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## Seasonal variation in benthic macroinvertebrate assemblages and water quality in an Afrotropical river catchment, northeastern Tanzania

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#### ABSTRACT

Population growth and economic development have resulted in increased water demands, threatening freshwater resources. In riverine ecosystems, continuous monitoring of the river quality is needed to follow up on their ecological condition in the light of water pollution and habitat degradation. However, in many parts of the world, such monitoring is lacking, and ecological indicators have not been defined. In this study, we assessed seasonal variation in benthic macroinvertebrate assemblages in a tropical river catchment in northeastern Tanzania, which currently experiencing an increase in agricultural activities. We examined the potential of instream environmental variables and land-use patterns to predict the river macroinvertebrate assemblages, and also identified indicator taxa linked to specific water quality conditions. Macroinvertebrate abundance, taxon richness and TARISS (Tanzania River Scoring System) score were higher in the dry season most likely due to higher surface runoff from agricultural land and poorer water quality in the wet season. In the wet season macro invertebrates seem to be limited by chlorophyll-a, oxygen and phosphorous while in the dry season, when water flow is lower, nitrogen and turbidity become important. Substrate composition was important in both seasons. Given the fact that different selective filters limit macroinvertebrate assemblages in both seasons, a complete picture of water quality can only be established by monitoring in both seasons. Riparian buffer zones may help to alleviate some of the observed negative effects of agricultural activities on the river system in the wet season while limiting irrigation return flows may increase water quality in the dry season.

## 1. Introduction

Human activities negatively affect the functioning of freshwater ecosystems globally, resulting in the deterioration of water quality, loss of biodiversity and loss of ecosystem services (Malmqvist and Rundle, 2002; Søndergaard and Jeppesen, 2007; Chakona et al., 2008; Dudgeon, 2010; Vorosmarty et al., 2010). Excessive nutrient inputs, flow alteration, loss of riparian buffer zone and sedimentation are among the major anthropogenic impacts on freshwater ecosystems (Hrodey et al., 2009; Nyenje et al., 2010; Dodds et al., 2013). Globally, it is estimated that about 65% of freshwater habitats are considered moderately to severely threatened (Dudgeon et al., 2005; Schowe and Harding, 2014). This is especially true for (sub) tropical developing countries where intensification of land use for agriculture and poor disposal of untreated waste have markedly degraded rivers and streams (Dudgeon, 1992; Beyene et al., 2009; Dlamini et al., 2010; Nyenje et al., 2010; Paisley et al., 2011; Bere and Nyamupingidza, 2014)

The quality of aquatic resources is usually assessed using physical, chemical and biological characteristics. However, impact assessment based on water chemistry alone is insufficient (Dalu et al., 2017a), since it does not integrate water quality temporally (Bellinger et al., 2006; Dalu and Froneman, 2016). Biological monitoring of freshwater ecosystems is acclaimed to be a quick and cost-effective method for assessing ecosystem conditions (Ollis et al., 2006; Dallas et al., 2010; Li et al., 2010). It allows long-term environmental effects to be detected, providing a broad measure of their synergistic impacts (Dalu and

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Froneman, 2016). Among the potential biotic component available for biomonitoring, benthic macroinvertebrates are the most commonly used in many regions (Dallas, 1995; Li et al., 2010; Aschalew and Moog, 2015; Siddig et al., 2016; Nhiwatiwa et al., 2017b). This is because they are ubiquitous and abundant even in small streams and form a dominant component of stream food webs (Rosenberg and Resh, 1993; Resh et al., 1995; Barbour et al., 1999; Hering et al., 2006). Benthic macroinvertebrates are regarded as good indicator organisms because they show taxon-specific differences in sensitivity to pollution, are taxonomically diverse, and have an aquatic life span long enough to provide a record of environmental quality (Metcalfe, 1989; Barbour et al., 1999; Nhiwatiwa et al., 2017a).

Rivers and streams often vary over time and exhibit seasonal variability in factors such as hydrology, water chemistry and habitat availability (Allan and Castillo, 2007; Dallas, 2004). Riverine organisms have specific habitat requirements and seasonal variation in these conditions will therefore affect the structure of benthic macro-invertebrate assemblage (Dallas, 2004; Zhang et al., 2012). As a result, to use macroinvertebrates as indicators and interpret the functionality of these communities in the light of ongoing environmental change, it is necessary to take this seasonal variation into account. (Dallas, 2004; Kilonzo et al., 2014). A major challenge is to separate potential effects of natural in-stream factors (e.g., flow rate, substrate) on biota and water quality from those linked to pollution and anthropogenic disturbance (e.g., agricultural activities) (Kilonzo et al., 2014; Jun et al., 2016).

Local in-stream factors such as water velocity, substrate type and water chemistry have been shown to primarily structure the assemblage of benthic macroinvertebrates by shaping local habitat characteristics (Richards et al., 1997; Statzner et al., 1988; Sandin and Johnson, 2004; Brooks et al., 2005; Allan and Castillo, 2007). Water velocity, for example, presents a direct physical force to the organisms and affects other in-stream factors such as food and sediment delivery, and oxygen content (Poff et al., 1997; Sandin and Johnson, 2004; Belmar et al., 2013; Pan et al., 2013). In addition, variation in substrate composition, in particular, is essential for the existence of many macroinvertebrate species because substrata provide shelter, food sources, and protection from predators (Ciutti et al., 2004; Li et al., 2012; Jun et al., 2016).

Moreover, landscape factors such as land-use patterns have a strong influence on river water and habitat quality, and subsequently on its biotic components. Agricultural activities, for example, can incite erosion and runoff of sediments, nutrients, and pesticides in river systems, consequently affecting macroinvertebrate assemblages (Kilonzo et al., 2014; Kalkhoff et al., 2016; Nhiwatiwa et al., 2017a). Riparian clearance and subsequent increased solar radiation can lead to higher water temperature and alter fundamental biogeochemical processes such as respiration and inputs of dissolved organic carbon (Paulo et al., 2019). Several studies have linked land use patterns to responses of benthic macroinvertebrate communities (Karaouzas et al., 2007; Zhang et al., 2012; Theodoropoulos et al., 2015; Bere et al., 2016). However, information from tropical regions and Africa in particular are still scarce (Masikini et al., 2018).

