

## Ecological importance of low-order streams to macroinvertebrate community composition in Afromontane headwater streams

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### ABSTRACT

Low-order streams contribute to the abiotic and biotic character of large rivers and are renowned for harboring unique forms of aquatic flora and fauna. However, most studies on headwater streams mainly focus on the mainstems and overlook the contribution of the tributary systems. Moreover, low-order streams are generally overlooked in legislation and bioassessment programs, and consequently not protected in many countries. To contribute to the recognition of the ecological importance of low-order streams, this study focused on determining whether river network characteristics and associated physico-chemical parameters can be used to effectively predict the variabilities in macroinvertebrate assemblage characteristics between first-order and second-order streams in the headwaters of the Nzoia River Basin, Kenya. The study quantified the structural and functional community composition, diversity, similarity, and richness of macroinvertebrate communities between the two river systems. Dissolved oxygen, coarse particulate organic matter, conductivity, stream width, depth, discharge and flow velocity were the main predictors of the diversity and distribution of macroinvertebrates in the first order and second-order streams. The first order streams recorded higher abundance of macroinvertebrates than the second-order streams. Taxa from families Ephyridae, Elmidae, Gomphidae, and genera *Euthraulus*, *Neoperla*, *Orthothrichia* and *Prosopistoma* were limited to the second-order stream sites while families Ceratopogonidae, Pisuliidae, Dytiscidae and genus *Trichosetodes* occurred exclusively in the first-order stream sites. Collector-filterers and collector-gatherers were the most abundant functional feeding groups (FFGs) in the two river systems. Scrapers were abundant in the second-order stream while shredders were abundant in the first order streams. The distinctness in the structural and functional composition of macroinvertebrates between the two river systems suggests that linkages among streams in a network as exemplified in the Nzoia River Basin, support and foster biodiversity.

### 1. Introduction

The diversity of life in headwater streams contributes to the overall diversity of aquatic communities throughout the river network, including the riparian zone (Clarke et al., 2008; Chakona et al., 2018; Matomela et al., 2021). Whereas large rivers play significant roles in contributing to the diversity of low-order streams (Gavioli et al., 2019), tributaries or low-order streams also fulfil important ecosystem functions that include structuring mainstem channel habitats, increasing ecosystem productivity, and enhancing network heterogeneity (Clarke et al., 2008; Finn et al., 2011; Ferreira et al., 2023), thus, playing major

ecological roles in fluvial networks (Rice et al., 2008; Finn et al., 2011). Low-order streams play these important roles by generating pathways for the transportation of organic and inorganic matter and nutrients by connecting aquatic and terrestrial ecosystems, contributing organisms (i.e., genetic reserves for endemic taxa, spawning sites), providing distinct habitats for aquatic biota, and frequently serving as refugia for aquatic organisms (Lepori et al., 2005; Clarke et al., 2008; Milner et al., 2019). The low-order streams contain a high diversity of species that are specialists and are restricted to these systems, and they may harbour undescribed and/or endemic species (Clarke et al., 2008; Samways and Sharratt, 2010; Finn et al., 2011; de Moor and Day, 2013). Similarly, the

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migration of aquatic biota from the river's mainstem to tributary habitats, and vice versa, further influences the population and dispersal patterns thus affecting meta-community dynamics and biodiversity in these ecosystems (Sor et al., 2021).

Biodiversity and community assembly processes in streams are influenced by several factors, including longitudinal changes in stream size, lateral interactions with the surrounding terrestrial landscape as well as changes in in-stream biotic and abiotic characteristics at the scale of stream sections or reaches (Vannote et al., 1980; Jawad and Waryani, 2021; Green et al., 2022; Graziano et al., 2022). Because of their close connection with the terrestrial environment, headwater streams provide a diverse range of unique habitats (such as dead wood, and leaf litter entering the stream) for a variety of uniquely specialized species such as macroinvertebrate shredders that utilize these resources (Meyer et al., 2007; Ferreira et al., 2023). Additionally, these systems link emerging and drifting insects with the main river channel and sub-sequentially the downstream reaches of these streams as well as surrounding terrestrial environments (Torgersen et al., 2008; Heino and Koljonen, 2022). Studies of macroinvertebrate diversity in headwater streams show that taxonomic richness is highly variable when stream systems are viewed as networks at the landscape scale. For instance,  $\alpha$ -diversity may be low in individual headwater streams but with a high gamma diversity among headwater streams (Clarke et al., 2008). Assessing the species composition in mainstem sites as well as the interconnected tributary sites is critical in understanding community assembly concerning species diversity and fluvial connectivity in these systems (Torgersen et al., 2008; Heino and Koljonen, 2022).

The patterns in macroinvertebrate assemblage structure are valuable in identifying community differences between tributaries and their mainstems. Macroinvertebrates usually vary in their diversity and occurrences along stream channels depending on the abiotic and biotic parameters and their specific ecological requirements of different species (Thirion, 2016; Masese et al., 2021). Abiotic factors such as substrate characteristics, water volume and velocity, water physico-chemistry, and riparian characteristics vary between tributaries and their mainstems (Vasconcelos et al., 2013; Ferreira et al., 2023), which in turn influences the processing of organic matter, nutrient cycling and the distribution and abundance of biotic communities (Liébault et al., 2008; Jawad and Waryani, 2021; Ferreira et al., 2023). Changes in macroinvertebrate functional feeding groups (FFGs) and taxonomic structure typically occur along a river continuum.

Based on the River Continuum Concept (RCC) (Vannote et al., 1980), tributaries receive higher allochthonous organic matter inputs, and thus the structure of the aquatic communities is different from larger mainstems. Shredders that feed on coarse particulate organic matter in these stream channels are most likely to occur in small headwater tributaries (Sor, et al., 2021) while filter-feeders and collector-gatherers are more dominant in sections where there are sufficient fine particulate food resources supplied from upstream sections (Masese et al., 2014a). Scrapers thrive in mainstems where the open canopy supports the primary production of algae, which is the main energy source for this group of macroinvertebrates (Vannote et al., 1980).

Studies on macroinvertebrate assemblage structure in streams view fluvial systems as linear, rather than as networks, thereby overlooking the contribution of these networks to community assembly in headwater streams (Heino et al., 2005; Longing et al., 2010; Jiang et al., 2011). Studies on the structural and functional composition of macroinvertebrate assemblages in stream networks are fundamental in understanding the contribution of each system to biodiversity management. Previous research has largely focused on the longitudinal zonation of macroinvertebrates in river systems (Vannote et al., 1980; Taybi et al., 2020; Englmaier et al., 2020), with only a few exceptions focusing on the tributary-mainstem channel linkages (e.g., Benda et al., 2004; Rice et al., 2008; Tavernini and Richardson, 2020).

