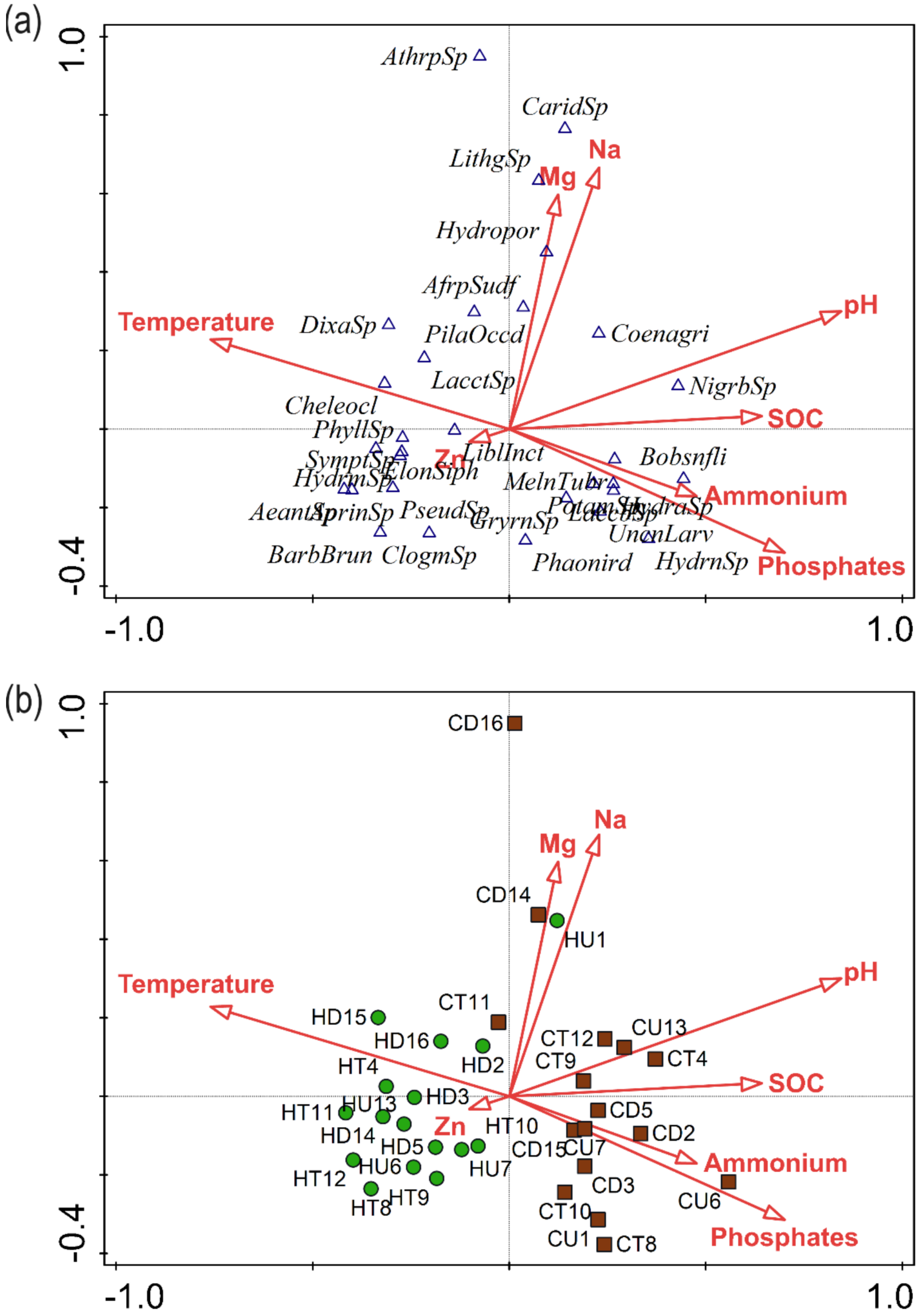

Fig. 3. The Canonical Correspondence Analysis plots highlight the relationship between measured significant environmental variables with macroinvertebrate communities between the cool–dry and hot–wet seasons across various river sections. Biplots for environmental variables with (a) taxon and (b) samples.