Although numerous studies on seasonal variations in benthic macroinvertebrate assemblages in river systems have been conducted in temperate region (e.g., Perona et al., 1999; Sporka et al., 2006; McCord and Kuhl, 2013), there is an increasing interest in tropical systems across the globe (Dudgeon, 2008; Qadir et al., 2008; Kilonzo et al., 2014; Jun et al., 2016). There is important variation in flow predictability in tropical rivers (Pearson, 2014) and stream macroinvertebrate assemblages can vary strongly both within and among catchments (Boulton et al., 2008; Pearson et al., 2017). As a result, it is difficult to generalize the relative importance of landscape and local factors affecting river ecosystems (Sandin and Johnson, 2004) and there is a need for more studies from different areas of the world, particularly from the tropics.

In Tanzania like many (sub) tropical African countries, assessment

of river quality is mainly based on the analysis of physico-chemical water quality parameters (e.g., Kihampa et al., 2013; Selemani et al., 2017). Biological assessment of river quality conditions using benthic macroinvertebrates has become established albeit relatively recently (Elias et al., 2014; Kaaya et al., 2015). Thus far, only a few studies on benthic macroinvertebrates have been published (Elias et al., 2014; Kaaya et al., 2015; Masikini et al., 2014; Kaaya et al., 2015; Shimba and Jonah, 2016; Masikini et al., 2018). But these are limited in resolution and most river systems have not been investigated. As a result, the association between macroinvertebrate assemblages and environmental conditions in river systems in Tanzania is still not fully understood and we are currently not able to assess potential anthropogenic impacts on river quality. Consequently, there is a need for integrated high-resolution studies supported by appropriate statistical models that try to achieve this goal.

The Ruvu River catchment (RRC) is a socio-economically important catchment in the upper Pangani River basin: a biodiversity hotspot area in northeastern Tanzania (IUCN Eastern Africa Programme, 2003). The catchment is experiencing increases in agricultural activities accompanied by overexploitation of water for irrigation (Shaghude, 2006; PBWO/IUCN, 2007). Despite the increasing anthropogenic pressure, thus far no study has addressed how land use could affect seasonal variation in the river quality (i.e., the physico-chemical and biological condition of the river system) in the catchment. At the moment, it is also not known which macroinvertebrate indicator taxa may be linked with specific water quality conditions.

In this study, we reconstruct seasonal variation in physico-chemical water quality and benthic macroinvertebrate assemblages in a tropical river catchment in Tanzania and build models to explain this variation. We used a combination of more quantitative indicator species analysis (IndVal) and multivariate analyses (variation partitioning of redundancy models) to study links between benthic macroinvertebrate assemblages and environmental factors over an entire catchment during two different seasons. We specifically aimed to (i) assess seasonal trends in physico-chemical water quality and macroinvertebrate assemblages and distributions, and (ii) identify key environmental factors (i.e., instream environmental variables and land-use factors) that can explain variation in macroinvertebrate community composition in the RRC. We also aimed (iii) to identify macroinvertebrate indicator taxa for the different water quality conditions in the RRC. We hypothesized that macroinvertebrate assemblages would vary seasonally due to differences in environmental conditions and rainfall surface run off patterns. We initially hypothesized that land use effects on river quality may be more pronounced in the rainy season due to higher surface runoff. On the other hand, lower flow rates might result in higher impacts in the dry season when pollutants could be present at higher concentrations. Given these contrasting conditions we expected that different taxa might be useful as indicator species in the dry season compared to the wet season.

## 2. Materials and Methods

## 2.1. Study area

The Ruvu River catchment (not to be confused with Ruvu River in the Wami basin in the Morogoro region, Tanzania) is located in the upper Pangani River basin in the Kilimanjaro region, Tanzania, and is one of the permanent rivers recharging the Nyumba ya Mungu dam (Fig. 1). The catchment lies between 3.0° and 4.2°S and 36.3° and 38.1°E in the northeastern part of Tanzania and covers approximately 25% of the total basin area. The area is drained by four main rivers and their tributaries: Ghona River (RH), Dehu River (RD), Soko River (RS), and Ruvu River (RV). The water in the catchment originates from natural springs (along the eastern slopes of Mt. Kilimanjaro) and Lake Jipe (recharges the Ruvu River). The altitude of the Ruvu River catchment ranges from 4000 meters to 650 meters a.s.l. Mean annual rainfall ranges from 2000 mm along the slopes of Mt. Kilimanjaro to



**Fig. 1.** Map of the Ruvu River catchment showing (a) the location of Ruvu River catchment in Tanzania and the location of the twenty-nine monitoring sites, and (b) the spatial distribution of seven land-use classes in the Ruvu River catchment in relation to the river monitoring sites. (Source: Kiptala et al., 2013). Abbreviations: RV = Ruvu River, RD = Dehu River, RH = Ghona River, RS = Soko River.

500 mm in the low lands (PBWO/IUCN, 2007). The rainfall has a bimodal pattern where long rains (*Masika*) are experienced in the months of March to May and the short rains (*Vuli*) which are less reliable normally coming in November and December (Kiptala et al., 2013). The river system is subjected to various sources of pollution including diffuse pollution from agricultural activities (e.g., soil erosion, fertilizer run-off), sewage and domestic waste (personal observations).

Economic activities follow the escarpment with Afrotropical montane rain forest (along the Mt. Kilimanjaro forest reserve) and multistrata agroforestry (with intercropped coffee and banana plantations as main crops, and livestock keeping including dairy cattle, goats and pigs) in the upper reaches (Mathew et al., 2016). The middle and lower reaches consist of savanna bushland, small and large scale irrigated agriculture (common grown crops are rice, maize, beans, sisal, sugarcane, vegetables and fruits), herding, fishing and small industries (UNDP, 2014; Mathew et al., 2016). Mbonile (2005) and PBWO/IUCN (2007) reported higher population density in the upper Pangani River Basin because of more favorable living conditions and the availability of fertile soils for agriculture, in particular. Approximately 80% of the population is engaged in agriculture and irrigation consumes most (up to 64%) of the available freshwater resources (Kiptala et al., 2013).