Studies focusing on aquatic communities in low-order streams have highlighted the threat they increasingly face from human activities

(Meyer et al., 2007; Matomela et al., 2021; Heino and Koljonen, 2022). The persistence of anthropogenic activities such as riparian clearance and introduction of exotic species, land use change and deforestation threaten these ecosystems due to their small size (Vörösmarty et al., 2010; Arthington, 2012; Ferreira et al., 2023). In the Afromontane region, there is a paucity of studies that have focused on disentangling the existing variabilities in the functional and structural composition of macroinvertebrates between the low-order streams and their mainstem channels. Previous studies in Afromontane streams have focused on river mainstems but not as networks (Kasangaki et al., 2008; Musonge et al., 2020; Yegon et al., 2021; Matomela et al., 2021). This knowledge gap formed the basis of our study.

We investigated differences in macroinvertebrate structural and functional composition between five first-order sites and 5 second-order sites in the Afromontane headwater streams of the Nzoia River Basin, Kenya. In the Nzoia River Basin, the water quality in mainstem sites in the lower reaches has been severely impacted by changing land use over the years while the upper reaches remain largely intact (Masese et al., 2009, 2023; Cumberlidge and Clark., 2010; Mugagga et al., 2012; Mwangi et al., 2020; Yegon et al., 2021). In this regard, headwater streams can preserve unique and native taxa that can be lost due to land use change or human disturbance (Masese et al., 2014a). The study aimed at determining whether stream size and associated physico-chemical water parameters arising from the evolution of channel systems can be used to describe and predict macroinvertebrate assemblages and diversity in these streams. We examined whether the composition and diversity of macroinvertebrates at first-order sites were distinct in terms of structural and functional composition (i.e., assemblage structure) from those in the second-order sites. We hypothesized that physical habitat heterogeneity and complexity within the first-order sites contribute to a higher taxonomic diversity than the second-order sites that have been largely homogenized by human activities. The higher habitat quality (characterized by the substrate composition and physico-chemical water variables) would favour the occurrence of a higher density and diversity of macroinvertebrates in the first-order stream sites than in second-order sites.

## 2. Materials and methods

### 2.1. Study area

The study was conducted in the montane streams draining Mt. Elgon in western Kenya. The first-order streams form the headwaters of the Nzoia River Basin. The Mt. Elgon landscape of Kenya is partly in Trans-Nzoia and Bungoma counties in western Kenya. It has a size of 72,874 ha and is located between latitudes 0° 47' N and 0° 54' N and longitudes 34° 34' E and 34° 45' E (Yegon et al., 2021). The Nzoia River is the largest in Kenya's Lake Victoria Basin. It drains the Afromontane forests in the upper reaches of the Mt. Elgon National Park (Musau et al., 2015), but extensive land use change from forestry to agriculture and grazing lands has significantly reduced native forest cover (Okeyo-Owuor et al., 2011; Petursson et al., 2013; Mwangi et al., 2020; Yegon et al., 2021). To avoid the confounding factors caused by changes in land use in the study area, only streams from forested catchments were used (Fig. 1). The sampled second-order stream sites were in the altitudinal range of 2239–2407 m asl, while the sampled first-order stream sites were within an altitudinal range of 2246–2435 m asl. Information on land use, climatic characteristics and community economic activities are presented in detail in the supplementary material.

### 2.2. Field sampling

We sampled a total of ten sites (five paired sites in first-order and second-order sites). The first-order sites were first-order streams while the second-order sites were second-order streams with all the streams being perennial. Sampling was done during the rainy medium discharge

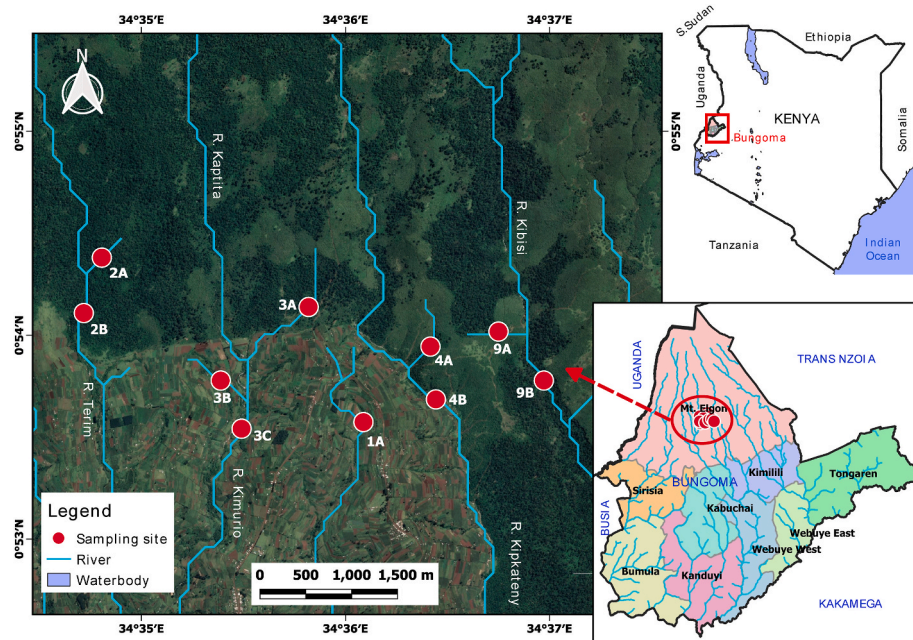


Fig. 1. Map of the study area and the sampled sites. Sites 2A, 3A, 3B, 4A, and 9A represent the first-order sites while 1A, 2B, 3C, 4B and 9B represent the second-order sites.

conditions (short rains) in October and November 2019. Macroinvertebrates were sampled and environmental variables were recorded within a 100-m representative reach at the sampling sites. At each sampling site, we recorded *in situ* physico-chemical variables that included dissolved oxygen (DO; mg L<sup>-1</sup>), temperature (°C), electrical conductivity (EC; μS cm<sup>-1</sup>) and pH using a YSI multiprobe water quality meter (556 MPS; Yellow Springs Instruments, Yellow Springs, OH, USA). The substrate types that constituted more than 5% coverage in each site were identified and recorded as presented in Table 1.

At each site, triplicate water samples of known quantity (500–1000 ml of water depending on water turbidity at each site) were filtered through pre-combusted (at 450 °C for 4h) and pre-weighed GF/F Whatman filters (diameter 47 mm, pore size = 0.7 μm). The filters were then transported to the laboratory in cooler boxers for analysis of total suspended solids (TSS) and particulate organic matter (POM).

Table 1

Sites description; sites, coordinates, altitude, distance from the river source and dominant substrate. RDS = rivers distance from source.