Macroinvertebrate community structure across the river sections and seasons was found to be significantly associated with temperature, Mg, Na, pH, SOC, ammonium and phosphates (Fig. 3). Canonical correspondence analysis further showed variables such as temperatures, Mg, Na, pH, SOC, ammonium and phosphates being important in structuring macroinvertebrates during hot–wet season, whereas, temperature and Zn were important during the cool–dry season (Fig. 3). Water temperature and Zn were positively associated with the first axis, while temperature, Mg, Na, pH, SOC, ammonium and phosphates was negatively associated with the second axis (Fig. 3). The hot–wet season was clearly separated along the first axis from the cool–dry season. Macroinvertebrate taxa such as Coenagrionidae are positively correlated with pH, while most of the Odonata were positively correlated with Zn.

Discussion

Macroinvertebrates community structure was found to be significantly different across the different river sections, thereby supporting the hypothesis that macroinvertebrates community structure will differ in various rivers sections (i.e., mainstream and tributaries) and across seasons. The communities structure is found to be dependent on water and sediment chemistry across sampling sites, and macroinvertebrates exhibited higher diversity during hot–wet season than in cool–dry season (see Table S1 for diversity and abundances), with a turnover from six distinct macroinvertebrates families (i.e., Odonata, Diptera, Coleoptera, Hemiptera, Trichoptera and Ephemeroptera) exhibiting richest taxas within the river systems. Furthermore, it seems that seasonality played a critical role in promoting spatio–temporal diversity of macroinvertebrates communities within the mainstem Luvuvhu River and its tributaries.

The present study found that collectively the macroinvertebrates across all systems (both mainstream and tributaries) was highly diverse with 1 973 individual macroinvertebrates belonging to 92 macroinvertebrates taxa and 11 orders being identified from the river system over the two seasons (see Table S1) being higher compared to that 24 families recorded in the low reaches of the Luvuvhu River, in the Kruger National Park[30]. The low diversity and abundance of macroinvertebrates in the mainstream can be attributed to low food supplies, seasonal dynamics and habitat loss as the river becomes more vulnerable to threats such as pollution[31], leading to the differences in taxon richness, diversity and abundances in the system and habitat deterioration[32,33]. Due to anthropogenic activities, rivers are prone to increases in metals and nutrient load, which may lead to unhealthy ecosystems that are not capable of supporting aquatic biodiversity, resulting in the loss of species and deterioration of water quality[34,35].

The Luvuvhu River system nutrient and metal concentrations in sediments showed variation during both cool–dry and hot–wet seasons. These results are similar to those observed in the Luvuvhu River catchment by Netshiongolwe et al.[31] and Edokpayi et al.[36]. Hence, only a few variables (i.e., pH, Temperature, Mg, Na and Zn) and nutrients (i.e., phosphates and ammonium) were found to be significant in structuring macroinvertebrate communities. Upstream sites showed high macroinvertebrate taxon richness, where sediment variable concentrations were relatively low in the Luvuvhu River. In similar studies, Dalu et al.[37], Keke et al.[38] and Strungaru et al.[39] found that macroinvertebrates in upstream sites with low sediment metal concentration had high taxon richness compared to those in downstream sites with high sediment metal concentration. While sediment metal concentrations, particularly Mn and Fe were found to be elevated in tributaries, the taxon richness of macroinvertebrates remained high compared to those in the mainstream. This suggests that sediment metals, such as Mn and Fe influenced the composition of the macroinvertebrate community.

Due to various activities such as car washing, bathing, brick making, sewage spillage, poor solid waste disposal, urban runoff and subsistence agriculture, water and sediment quality in the Luvuvhu River catchment is being compromised and degraded, leading to low aquatic biodiversity. Similarly, several studies[40–45]across the globe have reported the same activities being the major contributor to deterioration of aquatic life and water quality in rivers. The majority of sites in the upstream of the mainstem were located in rural settlements and agricultural areas, whereas river sites in the tributaries were situated in the urban areas with sewage spills prevalent[13,44,45]. Therefore, high nutrient loads at the downstream of the tributaries can be attributed to bursting and leaking of sewage pipes and leachates from agricultural lands in the upstream sites. The latter results are similar to Dalu et al.[37] study in the Bloukrans River system, which showed sewage, leachates and nutrient load from agricultural lands as major contributors of nutrient enrichment in rivers.