## 2.2. Study design

A total of 29 monitoring sites in the Ruvu River catchment (Fig. 1 and Table S1 (Supplementary Information) were sampled both in the dry (August–September 2015) and in the wet season (April–May 2016) to capture seasonal patterns in environmental variables and benthic macroinvertebrate assemblages. The sampled sites included four main rivers, namely Ghona River (RH), Dehu River (RD), Soko River (RS), and Ruvu River (RV) which drain to form the Ruvu River catchment (stream orders 1–3). The spatial gradient consisted of a  $\sim 51 \text{ km}$  longitudinal distribution of monitoring sites from upstream to downstream. Along this gradient, there was variation in land-use activities (Table S1).

## 2.3. Environmental variables sampling and analysis

Water samples were collected once in each season. At each sampling time, two water samples (n = 2) were taken across the river section at each monitoring site. Water samples were collected using high density polyethylene (HDPE) 1-litre bottles. The bottles were washed and rinsed with distilled water and left overnight with 5 % hydrochloric acid solution (HCL). Prior to sampling, the bottles were rinsed again three times with sample water on site. Samples were collected by inserting the bottles at mid-depth in the river in the opposite direction of the river current (APHA, 2012). The samples were transported in an ice cooler box to the laboratory of the Department of Water Environmental Science and Engineering (WESE), at the Nelson Mandela African Institution of Science and Technology (NM-AIST) Arusha, Tanzania for analysis. In the laboratory, samples were preserved at 4 °C to stop the metabolism and all activities of the organisms in the water prior the analysis (APHA, 2012).

On each sampling occasion, electric conductivity (EC), temperature, pH, chlorophyll-a (Chl-a), turbidity (Turb), and dissolved oxygen (DO) were measured in situ at each monitoring site. Turbidity was determined using a HANNA-portable turbidity meter (Model-HI93703). Chlorophyll-a was measured using an AquaFluor Handheld Fluorometer (Model-8000-010). Fluoride (F-) was determined using an ion selective electrode (Mettler Toledo SevenCompact™ pH/Ion S220). DO, pH, and EC, were measured using a HANNA multi-parameter instrument (Model-HI 9829). Chemical measurements of orthophosphate (PO<sub>4</sub><sup>3-</sup>), nitrate (NO<sub>3</sub>-N), ammonia (NH<sub>3</sub>-N), total phosphorus (TP), total nitrogen (TN), and chemical oxygen demand (COD) were carried out in the laboratory using a portable spectrophotometer (Model HACH-DR 2800). Orthophosphate concentration was measured using an ascorbic acid (PhosVer 3) method (range: 0.02 to 2.50 mg/L  $PO_4^{3-}$ ), nitrate concentration was measured using a cadmium reduction method (range: 0.01 to 30.0 mg/L NO<sub>3</sub>-N), ammonia was measured using the Nessler method (range: 0.02 to 2.50 mg/L NH<sub>3</sub>-N), total phosphorus was measured using PhosVer3 with acid persulfate digestion method (range: 0.06 to 3.50 mg/L  $PO_4^{3-}$ ), total nitrogen was measured using a persulfate digestion method (range: 0.1 to 25.0 mg/L N), and chemical oxygen demand was measured using a reactor digestion method (range: 0.7 to 150.0 mg/L COD). All chemical analyses followed the standard methods for the examination of water and wastewater by APHA (2012).

Depth, flow velocity and substrate composition were measured at each monitoring site. Flow velocity was measured using a Seba

Universal Current Flow Meter F1 positioned at a height of 0.60 (water depth) above the stream. Water depth was determined using graduated measuring rod. Substrate composition was visually assessed following Minshall (1984), based on the following size class categories: (silt/ mud < 0.06 mm), sand (0.06-2 mm), gravel (2-64 mm), cobbles (64-256 mm) and boulders (> 256 mm). The dominant substrate type at each monitoring site was noted. A digital Elevation Model (DEM) (30 m resolution) was used to delineate the catchment boundaries using the hydrology tools using ArcGIS 10.2 desktop GIS software (ESRI Company, Redlands, California, USA). Data on land-use categories in the catchment were obtained from Kiptala et al. (2013). In this study, land-use types were reclassified into seven major classes: (1) Water bodies: (2) Bare land: (3) Shrub land: (4) Agricultural land: (5) Afromontane forest; (6) Forest and cropland; and (7) Wetland and swamps (Fig. 1, Table S2; Supplementary information). Land-use information was derived as percentage (%) composition of each land-use type of a catchment area upstream of each monitoring site using ArcMap 10.2.

#### 2.4. Macroinvertebrate sampling, identification and counting

Macroinvertebrates were collected in a semi-quantitative way using a kick net of 1 mm mesh size on a 30-cm square frame following the TARISS sampling protocol (Kaaya et al., 2015). Samples were collected from the dominant habitat type present at each site (i.e., the habitat that covers about 70% of the 50 m stretch making up the river section at the site). Sampled habitat types included (i) stones in-current (cobbles, boulders and bedrock), (ii) vegetation/macrophyte, and (iii) gravel/ sand/mud (GSM). Stones and GSM habitats were sampled for one minute by kicking, turning or scraping them with the feet, whilst continuously sweeping the net through the disturbed area. The vegetation/ macrophyte habitat was sampled by pushing the net vigorously and repeatedly against and through the vegetation over an area of approximately two meters. All drifting material collected in the kick net was stored in a labeled plastic container with 70% ethanol and transported to the laboratory for sorting and identification. In the laboratory, samples were washed with tap water using a 0.5 mm mesh sieve then transferred into a white tray to sort out all macroinvertebrate specimens before preservation in 70% ethanol. The macroinvertebrates were identified with a dissecting microscope (10X magnification) to family level using different identification keys (Croft, 1986; Davies and Day, 1998; Gerber and Gabriel, 2002). It is well recognized that the relationship between macroinvertebrate assemblages and the environment is best performed using species-level identification (Fugère et al., 2016; Dalu et al., 2017b), however, family richness and species richness often correlate strongly in stream invertebrate communities, and the same key environmental factors seem to drive assemblage composition at the family species level (Fugère et al., 2016). Thus, family level identification has shown to be sufficient to detect effects of environmental disturbances such as pollution (Kaaya et al., 2015; Dalu et al., 2017b).