Site code	Channel System	RDS (km)	Coordinates		Altitude (m)	Dominant substrate (%)
			Latitude	Longitude		
2A	First-order	0.57	00° 54' 32" N	34° 36' 10" E	2380	85% CPOM
3B	First-order	1.8	00° 53' 44" N	34° 35' 16" E	2347	60% Macrolithal
3C	First-order	0.63	00° 54' 08" N	34° 35' 51" E	2435	55% Akal
4A	First-order	0.3	00° 54' 04" N	34° 36' 01" E	2350	50% Macrolithal
9A	First-order	0.26	00° 54' 01.3" N	34° 36' 54" E	2246	50% Woody Debris
1A	Second-order	1.36	00° 54' 25" N	34° 35' 15" E	2317	80% Microlithal
2B	Second-order	1.96	00° 54' 46" N	34° 36' 06" E	2407	70% Macrolithal
3C	Second-order	12.6	00° 53' 28.8" N	34° 35' 21.2" E	2239	65% Macrolithal
9B	Second-order	22.75	00° 54' 10" N	34° 37' 03" E	2298	60% Macrolithal
4B	Second-order	4.19	00° 53' 45.28" N	34° 35' 56.28" E	2293	85% Macrolithal

The biomass of coarse particulate organic matter (CPOM) was estimated by collecting triplicate benthic samples from each site. At each site, a quadrat (0.5 × 0.5 m<sup>2</sup>) was thrown haphazardly in the stream, and CPOM samples were collected from within the quadrat. The CPOM samples were placed in Ziploc™ bags and transported to the laboratory for processing.

At each sampling site, measurements of water depth and flow velocity were determined. Stream widths were taken using a measuring tape along several points of 10-m intervals over a 100-m reach. Transects were delineated at several points of the designated reach depending on channel shape and stream width. At each of the 10 m interval transects water depth and flow velocity were also measured. Water velocity was measured with a velocity plank (Matthews, 2018) and depth was measured with a meter rule. Stream discharge was calculated using the velocity–area method (Wetzel and Likens, 2000).

### 2.3. Sampling of macroinvertebrates

Sampling was done once in each of the sampling sites. At each site, one macroinvertebrate composite sample was taken following the multi-habitat sampling approach (AQEM sampling manual, 2002). Following this protocol, 20 sub-samples from different habitat types constitute one Multi-Habitat-Sample (MHS). The sampling of available habitats depends on the proportion of their presence within a sampling reach. At each site, 20 sampling units were collected from substrate types with more than 5% coverage within a representative reach that was 100 m long. The selection of defined habitats is based on the principle that each habitat is colonized by a unique assemblage of macroinvertebrates (Rosenberg and Resh, 1993). The proportion of each substrate type per site (Table 1) determined the number of units among the 20 MHS samples that were collected from that particular substrate type. Therefore, the initial identification of the substrate types and percentage coverage of the streambed was necessary. The sampling of macroinvertebrates was done by disturbing the substrates and collecting the dislodged organisms using a multi-habitat sampling net (1000 μm mesh size). In stony substrates where benthic invertebrates tend to attach, the substrate was scrubbed with a brush and organisms washed into the net. An area of 0.0625 m<sup>2</sup> was sampled for each sampling unit. Sampling was done from downstream to upstream within a reach to minimize drift.

The macroinvertebrate samples were preserved in 95% ethyl-ethanol packaged and stored in cooler boxes for transportation to the laboratory for further processing.

#### 2.4. Laboratory analyses

Total suspended solids (TSS) and POM were determined by drying the GF/F filters with embedded sediments at 60 °C for 72 h to attain constant weight. The filters were then reweighed using an analytical balance (Secura 124-1S; Sartorius; 0.0001 g) for gravimetric determination of TSS. The filters were then ashed at 450 °C for 4 h in a muffle furnace and reweighed for the determination of POM as the difference between the TSS and ash-free dry mass (APHA, 2005). The CPOM fractions were dried in an oven at 60 °C until a constant weight was attained and weighed for biomass estimation.

Macroinvertebrates were identified under a dissecting microscope to family or genus level with the aid of several keys and guides (Gerber and Gabriel 2002; Day and de Moor 2002a, 2002b; de Moor et al., 2003a, 2003b; Merritt et al., 2008). Functional feeding groups (FFGs) were then assigned to the identified taxa based on Merritt et al. (2017) and Masele et al. (2014a) and references therein. Five major FFGs were identified: shredders, scrapers, predators, collector-filterers and collector-gatherers. Complex and large samples were sub-sampled into smaller fractions for easier and thorough sorting. This was done by dividing the larger samples into equivalent fractions of (1/2, 1/4, and 1/8) and working on each fraction at a time. All animals in each of the fractions were sorted and identified and used in the analysis.

#### 2.5. Data analysis

Descriptive statistics (means  $\pm$  standard deviation) were used to present spatial variation in water quality variables in the different sites. Dissolved oxygen (DO), temperature and EC were expressed as means  $\pm$  SE in the first-order and second-order sites. pH at each of the sites was expressed as a range between 1 and 14 and not calculated as means. Independent t-tests were used to test for differences in the physico-chemical water variables and stream size variables between first-order and second-order sites. Principal component analysis (PCA) was used to reduce the dimensionality of the physico-chemical and stream size data. We included two principal components in describing the water quality and stream size variables in the second-order and first-order sites. The PCAs were statistically assessed using permutational multivariate analysis of variance (PERMANOVA) based on Bray-Curtis dissimilarity matrices (McArdle and Anderson, 2001).

The structure and composition of the macroinvertebrate community in the different channel systems were described using abundance and richness metrics. Independent t-tests were used to test for significant differences in the macroinvertebrate abundance and richness data between the channel systems. The diversity, richness and composition of macroinvertebrates were summarized using structural diversity indices. Our richness metrics were restricted by our taxonomic resolution, which was mainly done at the family level. Shannon's diversity index ( $H'$ ) was derived as a measure of diversity (Magurran, 2004), and an associated  $H'/H'$  max index (Pielou, 1975) was used as a measure of evenness. The reciprocal form of the Simpsons index (1-Ds) (Simpson, 1949) was used as a measure of species richness.

Indicator species analysis was employed to identify the most discriminant taxa for first-order and channel sites. The statistical significance of the indicator species values was evaluated using a Monte Carlo test randomization procedure with 4999 permutations (Dufrene and Legendre, 1997). The test statistic (an Indicator Value [IV]), which has a range from 0 (zero) to 100, with a higher value showing perfect indication), was calculated. The species-indicator value index reflects the predictive association value of a species as a bio-indicator of a given environmental condition (De Cáceres and Legendre, 2009). A higher indicator value shows a higher preference for a variable to belong to a

particular group by combining the information on the concentration of species abundances in a particular group and the degree of occurrence in that particular group. These tests were implemented in PCORD 5 (Grandin, 2006).

A one-way analysis of similarities (ANOSIM) was used to compare average rank similarities in macroinvertebrates' taxa composition between the two channel systems (first- and second-order sites). This analysis was performed to check if macroinvertebrates changed in composition between the two channel systems (first- and second-order sites). ANOSIM calculates the R-statistic, which is a test statistic that varies between 0 and 1, with higher values indicating bigger differences between the factors being compared.