Environmental variables also changed with sampled sites and seasons, for example, water temperature was generally found to be high in the hot–wet season and low during cool–dry season. The fluctuations in water temperature observed can be attributed to seasonal climatic variations, whereby during cool–dry (winter season), the temperature in the catchment drops, while during hot–wet, the temperature rises, with changes in air temperature[23]. The changes in temperature caused macroinvertebrate community shift whereby species that cannot survive low temperatures die off, while those that can withstand low temperatures survive and reproduce. Similar to a review done by Bonacina et al.[46], the present study attest that temperature plays a critical role in structuring macroinvertebrates diversity and abundances in a river system. The water pH in the tributaries was found to be neutral in most sites, suggesting that pH did not change with water quality impacts while no significant changes on macroinvertebrates community structure. However, from CCA analysis, an increase in pH correlated with an increase in macroinvertebrates for most of the tributaries and selected up– and downstream sites during the cool–dry season, suggesting that pH influence is closely related to seasonal dynamics of a river system. Ammonium presence in this particular river may be attributed to blockage of sewage pipes and industrial effluents which spills and enters the stream, thus leading to contamination of the rivers[47].

In the sampled sites, taxonomic diversity was high. Furthermore, we identified some pollution–tolerant taxon such as Corydalinae, *Caridina nilotica*, and Chironomidae which dominated the river ecosystem[48]. The macroinvertebrate assemblages found in this system were high in abundance and diversity compared to those identified by Munyai et al.[49] in the Mutshundudi River and Kekana et al.[50]in the Greater Letaba River. The majority of pollution–sensitive species, such as Ephemeroptera, Odonata and Trichoptera were found in the studied sites, with high abundances and diversity being observed in the tributaries. The findings from this study indicate that the abundance and diversity of Ephemeroptera, Odonata, and Trichoptera may suggest that a significant portion of these taxa in rivers is unique to particular sites and seasons, with the highest complexity linked to land use activities[51–53].

Anthropogenic activities, including water abstraction and sewage discharge are likely exerting a direct influence on the water and sediment quality of the Luvuvhu River system. Different macroinvertebrates exhibit distinct preferences for specific environmental qualities, so implying that even slight modifications in their preferred environmental ranges resulting from contaminants can have a significant impact on their community structure[54,55]. Similar to several studies[37,56,57]conducted in rivers of South Africa, the findings of this study suggests that the seasonal variation played a significant role in shaping macroinvertebrate communities. This study found that macroinvertebrate diversity was low during cool–dry season, and this was caused by low rainfall which led to cold temperatures and reduce sunlight limiting the growth of aquatic plants and algae, which are important food source for macroinvertebrates[58]. Furthermore, the concentration of dissolved metals on the low–flow season (cool–dry) could have caused increased mortality as compared to potential dilution in the high–flow season (hot–wet). Alternatively, some taxa may have retreated into the hyporheos during cold condition (cool–dry), making them harder to access through benthic sampling in the river.

Conclusions

Different human activities have been found to have a notable adverse impact on the rivers, and this has affected the macroinvertebrate diversity and their community structuring and distribution in the Luvuvhu River system. It can be noted that both environmental variables (water and sediment chemistry) and seasons had significant effects on macroinvertebrates community composition, however, spatial dynamics captured more variations in the Luvuvhu River and its tributaries. This study offers valuable insights for the management and conservation strategies of river systems that are potentially affected by anthropogenic activities such as agriculture, solid waste disposal, wastewater discharge, sand mining and fishing, through municipal initiatives. Furthermore, these findings could be useful in future studies for habitat management and river monitoring programs.

Data availability

The datasets generated during and/or analysed during the current study are not publicly available due as its part of an ongoing large study that involve students but are available from the corresponding author on reasonable request. These data will be linked to manuscript after 2 years from the date of publication.

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Author contributions

L.F.M: Conceptualization, Methodology, Investigation, Data curation, Funding, Supervision, Writing – original draft, Writing – review and editing. B.P.G: Methodology, Data curation, Formal analysis, Investigation, Writing – original draft, Writing – review and editing. F.D: Visualization, Methodology, Investigation, Writing – original draft, funding, Writing – review and editing. T.D: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Funding, Supervision, Writing – original draft, Writing – review and editing.

Declarations

Competing interests

The authors declare no competing interests.

Conflict of interest

All authors have no relevant financial or non–financial interests to disclose.

Ethical and permit considerations

The study was ethically approved by University of Mpumalanga School of Biology and Environmental Sciences Research Ethics Committee (number: AS/UMP /Gumede/ ADPCON/ 2023), with permissions to sample being granted by Limpopo Economic Development, Environmental and Tourism (permit number CPM1753). All research work were performed in accordance with relevant ARRIVE guidelines and regulations as per ethical approvals.

Additional information

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Correspondence and requests for materials should be addressed to L.F.M.

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