## 2.5. Data Analysis

As macroinvertebrate biotic indices, we used taxon richness (number of Taxa (Taxa\_S)) and the TARISS score. The latter index was developed specifically for river macroinvertebrates in Tanzania: designed for assessing ecological condition of river systems in the country. It takes high values when a site contains many sensitive taxa. The index was calculated following Kaaya (2014) by summing up taxon specific sensitivity weighting scores for each site. This index therefore does not consider the abundance. The sensitivity weighting ranges from 1 to 15, with values > 10 indicating taxa less tolerant to pollution.

Given that several variables could not generate acceptable normal distributions of residuals necessary for parametric tests (Shapiro Wilk test,  $p \ge 0.05$ ), therefore, we opted for the non-parametric Wilcoxon signed rank test (Wilcoxon, 1945) at 95% confidence level to test for

significant differences in macroinvertebrate biotic indices and environmental variables between seasons.

Generalized linear models with a Poisson error distribution were used to study the relationship between environmental variables (i.e., land-use and in-stream environmental variables) and macroinvertebrate biotic indices (i.e., taxon richness and TARISS score). The Poisson error distribution is appropriate for modelling community count data with many zeros (O'Hara and Kotze, 2010). A backward selection followed by a forward selection was computed to eliminate non-significant environmental variables from the models using the function step in the vegan package (Oksanen et al., 2016) in R (version 3.1.2. R Core Team. 2014). The procedure aimed to maximize the potential variation in macroinvertebrate biotic indices that can be explained by environmental variables. The Akaike information criterion (AIC) and Mc Fadden's pseudo R<sup>2</sup> coefficient (R<sup>2</sup><sub>pseudo</sub>) were used to determine the model with the best subset of environmental predictor variables. The AIC is an estimator of the relative quality of statistical models for a given set of data; it estimates the quality of each model relative to each of the other models (Akaike, 1974). The chosen 'best' model is the one that minimizes the Kullback-Leibler distance between the model and the data, and has a minimum AIC (most parsimonious model) compared to all the other models (Burnham and Anderson, 2002). The latter (i.e.  $R^2$  <sub>pseudo</sub>) is a simple measure for model fit for generalized linear models.  $R^2_{\mbox{\scriptsize pseudo}}$  coefficients are typically much smaller than conventional R<sup>2</sup> coefficients, values between 0.2 and 0.4 already indicate excellent model fit. Prior to this analysis, the explanatory variables were tested for correlation using Spearman rank correlations to prevent multicollinearity in the models. For example, total-P and orthophosphate were strongly correlated so we only included total-P in the model, while acknowledging in our interpretation that this gradient also reflects orthophosphate.

We tested for the effect of environmental variables (i.e., land-use and in-stream environmental variables) on macroinvertebrate community composition using separate redundancy analyses (RDA), a multivariate extension of multiple regression. Prior to analysis, macroinvertebrate count data were Hellinger transformed to improve the performance of ordination with community composition data containing many zeros (Legendre and Gallagher, 2001; Zuur et al., 2007). Rare taxa that occurred in less than three sites were not included in the analysis as this is insufficient to model their distributions. The significance of the RDA models was assessed with Monte-Carlo permutations (nperm = 999). A forward selection procedure was performed to retain only significant variables in the models. The relationships between the most important explanatory variables (retained in the model by forward selection) and macroinvertebrate community composition (Hellinger transformed taxon abundance data) were visualized using a Principal Component Analyses (PCA) ordination plot. The environmental variables were added in the plot as supplementary variables that do not affect the ordination. Taxa for which less than 30 percentage of variation was captured by the plot were not shown.

In addition, the relative importance of local in-stream environmental variables and land-use variables in terms of explaining macroinvertebrate assemblages was quantified using a variation partitioning procedure. This procedure based on partial redundancy analyses (pRDA) (Legendre and Lengendre, 2012) allows to partition the total amount of variation in macroinvertebrate assemblages, and to be decomposed into fractions explained by different sets of explanatory variables (Legendre and Lengendre, 2012). It defines a fraction of unexplained variation, fractions that are uniquely explained by land use or in-stream environmental variables, respectively, and a third fraction that captures the variation explained by the covariation between land use and local in-stream environmental conditions.

Finally, the IndVal i.e., indicator species method was used to detect indicator taxa linked to different water quality classes (Dufrêne and Legendre, 1997; De Cáceres et al., 2010). The studied sites were categorized *a priori* in good, intermediate/fair and poor water quality

categories using the TARISS score (Kaaya et al., 2015); good: TARISS > 80, intermediate/fair: TARISS 50–80; poor: TARISS < 50. The classification was based on the Gower distance matrix and a hierarchical clustering analysis (Ward method) (Dufrêne and Legendre, 1997; Borcard et al., 2011). A good indicator taxon is mostly found in one site class and is present in most sites belonging to that class. The indicator value of a taxon varies between 0 and 1, attaining its maximum value when all individuals of one taxon occur in all sites of a single site class (Dufrêne and Legendre, 1997; Heino et al., 2005; Lumbreras et al., 2016). The significance of the indicator values for each taxon were tested via Monte-Carlo permutations (nperm = 999). The indicator value has two components: (i) a specificity/predictive value (component A) and (ii) a sensitivity/fidelity (component B). Specificity (A) is the probability that the surveyed site belongs to the target site class given the fact that the taxon has been found, while sensitivity (B) is the probability of finding the taxon in sites belonging to the site class.

All analyses of macro invertebrate assemblages were performed separately for the dry and the wet seasons to be able to contrast different drivers of diversity and assemblage structure. All statistical tests were performed in R version 3.1.2 (R Core Team, 2014) using the packages vegan, permute, packfor and indicspecies.

## 3. Results

## 3.1. Environmental variables

The summary statistics of the measured water quality variables are presented in Table S3 (Supplementary Information) as mean  $\pm$  standard deviation (SD). Significant differences between dry and wet seasons (Wilcoxon signed rank test, p < 0.05) were observed for chemical oxygen demand, total nitrogen, chlorophyll-a, turbidity and fluoride, Table 1 and Fig.S1 (Supplementary Information). Concentrations of phosphate, ammonia and nitrate showed no significant seasonal variation (Wilcoxon signed rank test, p > 0.05), however, the concentrations were higher in the wet than in the dry season, Table 1. Wilcoxon signed rank tests also showed significant differences in average velocity and average water depth (Wilcoxon signed rank test, p < 0.05) between wet and dry seasons (Table 1).