We used beta diversity to examine patterns in local site contributions (LCBD) to the overall beta diversity for the first-order and second-order sites. We further partitioned beta diversity into nestedness and turnover components. Nestedness describes the dissimilarities arising because of one community having more species than the other while turnover describes species replacement where species replace each other along the gradient. Following Legendre and De Cáceres (2013), we first Hellinger-transformed species abundance or presence-absence community matrix, and subsequently calculated the total beta diversity (BD total) and LCBD value for each site using the "adespatial" package in R (Dray et al., 2018). Sorensen similarity index (Sorensen, 1948) was calculated as a measure of species similarity between the two systems (first-vs second-order sites). Non-metric multidimensional scaling (nMDS) was thereafter used to visualize the macroinvertebrate community composition in the first- and second-order sites. Dissimilarity matrices based on the Bray-Curtis coefficients (Bray and Curtis, 1957) were employed and the goodness of fit of the ordination was assessed by the magnitude of the associated stress value. Permutational multivariate analysis of variance (PERMANOVA) was used to test for significant differences in the nMDS ordination.

Canonical correspondence analysis (CCA) was used to elucidate relationships between the structural and functional composition of macroinvertebrates and environmental variables. The outputs were displayed as triplot in which the plotted points for the macroinvertebrates in the first- and second-order sites could be related to the physico-chemical and habitat variables that were represented as rays. Statistical analyses were performed with PCORD (ver. 5.0; Grandin, 2006) and R version 3.3.3 (R Development Core Team, 2017).

### 3. Results

#### 3.1. Variation in water physico-chemical and stream size variables between the two channel systems

There were significant differences ( $p < 0.05$ ) observed in some of the physical and chemical characteristics between the first- and second-order sites. The main substrate type recorded in the second-order sites was macrolithal (of grain size 20–40 cm) while coarse particulate organic matter (CPOM) dominated the substrate in the first-order sites (Table 1). There were significant differences in the channel width, water depth, stream discharge, water velocity and dissolved oxygen concentration (DO) between the two systems. As expected, stream discharge, width and water depth were higher in the second-order sites than in the first-order sites. However, DO was higher in first-order sites than in the second-order sites (Table 2).

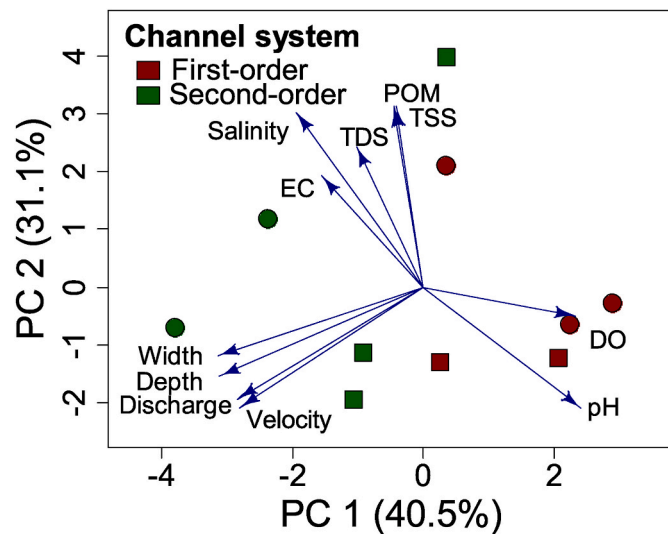
The first axis (PC1) in PCA ordination of water physico-chemical and stream size variables explained 40.5% of the total dataset variance, whereas the second axis (PC2) explained 31.1% of the total variance (Fig. 2). PCA loadings showed that the higher values in PC1 were explained largely by the stream size variables which were higher in second-order sites. The PC2 was a water quality gradient that separated sites according to the amount of total suspended solids (TSS), electrical conductivity and POM, which were higher in the second-order sites. High discharge and velocities also characterized second-order sites

**Table 2**

The mean ± SE of physico-chemical characteristics of the first-order and second-order stream sites. DO = Dissolved Oxygen, TSS = Total Suspended Solids, TDS = Total Dissolved Solids. Altitude and pH have been provided in ranges (Min-Max).

Variable	First-order	Second-order	t-value	p-value
Altitude (m)	2246–2435	2239–2407		
pH	7.0–8.0	7.0–7.7		
Channel width (m)	2.30 ± 0.94	6.54 ± 1.24	2.57	0.029*
Water depth (m)	0.12 ± 0.01	0.26 ± 0.01	2.55	0.034*
Discharge (m <sup>3</sup> /s)	0.32 ± 0.02	0.96 ± 0.02	2.31	0.048*
Current velocity (m/s)	0.42 ± 0.06	0.76 ± 0.04	2.37	0.030*
Temperature	14.80 ± 0.44	15.10 ± 0.48	2.57	0.406
DO (mg/L)	10.62 ± 0.04	8.02 ± 0.03	2.78	0.054*
Conductivity (µS/cm)	66.30 ± 1.26	76.50 ± 1.26	2.45	0.330
TSS (mg/L)	25.32 ± 2.56	39.76 ± 4.24	2.54	0.361
TDS (mg/L)	0.072 ± 0.005	0.088 ± 0.001	2.36	0.467

\* Significance  $p < 0.05$ .



**Fig. 2.** PCA biplot for physico-chemical and abiotic stream variables of the streams within the network. DO = dissolved oxygen concentration, TSS = total suspended solids, TDS = total dissolved solids, POM = particulate organic matter, EC = electrical conductivity.

while higher DO concentration and pH characterized the first-order sites (Fig. 2).

**3.2. Structural composition of the macroinvertebrates between first- and second-order sites**

A total of 185,504 ind/m<sup>2</sup> macroinvertebrate individuals were collected from the ten sites. Of these, 115,312 ind/m<sup>2</sup> were collected from the first-order sites which was significantly different ( $t_5 = 1.80, p = 0.03$ ) than the 70,192 ind/m<sup>2</sup> taxa collected from the second-order sites (Supplementary Table 1). The collected and identified taxa were taxonomically classified and comprised taxa from 11 orders and 42 families. The order Diptera, represented by eleven families, had the highest number of families. Trichoptera was represented by a total of nine families, Ephemeroptera by six families, Coleoptera by five families, Mollusca by three families, Odonata by two families, Plecoptera by one family and genus (Perlidae; *Neoperla*), Decapoda by one family and genus (Potamonautidae; *Potamonautes*). Hirudinea (Glossiphoniidae), Tricladida (Planariidae) and Oligochaeta (Lumbriculiidae) were each represented by one family.

Indicator species analysis performed on the abundance of taxa showed that the families Tipulidae, Scirtidae and Lepidostomatidae

were commonly found in the first-order sites while the genus *Cheumatopsyche* (Order: Trichoptera, Family: Hydropsychidae) and *Neoperla* sp. (Order: Plecoptera, Family: Perlidae) were more abundant in the second-order sites (Table 3). Ephydriidae, *Euthraulius* sp., Elmidae, Gomphidae, *Neoperla* sp., *Orthothrichia* sp. and *Prosopistoma* sp. only occurred at the second-order sites. Ceratopogonidae, Pisuliidae and *Trichosetodes* were rare taxa only observed in the first-order sites.