#### 3.2. General patterns of macroinvertebrate community structure

A total of 7530 macroinvertebrates corresponding to 54 families were collected in both the dry and the wet seasons (Table S4; Supplementary Information). The main taxonomic groups were Trichoptera, Ephemoroptera, Coleoptera, Hemiptera, Plecoptera Odonata, Decapoda, and Gastropoda. Diptera was the most diverse taxon with nine families, followed by Hemiptera with eight families, Coleoptera with seven families, and Ephemeroptera, Trichoptera and Odonata with five families each. Macroinvertebrate abundances, taxon richness and TARISS score were all higher in the dry season than in the wet season (Wilcoxon signed rank test, p < 0.05 (Table 1, Fig. S2; Supplementary Information).

# 3.3. The effect of environmental variables on macroinvertebrate biotic indices

Environmental variables significantly explained variation in taxon richness (dry: AIC = 175.68,  $R_{Pseudo}^2 = 0.27$ ; wet: AIC = 111.71,  $R_{Pseudo}^2 = 0.21$ ) and TARISS scores (dry: AIC = 578.94,  $R_{Pseudo}^2 = 0.51$ ; wet: AIC = 233.27,  $R_{Pseudo}^2 = 0.69$ ), Table 2. A back ward followed by forward selection identified different sets of significant environmental variables for taxon richness and TARISS scores, but land-use (forest and cropland, agricultural land, dense forest, and shrub land and thickets) and substrate composition had a significant effect on all the biotic indices in both seasons, Table 2. In the dry season communities were

#### Table 1

Results of Wilcoxon signed rank tests for environmental variables and macroinvertebrate biotic indices between dry and wet seasons in the Ruvu River catchment showing the z-statistics and p-values. Significance levels are indicated as follows: \*\*\* = p < 0.001, \*\* = p < 0.01, and \* = p < 0.05.

Variables		Mean Values	Mean Values		Wilcoxon rank sum test	
		Dry season	Wet season	Z	р	
Environmental	рН	7.67	7.71	-0.144	0.895	
	Electric Conductivity (µS/cm)		523.55	-0.137	0.899	
	Dissolved oxygen (mg/L)	6.64	6.85	-0.216	0.838	
	Temperature (°c)	23.81	20.26	-0.174	0.651	
	Chlorophyll a (µg/L)	90.05	32.04	-4.541	0.001***	
	Turbidity (ftu)		40.79	4.197	0.001***	
	Fluoride (mg/L)	0.24	0.16	-2.739	0.006**	
	Ammonia (mg/L)	0.15	0.18	-1.270	0.209	
	Nitrate (mg/L)	0.59	0.91	0.483	0.638	
	Orthophosphate (mg/L)	1.72	2.22	-0.745	0.462	
	Total Phosphorus (mg/L)	0.81	1.37	0.985	0.331	
	Total Nitrogen (mg/L)	0.89	1.53	1.754	0.042*	
	Chemical Oxygen Demand (mg/L)	15.90	30.10	2.823	0.005**	
	Average velocity (m/s)	0.38	1.29	4.541	0.001***	
	Average depth (m)	0.42	0.76	4.469	0.001***	
Macroinvertebrates	Abundance	237.48	41.41	1.714	0.046*	
	TARISS Score	64.15	33.33	-3.400	0.001***	
	Taxon richness	10.0	4.74	-3.747	0.001***	

limited by nitrogen, water velocity and turbidity while in the wet season chlorophyll-a, oxygen and phosphorous become important in explaining variation in taxon richness and TARISS scores.

# 3.4. The influence of environmental variables on macroinvertebrate assemblages

Generally, there was a significant effect of environmental variables on the macroinvertebrate assemblages both in the dry (in-stream variables: F = 3.38, p = 0.001; land-use: F = 3.165, p = 0.002) and in the wet (in-stream variables: F = 5.22, p = 0.001; land-use: F = 4.95, p = 0.01) seasons, Table 3. Local in-stream factors explained 36% and 32% of the total variation in the composition of macroinvertebrates in the dry and wet seasons respectively. Land-use explained 23% and 21% of the total variation in the composition of macroinvertebrates in the dry and wet seasons respectively. Forward selection identified different sets of significant environmental variables for macroinvertebrates between seasons, but substrate type (in-stream environmental factor) and land-use (agricultural land and shrub land and thickets) had a significant effect on macroinvertebrate assemblage in both seasons, Table 3. The PCA ordination plot for the visualization of the relationship between macroinvertebrates abundance data) and the most important explanatory variables retained in the forward selection plotted as supplementary variables (separately for the in-stream and land-use variables) for the dry and wet seasons are presented in Fig. 2.

Variation partitioning analyses revealed that the overall effect of instream environmental variables and land-use on macroinvertebrate

#### Table 2

Results of the generalized linear models with AIC and coefficients of determination  $R_{pseudo}^2$ , z-statistic and p-value of the most important explaining variables of the selected models for macroinvertebrate taxon richness and TARISS score in the Ruvu River catchment. Models are based on a backward followed by forward selection procedure aimed to maximize the potential variation in macroinvertebrate indices that can be explained by environmental variables. Significance levels are indicated as follows: \*\*\*= p < 0.001, \*\* = p < 0.01, and \* = p < 0.05. A (+) sign refers to a positive association, (-) sign refers to a negative association, and (ns) refers to not significant.

Explaining Variables	Season								
	Dry	Wet							
	Richness AIC = 175.68 $R_{Pseudo}^2 = 0.27$		TARISS AIC = 578.94 $R_{Pseudo}^2 = 0.51$		Richness AIC = 111.71 $R_{Pseudo}^2 = 0.21$		$TARISS$ $AIC = 233.27$ $R^2_{Pseudo} = 0.69$		
	z value	p value	z value	p value	z value	p value	z value	p value	
Dissolved oxygen	ns	ns	ns	ns	ns	ns	2.625	0.008 **	
Chlorophyll-a	ns	ns	6.281	0.0001***	1.858	0.036*	ns	ns	
Turbidity	-4.385	0.0001***	-2.354	0.038*	ns	ns	ns	ns	
Ammonia	-2.691	0.007 **	-4.982	0.012*	ns	ns	ns	ns	
Nitrate	ns	ns	ns	ns	-1.761	0.044*	ns	ns	
Total nitrogen	2.445	0.014*	ns	ns	ns	ns	ns	ns	
Total phosphorus	ns	ns	ns	ns	ns	ns	-2.628	0.008 **	
Velocity	-2.574	0.01*	-2.830	0.005**	ns	ns	ns	ns	
Substrate: GSM (gravel/sand/mud)	-4.466	0.0001***	-6.622	0.0001***	-1.511	0.028*	-3.655	0.0001***	
Substrate: Stones (cobbles/boulders/bedrock)	ns	ns	9.395	0.001***	ns	ns	2.669	0.007 **	
% Agricultural land	-4.168	0.001**	-5.354	0.0001***	-3.386	0.002**	-3.441	0.003**	
% Dense forest	ns	ns	1.967	0.034*	2.936	0.006**	ns	ns	
% Forest and cropland	4.239	0.001**	2.132	0.004**	ns	ns	2.271	0.001**	
% Shrub land	-1.697	0.032*	ns	ns	ns	ns	-1.914	0.018*	

#### Table 3

Results of the RDA analyses showing the global F, p-value and coefficients of determination ( $R^2_{Adjusted}$ ) of the full models, and F-statistic and p-value of the selected important environmental variables explaining macroinvertebrate assemblages in the Ruvu River catchment. Models are based on a forward selection procedure aimed to maximize the potential variation in macroinvertebrate assemblages that can be explained by environmental variables. Significance levels are indicated as follows: \*\*\* = p < 0.001, \*\* = p < 0.01, and \* = p < 0.05.

Season	Explaining Variable	F	p value	Global F	p value (global F)	${\rm R}^2_{\rm Adjusted}$
Dry	Instream variables			3.38	0.001 **	0.36
	Substrate	4.99	0.002**			
	Turbidity	2.62	0.011*			
	Ammonia	2.64	0.015*			
	Velocity	2.32	0.021*			
	Land-use variables			3.165	0.002**	0.23
	% Agricultural land	7.56	0.002**			
	% Shrub land and thickets	2.56	0.021*			
	% Forest and cropland	2.03	0.034*			
Wet	Instream variables			5.22	0.001 **	0.32
	Substrate	2.01	0.040*			
	Chlorophyll-a	11.97	0.001**			
	Land-use variables			4.95	0.01*	0.21
	% Agricultural land	6.32	0.001**			
	% Shrub land	2.53	0.018*			

community composition was similar in the dry season, Fig. 3. In the wet season, the direct effect of land-use on macroinvertebrate community composition was relatively higher than the in-stream environmental conditions, Fig. 3. A considerable fraction of compositional variation in macroinvertebrate community was explained by shared effects between in-stream environmental conditions and land-use factors.

## 3.5. Indicator taxa

Hierarchical clustering diagrams for site classification are presented in Fig. S3 (Supplimentary Information). In the dry season, three families (Baetidae, Potamonautidae and Heptageniidae) were indicators of good water quality (see Table 4). Baetidae had the highest indicator value (IndVal = 0.929), occurring in all good quality sites (B = 1.000) and was largely restricted to it (A = 0.8628). Potamonautidae and Heptageniidae occurred only in good water quality sites (A = 1.000). Thiaridae was an indicator taxon for intermediate water quality while Hirudinea and Hydrophilidae were indicators for poor water quality. Thiaridae occurred in all sites belonging to the intermediate water quality class (B = 1.000) and was largely (but not completely) restricted to it (A = 0.9541). Hirudinea and Hydrophilidae occurred in poor water quality sites only (A = 1.000), though not all poor water quality sites were housing these families (B = 0.444). In the wet season, three families (Baetidae, Hydropsychidae and Heptageniidae) were indicators of good water quality. Baetidae occurred in all good water quality sites (B = 1.000) and they were almost completely restricted to these sites (A = 0.9763). Hydropsychidae and Heptageniidae occurred only in sites with relatively good water quality (A = 1.000). Chironomidae were an indicator of poor water quality. Chironomidae occurred in all sites with relatively poor water quality (B = 1.000) but were not completely restricted to these sites (A = 0.6079).

## 4. Discussion

In this study, we assessed seasonal variation in benthic macroinvertebrate assemblages and water quality in the Ruvu River catchment in northeastern Tanzania. Our findings indicate that physicochemical water quality and macroinvertebrate community composition varied between seasons and that different sets of indicator taxa for water quality emerge in different seasons. Both in-stream environmental conditions and land-use factors influenced macroinvertebrates, but their relative importance also depended on the season. In the wet season macroinvertebrates seem to be limited by chlorophyll-a, oxygen and phosphorous while in the dry season, when water flow is lower, nitrogen and turbidity become important.

We initially hypothesized that land use effects on river quality may be more pronounced in the rainy season due to higher surface runoff from agricultural land. This seems to be confirmed as macroinvertebrates were less abundant and showed lower diversity in the wet season. This may seem counter intuitive given that water volumes, and thus available habitat is larger in the wet season. However, most likely the higher nutrient concentrations (particularly phosphate) and turbidity levels eliminate some sensitive taxa during the wet season (e.g., Heptageniidae and Perlidae). This trend is confirmed in other studies of tropical rivers (Harding et al., 1999; Ndaruga et al., 2004; Bere et al., 2016; Nhiwatiwa et al., 2017a). Although diversity was higher and water quality was better, also in the dry season there was substantial variation in macroinvertebrate assemblages among sites, but these were driven by different variables. Lower water flow in this season due to lower precipitation and increased water abstraction via irrigation may lead to accumulation of nitrogen containing nutrients in some river sites and low oxygen conditions that can locally decrease water quality and exclude some taxa. Some taxa such as Hirudinea and Potamonautidae emerge as indicator species for poor and good water quality respectively in this season but not in the wet season. This is consistent with our second hypothesis and indicates that different selection pressures limit the occurrences and resulting diversity of macroinvertebrates in both seasons. Seasonal variation in discharge leads to differences in wetted perimeter, hydraulic conditions, and habitat availability which may also affect benthic macroinvertebrates (Dallas, 2004). Furthermore, the differences in macroinvertebrate community composition between seasons can also be partly due to the fact that different taxa show differential success between seasons according to their particular resilience or resistance traits (such as colonization and establishment abilities) (Blanchette and Pearson, 2013; Botwe et al., 2015).