Shannon diversity index displayed that second-order sites had higher diversity compared to the first-order sites (Table 4). Simpson richness index also indicated that the second-order sites had a higher richness than the first-order sites. However, there were more taxa and number of individuals ( $t_5 = 1.80, p = 0.03$ ) in first-order sites than in second-order sites. Similarly, the dominance index indicated that first-order sites had higher dominance values than the second-order sites (Table 4).

There was higher beta diversity in the first-order sites than in the second-order sites (Fig. 3). Local site biodiversity contributions (LCBD) data for both abundance-based and presence-absence-based data showed that second-order and first-order sites were not significantly different, but the means of the first-order sites were marginally higher than second-order sites (Fig. 3).

**3.3. Patterns of macroinvertebrates functional feeding groups (FFGs) in the two systems**

Collectors dominated other FFGs in the first- and second-order sites. Collector-filterers (CF) were however more abundant in the first-order streams (58.4%) while the collector-gatherers (CG) dominated the second-order sites (44.9%). Whereas scraper abundance was higher in the second-order (6.6%) than in the first-order (2.9%) sites, the first-order sites had a higher abundance (6.9%) of shredders than the second-order sites (Fig. 4a). The second-order sites however had a

**Table 3**

Abundance-based indicator species analysis for macroinvertebrates taxa with indicator values  $\geq 50$  in first-order and second-order stream sites.

Taxa	First-order system			
	Indicator Value	Mean	SD	p-value
Tipulidae	93	59.8	15.72	0.0304*
Scirtidae	91.9	57.7	14.87	0.0304*
<i>Lepidostoma</i> sp.	86.2	65.8	13.73	0.0584*
Oligochaeta	96.4	87.7	7.4	0.1708
Chironomidae	82.2	64.4	8.93	0.1194
Simuliidae	81.2	70.8	10.39	0.2879
<i>Pisidium</i> sp.	77.3	75.7	6.5	0.4341
<i>Tinodes</i> sp.	76.4	60.7	7.41	0.0538
<i>Caenis</i> sp.	75.6	63.6	9.6	0.1742
<i>Planaria</i> sp.	71.4	58.6	11.1	0.197
<i>Wormaldia</i> sp.	70.7	52.3	18.31	0.2651
Limoniidae	70.5	61	12.57	0.2837
<i>Afrocaenis</i> sp.	70	44.7	17.23	0.141
<i>Anisocentropus</i> sp.	69.2	46	17.19	0.148
<i>Trienodes</i> sp.	57.4	69.7	13.03	0.7167
Ceratopogonidae	50	31	16.5	0.4291
Dytiscidae	50	34.6	13.43	0.4323
Leptophlebiidae	50	28.3	18.52	0.4207
Physidae	50	28.6	18.56	0.4291

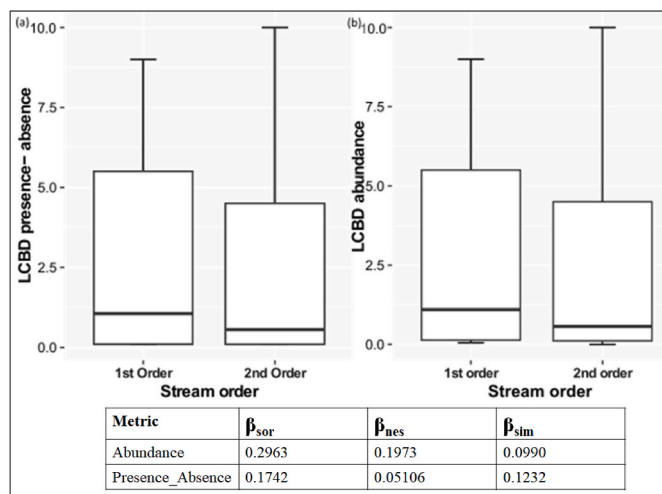
  

Taxa	Second-order			
	Indicator Value	Mean	SD	p-value
<i>Cheumatopsyche</i> sp.	85.8	62.8	13.55	0.0566*
<i>Neoperla</i> sp.	83	50.7	16.14	0.0119*
<i>Afronurus</i> sp.	71.2	54.7	13.61	0.1782
Potamonautidae	61.9	62.2	9.46	0.4859
<i>Oligoneuriopsis</i> sp.	59.5	51.3	15.82	0.3171
<i>Baetis</i> sp.	51.2	58	5.91	0.945
Ephydriidae	50	32.4	15.51	0.4381
<i>Prosopistoma</i> sp.	50	34.3	13.78	0.4357

\*Indicate statistically significant difference between first-order and second-order stream sites ( $p < 0.05$ ).

**Table 4**  
Community indices of macroinvertebrates in the first-order and second-order stream sites.

Indices	First-order	Second-order
<b>Diversity indices</b>		
Shannon_H	2.000	2.103
<b>Evenness indices</b>		
Evenness_e <sup>H/S</sup>	0.172	0.195
<b>Richness Indices</b>		
No. of taxa	43	42
Simpson_1-D	0.720	0.774
<b>Abundance indices</b>		
Sum of individuals/m <sup>2</sup> for all the sampled sites	115,312	70,192
<b>Dominance indices</b>		
Dominance_D	0.280	0.226



**Fig. 3.** Difference in local contributions to beta diversity (LCBD), (a) presence-absence data, and (b) abundance data among the first-order and second-order sites.

higher abundance of collector taxa than the first-order sites, which had a higher abundance of predator and shredder taxa (Fig. 4b).

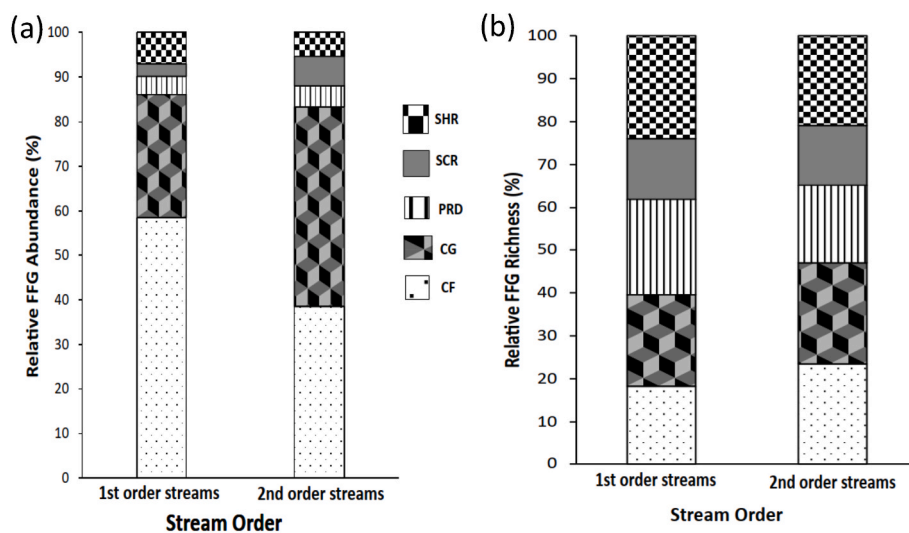
Analysis of similarity (ANOSIM) indicated significant differences in macroinvertebrate assemblages for un-transformed abundance data between the two channel systems (R-statistic = 0.74, p = 0.0001). We

used both presence-absence data and abundance data separately in non-metric multidimensional scaling (NMDS) to show the influence of taxon composition on the ordinations. Even though the within-channel group points were distant from one another, there was a separation between the first- and second-order sites (Fig. 5a and b). The NMDS based on taxon presence-absence data (Fig. 5a) showed no differentiation between channel systems (PERMANOVA  $F = 0.346$ ,  $df = 2$ ,  $p = 0.17$ ). Both NMDS outputs grouped macroinvertebrates according to the channel system (first-vs second-order sites) with minimal overlaps, which provided further evidence that the macroinvertebrate communities in the second order and first-order sites differed in terms of taxon richness and community composition.

The ordinations of canonical correspondence analysis (CCA) showed spatial patterns in macroinvertebrate structural and functional composition associated with water quality and stream size variables between the two channel systems (Fig. 6). The first CCA Axis (CCA 1) accounted for a higher variance of 32.0% whereas the second CCA axis accounted for 24.8% of the variation in the functional and structural composition. The two CCA ordinations explained 56.8% of the associations for the functional and structural composition of macroinvertebrates with environmental variables. The CCA ordinations showed that the first-order sites were associated with high levels of DO and POM, and were correlated with *Oecetis* sp., Chironomidae and *Lepidostoma* sp. The second-order sites were characterized as deeper and wider, and with higher water velocity and discharge, and were associated with the presence of *Baetis* sp., *Prosopistoma* sp. and Leptophlebiidae Gen. sp. (Fig. 6). In terms of FFGs, scrapers and predators were associated with in the second-order sites while shredders, collector-gatherers, collector-filterers and predators were associated with in the first-order sites.

#### 4. Discussion

This study investigated whether stream size and the associated habitat quality and water physico-chemical differences arising from the evolution of channel systems could be used to explain the variability in structural and functional characteristics of macroinvertebrate assemblages between tributaries and mainstems of Afrotropical stream networks. Dissolved oxygen concentration, coarse particulate organic matter, conductivity, stream width, depth, discharge and water velocity shaped the diversity and functional composition of macroinvertebrates in the two systems.



**Fig. 4.** (a) Relative abundance – number of individuals and (b) richness – number of taxa of FFG taxa in the first-order and second-order stream sites. CF = collector filterers, CG = collector gatherers, PRD = predators, SCR = scrapers, SHR = shredders.

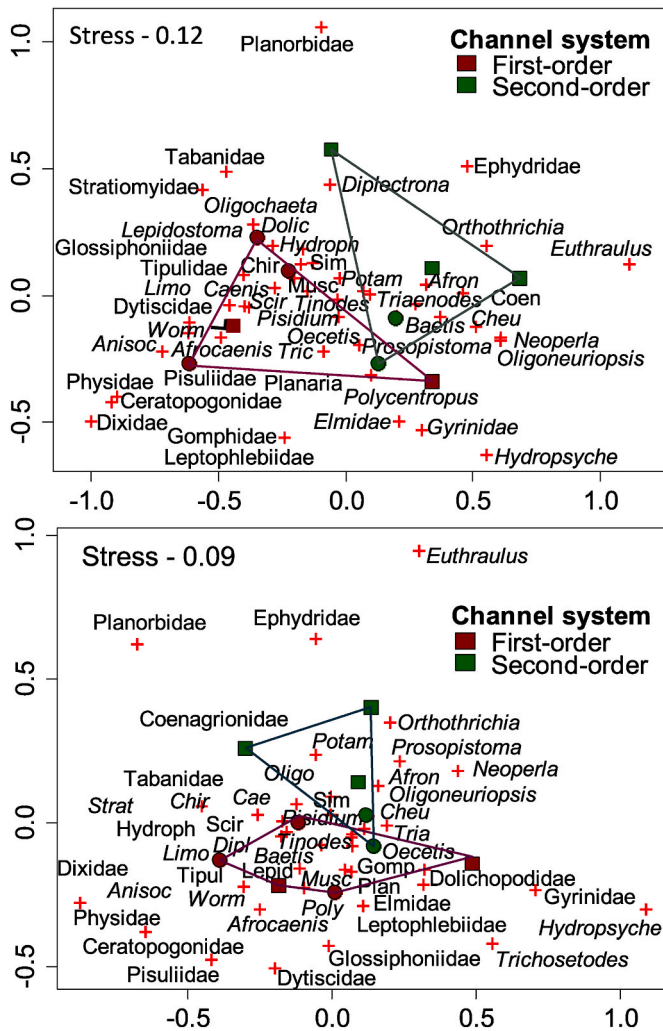


Fig. 5. (a) nMDS based on abundance and (b) presence-absence data of macroinvertebrate structural composition in the river network. Afron-Afronurus, Anisoc-Anisocentropus, Cae-Caenis, Cheu-Cheumatopsyche, Chir-Chironomidae, Coen-Coenagrionidae, Diplo-Dipteronella, Dolic-Dolichopodidae, Hydroph-Hydrophilidae, Lepid-Lepidostomatidae, Limo-Limoniidae, Musc-Muscidae, Oligo-Oligochaeta, Plan - Planorbiidae, Poly -Polycentropus, Potam-Potamonautes, Sim - Simuliidae, Strat - Stratiomyidae, Scir-Scirtidae, Strat-Stratiomyidae, Tria-Triaenodes, Tric-Trichosetodes, Worm-Wormaldia.

4.1. Patterns in environmental variables and macroinvertebrate structural and functional composition in headwater streams

Our findings show that first-order sites had higher abundances and taxonomic richness of macroinvertebrates than second-order sites, which can be attributed to the high habitat heterogeneity in the first-order sites. High discharge levels in second-order sites result in constant shifting and dislocation of the substrate making them less attractive and unfavourable to colonization by benthic invertebrates (Thirion, 2016). Macroinvertebrate assemblage structure and distribution patterns have been observed to be intimately interconnected with the trophic food resources and physico-chemical characteristics of streams on a local scale (Vannote et al., 1980; Minaya et al., 2013; Lubanga et al., 2021; Masese et al., 2021, 2023).