The RDA and generalized linear models showed that water velocity, substrate type, turbidity and nutrients were the most important local instream environmental variables that explained macroinvertebrate community structure. Stream water velocity has been indicated in many studies to be strongly related to the community composition of benthic macroinvertebrates (e.g., Poff et al., 1997; Sandin and Johnson, 2004; Allan and Castillo, 2007; Belmar et al., 2013; Pan et al., 2013). This is because, the flow velocity configures stream morphology, bed stability, and consequently the availability of aquatic habitats for in-stream or-ganisms (Belmar et al., 2013). For example, high flow velocity is re-garded not only to scour macroinvertebrates directly but also determines other habitat conditions by influencing the transport of



Fig. 2. Principal component analysis ordination bi-plots illustrating the relationship between macroinvertebrate assemblages and the most important explanatory variables plotted as supplementary variables: (a and b) in-stream environmental variables, and (c and d) land use variables in the dry and wet seasons. Only taxa with more than 30% contribution to the total variation are plotted. Baet = Baetidae, Caen = Caenidae, Heptag = Heptageniidae, Ephem = Ephemerythidae, Leptop = Leptophlebidae, Hydrop = Hydropsychidae, Ecnom = Ecnomidae, Leptoc = Leptoceridae, Philop = Philopotamidae, Hydrop = Hydroptilidae, Aesh = Aeshinidae, Libell = Libellulidae, Coenag = Coenagrionidae, Chlorocy = Chlorocyphidae, Olig = Oligochaeta, Hirud = Hirudinea, Physid = Physidae, Lymnae = Lymnaenidae, Planor = Planorbidae, Thiar = Thiaridae, Sphaer = SphaeriidaeHydrach = Hydrachnidiae, Gyrin = Gyrinidae, Elmidae, Elmidae, Psephen = Psephenidae, Helod = Helodidae, Hydroph = Hydrophilidae, Torridin = Torridincolidae, Noter = Noteridae, Potamon = Potamonautidae, Atheric = Athericidae, Taban = Tabanidae, Culic = Culicidae, Chiron = Chironomidae, Tipul = Tipulidae, Simul = Simuliidae, Atvid = Atvidae.Scyomyz = Scyomyzidae, Dixid = Dixidae, Psychod = Psychodidae, Ceratop = Ceratopogonidae, Empid = Empididae, Gerrid = Gerridae, Veliid = Veliidae, Naucor = Naucoridae. Pleid = Pleidae, Nepid = Nepidae, Hebrid = Hebridae, Corixid = Corixidae, Pyralid = Pyralidae,Perlid = PerlidaeNotonem = Notonemouridae, Planar = Planaridae, Turb = Turbidity, GSM = Gravel/Sand/Mud, RV = Ruvu River, RD = Dehu River, RH = Ghona River, RS = Soko River.

sediments (Poff et al., 1997; Sandin and Johnson, 2004; Belmar et al., 2013; Pan et al., 2013). As such, habitats are more stable during the dry season, thereby allowing longer time macroinvertebrates colonization and subsequently increment of the species number and abundance (Principe et al., 2007). In addition, water velocity also affects in-stream food delivery and oxygen content (Sandin and Johnson, 2004; Allan and Castillo, 2007) which directly affects the existence of in-stream biota.

Substrate type was important both in the dry and the wet season. Macroinvertebrate biotic indices were positively correlated with the stone substratum while negatively associated with GSM substratum. Stone substratum, particularly cobbles, has been indicated to support a large number of benthic macroinvertebrate taxa due to the availability of diverse microhabitats that provide refuge from currents and predation, attachment for filter-feeding taxa, food for herbivores and detritivores, and exit points for emerging insects with aerial adult stages (Brooks et al., 2005; Allan and Castillo, 2007; Pan et al., 2013; Jun et al., 2016). In contrast, habitats with fine substrate support few macroinvertebrate taxa: this is related to habitat instability, detritus shortage, and unavailability of refugia (Principe et al., 2007; Chakona et al., 2008). Similarly, several other studies conducted elsewhere have reported a strong correlation between substrate type and



**Fig. 3.** Unique and shared contributions of in-stream environmental variables and land-use variables on the macroinvertebrate assemblages in the (a) dry and (b) wet seasons. Significance levels are indicated as follows: \*\*\* = p < 0.001, \*\* = p < 0.01, and \* = p < 0.05. Percentages represent explained variation by each component.

## Table 4

Results of the macroinvertebrate indicator taxon analysis listing indicator species for each water quality class (G = good, IM = intermediate and PR = poor) and for each season. Indicator values and associated P values are provided as well as the specificity (A) and sensitivity (B) scores. Significance levels are indicated as follows: \*\*\* = p < 0.001, \*\* = p < 0.01, and \* = p < 0.05.

Season	Water Quality	Indicator taxa	Indicator Value	p value	Α	В
Drv	G	Baetidae	0.929	0.008**	0.8628	1
5		Potamonautidae	0.816	0.016*	1	0.6667
		Heptageniidae	0.73	0.043*	1	0.5333
	IM	Thiaridae	0.977	0.003**	0.9541	1
	PR	Hirudinea	0.667	0.044*	1	0.4444
		Hydrophilidae	0.667	0.045*	1	0.4444
Wet	G	Baetidae	0.988	0.001 **	0.9763	1
		Hydropsychidae	0.886	0.003 **	1	0.7857
		Heptageniidae	0.845	0.035 *	1	0.7143
	PR	Chironomidae	0.78	0.029*	0.6079	1
	IM + PR	Chironomidae	0.928	0.002**	0.9849	0.875

macroinvertebrate communities (Ciutti et al., 2004; Sandin and Johnson, 2004; Li et al., 2012; Jun et al., 2016). Li et al. (2012) indicated that substratum degradation can perturb the macroinvertebrate community even when water quality remains good. In our study area fine sediment from agricultural land can cover valuable gravel and cobble habitat for macroinvertebrates. Hence, part of the substrate effect we detected in this paper may still be traced to negative impacts of agriculture.