According to the macroinvertebrate structural indices used, the sampled sites had a high richness and diversity of species, which is typical of forested high-altitude Afromontane streams in the region (Musonge et al., 2020; Yegon et al., 2021). There was higher dominance of a few taxa in the first-order compared to the second-order sites

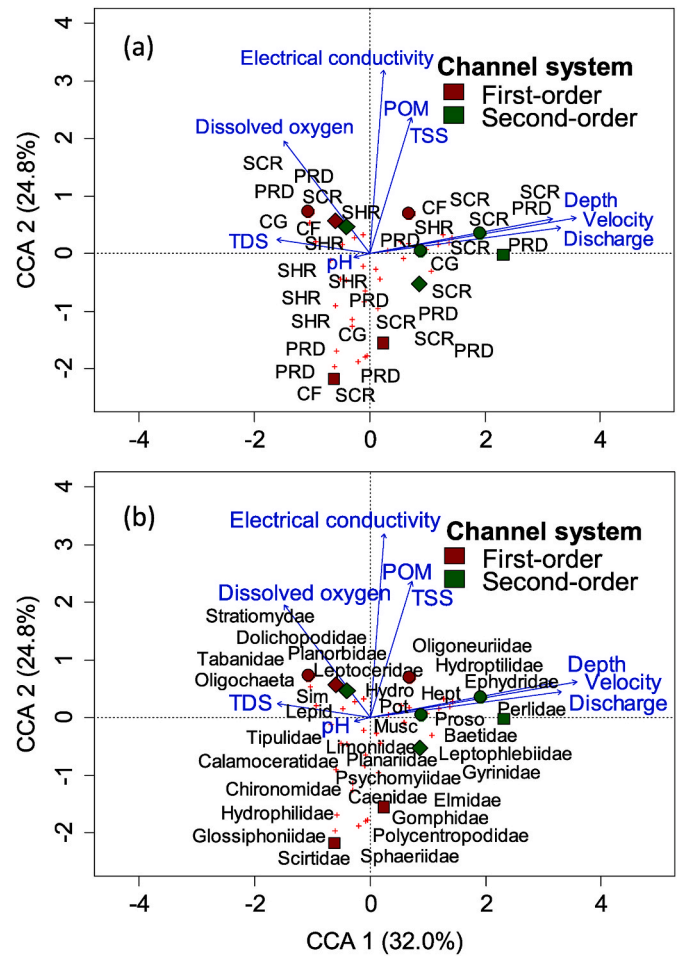


Fig. 6. CCA triplots of macroinvertebrate functional composition (a) and structural composition (b) based on abundance data in relation to physico-chemical water quality variables and stream size variables in first-order and second-order sites. Functional feeding groups: CF-Collector-filterers, CG-Collector-gatherers, PRD-Predators, SCR-Scrapers, SHR-Shredders. Taxa: Hept-Heptageniidae, Hydro-Hydropsychidae, Lepid-Lepidostomatidae, Musc-Muscidae, Pot-Potamonautidae, Proso-Prosopistomatidae, Sim-Simuliidae.

(Table 4). This can be attributed to the narrow ranges of environmental variables in the tributaries, i.e., shallow depths, low flow velocity, high amounts of CPOM and high concentrations of dissolved oxygen. The few specific taxa inhabiting the tributaries are adapted to the river network characteristics and physico-chemical parameters in these stream sections. Other studies (Dallas, 2007; Masese et al., 2021; Ntoko et al., 2021) have indicated that habitat characteristics and site variables play important roles in the distribution of macroinvertebrates as observed in the present study.

Beta diversity analyses showed that there was species replacement from first-order to second-order sites. Using the presence-absence data, the  $\beta$ -turnover (replacement) was higher than the beta nestedness (species richness) indicating the higher beta diversity in the first-order sites was contributed by the presence of rare species only present in these sites. The abundance data showed that most of the differences in the macroinvertebrate communities between the first-order and second-order sites arose from richness differences (nestedness). However, the taxonomic resolution, which is a common constraint in the identification of most Afro-tropical stream invertebrates, limits these differences, typically up to the family level (Ochieng et al., 2019). It has been shown that second-order sites are characterized by a more diversified macroinvertebrate assemblage because of the vast range of environmental factors that are present at these locations and provide

macroinvertebrates with transitional habitats (Heino and Mykrä, 2006). This higher diversity can also have been contributed from invertebrate drift from tributaries flowing into them (Jost, 2007).

Among a suite of physico-chemical characteristics, the river continuum concept (RCC) predicts that macroinvertebrate community composition changes gradually from the headwaters to the lower reaches (Vannote et al., 1980). For instance, the relative proportions of functional feeding groups change from shredder dominance in headwaters to collector dominance in the lower reaches of large rivers. The findings of our study are largely in agreement with the RCC predictions. The abundance of shredders and collector-gatherers was higher in first-order compared to the second-order sites while scrapers were more abundant in the second-order sites, which were wider with an open canopy supporting abundant primary production. Even though the RCC model predicts these changes in the transition from low order (first to third) to medium order (fourth to sixth) rivers, our observations of the change in the patterns from the first-to the second-order streams is an indication of disturbance. Reduced canopy cover in the second-order streams increased light intensity and primary production for the scrapers. This observation is important not only for this study but also for other river systems experiencing threats to biodiversity and ecosystem functioning caused by riparian deforestation and other human activities.

The indicator species analysis showed that certain taxa preferred either first-order or second-order sites based on their requirements for habitat, flow and food resources. Despite many taxa occurring across the two stream networks, there were a few rare taxa that were only found in the first-order systems, which could make them potentially efficient indicator species for these small systems. The families Tipulidae, Scirtidae, Lepidostomatidae, Ceratopogonidae, Pisuliidae, and Dytiscidae and genus *Trichosetodes* were significantly associated with the first-order sites while families Ephydriidae, Elmidae, Gomphidae, and genera *Euthraulus*, *Orthothrichia*, *Cheumatopsyche* and *Prosopistoma* were associated with sites. Most of the taxa associated with first-order sites are detritivores or herbivores (e.g., Tipulidae, Lepidostomatidae, Pisuliidae and *Trichosetodes*) which prefer cooler streams with enough supply of coarse plant material (Yule et al., 2009; Masese et al., 2014a, 2014b; Tomczyk et al., 2022). Taxa like *Baetis*, *Caenis*, Heptageniidae, Simuliidae, Potamonautidae and Chironomidae were common across the two systems. A study by Williams and Hynes (1971) in the same region showed that the macroinvertebrate communities of Mt. Elgon streams were dominated by *Baetis*, *Centroptilum*, *Cheumatopsyche*, Simuliidae and Chironomidae together with considerable numbers of *Dugesia*, Oligoneuriidae, *Euthraulus*, *Caenis*, *Neoperla* and *Hydropsyche*. In addition, substantial numbers of *Prosopistoma* and *Potamonautes* sp. were also prevalent at higher elevation and forested streams.

Among the most cosmopolitan taxa, *Baetis*, Hydropsychidae and Simuliidae have short regeneration times and rapid colonization rates, enabling them to cope with fluctuating environments, and thereby build up large populations opportunistically (Hynes, 1975; Rivers-Moore et al., 2007). The occurrence of freshwater crabs (*Potamonautes* sp.) in both systems indicates that both systems had sufficient CPOM that is food for these omnivorous macroconsumers. Similar observations have been made in Afrotropical streams in the region (Kibichii et al., 2007; Masese et al., 2014a; Lubanga et al., 2021; Sitati et al., 2021a; Yegon et al., 2021). Cumberlidge and Clark (2010) reported the occurrence of the endemic crab *Potamonautes elgonensis* in the upper reaches of rivers in the highlands of western Kenya and eastern Uganda, including Mt Elgon where the present study was done. The occurrence of most species across all streams could be attributed to zoogeographical aspects, environmental conditions, geographic location and the similar altitudinal range. Studies report the existence of species that occur in both small tributaries as well as in larger streams, although their abundance may vary with stream size (Heino and Mykrä, 2006; Gabbud et al., 2019). For species-rich families, disentangling the differentiation in the abundance and occurrence of these taxa in Afrotropical streams is hard,

because classification and identification of taxa is mostly limited to higher taxonomic levels of family and genus.