Our study showed that agricultural and shrub land and thickets land-use types were the most important land use variables influencing macroinvertebrate assemblages in both seasons. The responses were reflected in the declines in taxon richness and TARISS scores with agricultural land-use. These relationships were consistent with other studies describing changes in macroinvertebrate communities in agricultural catchments (Richards et al., 1996; Allan, 2004; Collier, 2008; Magierowski et al., 2012; Nhiwatiwa et al., 2017a). These patterns may be driven by multiple mechanisms common to all agricultural land-use, such as changes in water quality, habitat alteration, loss of riparian zones and dominance of fine sediments (Magierowski et al., 2012; Botwe et al., 2015). The remaining unexplained variation in macroinvertebrate assemblages might be a result of other gradients such as pesticide and heavy metal concentrations, biotic interactions (Al-shami et al., 2011; Nhiwatiwa et al., 2017a), or a set of multiple stressors (Dalu et al., 2017b) which were not quantified in our study.

The variation partitioning showed that in-stream environmental variables and land-use manage to explain similar amounts of variation in macroinvertebrate communities in the dry season. In contrast, the unique effect of land-use explained a substantial fraction of variation in macroinvertebrate assemblages in the wet season. This pattern could be realized through effects of land-use on macroinvertebrate communities via run-off (carrying sediment and various nutrients from the catchment area into the river system) in the wet season (Karaouzas et al., 2007). In addition, agricultural activity which appeared to be the main stressor for macroinvertebrates in the catchment, further intensify during the wets season. The variation partitioning shows that a substantial fraction of the variation is jointly explained by these two sets of variables. This fraction reflects that some land-use variables and instream environmental variables tend to be correlated in the field and thus their contribution to biotic responses cannot be separated. Hence it represents variation in macroinvertebrates that is explained by the variables, but we cannot statistically attribute the effect to

environmental or land-use variables, respectively.

Although the indicator taxon analyses showed that Baetidae and Heptageniidae were indicator taxa of good water quality conditions in both seasons, but there was a substantial variation in indicator taxon between seasons. For example, Hydropsychidae and Potamonautidae showed to be an indicator taxon of good water quality conditions in the wet and dry seasons respectively. Several studies have shown that Ephemeroptera, Plecoptera, and Trichoptera (EPT) taxa are sensitive to pollution (Rosenberg and Resh, 1993; Barbour et al., 1999; Soininen and Kononen, 2004; Masese et al., 2009; Al-shami et al., 2011). Therefore, their presence is often an indication of good water quality conditions, similar to the present study findings. A study of Kaaya et al. (2015), however, indicated Potamonautidae to be tolerant of pollution with a lower TARISS score, while we found them to be indicative of good water quality conditions. This family likely comprises both pollution tolerant and sensitive species. Judging water quality in the region based on the presence of members of this family is therefore not possible unless differential responses of genera and species within this family are known. Chironomidae was an indicator of poor water quality conditions in the wet season while Hirudinea showed to be indicator taxa of poor water quality condition in the dry season. When streams are disturbed, taxa that are sensitive to pollution will be eliminated, leaving communities to be dominated by only taxa that are resistant (i.e., able to survive the impacts) or resilient (i.e., having efficient recovery mechanisms). Chironomids, for example, are capable of surviving low dissolved oxygen levels and high turbidity and can exploit excess nutrients, hence dramatically increase in abundance in polluted water (Margues et al., 1999; Özkan et al., 2010). This explains why they appear as indicator taxa in the wet season but not in the dry season, when nutrients and turbidity are not an issue.

The macroinvertebrate biotic index developed for Tanzania (i.e. TARISS) was shown to be a complementary source of information compared to richness and analyses of composition. In the predictive model, TARISS was significantly affected by several variables that had no effects on richness. Hence, it does what it was intended for: providing a metric that integrates both richness and known sensitivity of taxonomic groups. A limitation of this study is that only two seasons were studied and, although they were quite representative in terms of typical weather conditions, longer-term monitoring would be required to validate to what extent the reported dynamics are indeed general. It would also be valuable to know to what extent water quality responds to particularly dry or wet years and to what extent variation in agricultural runoff among years has strong effects on biota.

The study highlights that current agricultural practices are indeed in all likelihood affecting the macroinvertebrate assemblages in this river and that monitoring them via their indicator species may help to identify sites with poor water quality where remediation actions can be taken. Riparian buffer zones may help to alleviate some of the observed negative effects of agricultural activities on the river system in the wet season while limiting irrigation return flows may improve water quality in the dry season. In addition, macroinvertebrate monitoring may also help to detect effects of extreme weather events expected under current scenarios of global climate change. Many perennial rivers are likely to be become non perennial and this risk also exists for the Ruvu river catchment. During the dry parts of the year some sections of the river no longer have flowing water with stagnant pools remaining with poor water quality. In fact, many rivers in the area currently suffer potential degradation due to increased water abstraction, nutrient enrichment and siltation resulting from land-use change mainly for agricultural activities and settlement. The combination with climate change may lead to further deterioration of water quality with serious consequences for the growing population that is predominantly reliant on river water as drinking water and for irrigation purposes.

#### 5. Conclusion

The study generated some new generic insights into the ecology of this type of tropical rivers by showing that different indicator species as well as different drivers of water quality and macroinvertebrates can be important during dry and wet seasons. Using a combined multivariate approach of indicator species analysis and biological indices allowed a more profound ecological diagnosis of the ecological condition of the Ruvu River and (re)confirmed the usefulness of benthic macroinvertebrates in monitoring schemes of river systems. Overall, this study advocates for a reinstatement of an effective nation-wide river monitoring system in Tanzania with monitoring taking place both in the dry and in the wet season. In addition, the poor current state of many river sites urges for the development of awareness programs coupled to possible financial compensations for farmers to reduce surface runoff via the establishment of effective riparian buffer zones.

## CRediT authorship contribution statement

**Grite Nelson Mwaijengo:** Conceptualization, Methodology, Investigation, Formal analysis, Writing - original draft, Data curation, Writing - review & editing. **Bram Vanschoenwinkel:** Methodology, Formal analysis, Conceptualization, Writing - review & editing, Supervision. **Trevor Dube:** Formal analysis, Writing - review & editing. **Karoli Nicholas Njau:** Supervision, Funding acquisition, Writing - review & editing. **Luc Brendonck:** Conceptualization, Supervision, Funding acquisition, Writing - review & editing.

## **Declaration of Competing Interest**

The authors declare that they have no conflicts of interest to disclose

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.plantsci.2004.08.011

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