Within drainage systems, tributary position, stream size and the distance from the source have been reported to be important determinants of ecological processes and assemblage structure in streams and rivers (Vannote et al., 1980; Milner et al., 2019; Englmaier et al., 2020; Masese et al., 2022). Additionally, substrate structure in streams and rivers is a primary organizing variable for aquatic communities in many studies (Masese et al., 2021, 2023; Elgueta et al., 2021). We found substrate type in the fluvial continuum to be an important predictor of macroinvertebrate assemblage characteristics in our study streams. For instance, most shredders (e.g., Lepidostomatidae and Pisuliidae) preferred headwater first-order sites with abundant CPOM while Perlidae and Oligoneuriidae preferred second-order sites that were characterized by macrolithal substrate with fast flows. These results are corroborated by results in other studies (Arimoro and Muller 2010; Principe et al., 2019).

The influence of substrate type on macroinvertebrate community composition was evident in the distinct occurrence of some taxa in either of the two systems. The main substrate recorded in the second-order sites was macrolithal (size class 20 cm–40 cm) while in the first-order sites detritus (mainly CPOM) dominated the streambeds. Consequently, the occurrence of taxa such as *Euthraulus*, *Cheumatopsyche*, *Oligoneuriopsis*, *Neoperla* and *Prosopistoma* in the second-order sites indicate the importance of coarse substrate that dominated these sites. Most of these taxa are rheophilic and reside in areas with fast flows and stable coarse substrates that they use for attachment (Thirion, 2016; Masese et al., 2021). In streams with fast water currents, the underside of pebbles or cobbles on the streambed often provides interstitial space that can be colonized by macroinvertebrates such as net-spinning organisms. Hydropsychidae larvae, such as *Cheumatopsyche* spp., typically reside on the surface of substrate exposed to fast flows, but they also build retreats in interstitial spaces in the benthos and cementing the gravel together with their silk, which makes them less vulnerable to aquatic predators (Natsumeda and Iguchi, 2019).

Coarse particulate organic matter (CPOM), in the form of twigs, leaves, and detritus, also influenced macroinvertebrate distribution patterns. Most of the taxa found in the first-order sites (Pisuliidae, Lepidostomatidae and Tipulidae) are those utilizing CPOM either as a habitat or food resources. The availability, quality and quantity of CPOM have been reported to be an important variable influencing the macroinvertebrate shredder groups (Akamagwuna et al., 2019; Guo et al., 2021) through their utilization of CPOM as food, as well as for making cases. Consequently, the presence of shredders can influence the distribution of collectors (gatherers and filterers), as shredders break down CPOM into fine particulate organic matter (FPOM) used by the collectors. The high number of scrapers in the second-order sites is a factor of the widened channel width, which favors the establishment of biofilm on the coarse substrate that is utilized by scrapers.

Stream size seemed to be a major factor influencing the richness and abundance of functional feeding groups of macroinvertebrates in our study (Fig. 6), concurring with earlier findings from other parts of the world (Heino and Mykrä, 2006). The multivariate analyses showed the existence of distinct patterns in the grouping of the sites in the two channel systems based on environmental variables. Changes in water quality and stream size variables with channel type, as noted for POM, DO, depth, width, velocity and discharge, played a significant role in describing the patterns observed in macroinvertebrate assemblages.

Canonical correspondence analysis (CCA) indicated that scrapers and predators were associated with in the second-order sites while shredders, collectors and predators were associated with the first-order sites. In both systems, there was a high abundance of macroinvertebrate predators. This is attributed to the high diversity of other macroinvertebrate FFGs that are prey for the predators. Studies in other Afrotropical stream and rivers have recorded high abundance of predators on stable substrate (Rivers-Moore et al., 2007; Sitati et al., 2021b).



Similarly, rheophilic taxa such as Simuliidae, Oligoneuriidae, Heptageniidae, Leptophlebiidae, *Prosopistoma* and Baetidae were associated with the second-order sites characterized by high and fast flows. On the contrary, CPOM and flow type were the predictor variables affecting invertebrate assemblages in the first-order system. The first-order sites were associated with taxa that preferred low flows such as *Lepidostoma* sp., Oligochaeta, Planorbidae, Calamoceratidae, Tipulidae and Pisuliidae. Other works such as those of Gabbud et al. (2019) describe the variability in macroinvertebrate composition between similar altitude sites in tributaries and their mainstems to be related to channel characteristics. The results of similar studies in the Afrotropics (e.g., Wolmarans et al., 2017; Edegbene et al., 2021; Masese et al., 2021) corroborate the findings of our study.

#### 4.2. Biodiversity conservation for sustainability and management of Afromontane tropical streams

The high and unique biodiversity in most Afromontane river systems is being threatened by the increasing human population pressure which has exerted pressure on natural systems (Cumberlidge and Clark., 2010; Minaya et al., 2013; Sitati et al., 2021a). These conversions have huge consequences on river systems that drain these catchments through deterioration in abiotic and biotic characteristics essential healthy ecosystems. Habitat degradation because of land-use change (i.e., from forested to cultivated lands) in this catchment likely corresponds to flow alteration, erosion and sediment input (sedimentation). Small headwater tributaries are more susceptible to the magnitude of the effects of these land-use changes due to their small size (Ferreira et al., 2023). Without maintaining the ecological integrity in these systems, aquatic organisms as well as native biodiversity become stressed, and populations of vulnerable species can decline or, in extreme cases, face extinction before they are recognized.

In the African systems, stream networks and their associated biodiversity are less studied. This is worsened by low investment in biodiversity research and a lack of harmonized indicators and databases to assess conservation needs and monitor biodiversity losses (Achieng et al., 2023). In the current times of environmental and climate changes, understanding the threats and levels of biodiversity losses might be harder given that the aquatic communities residing in these systems are poorly known with many undescribed species. The changes in community composition along the stream network witnessed in this study suggest that conservation of aquatic biodiversity might be achieved by maintaining habitats both in first-order as well as in second-order systems. Both systems host diverse macroinvertebrate communities that are distinct in their requirements for habitats and environmental conditions. In cases where river mainstems face degradation, tributaries can be utilized to maintain biodiversity and can serve vital roles in the restoration of faunal assemblages in entire river networks. Moreover, tributaries act as refugia for the rivers' mainstems in case of both natural and human-induced disturbances in the mainstems.

## 5. Conclusion

The results of this study reveal that tributaries played an important in their contribution to the overall biodiversity species pool of the Nzoia River headwaters. Even though many taxa occurred across all the two channel types, some species showed high fidelity to the small first-order systems. The unique biota prefers small streams (tributaries) due to their inherent characteristics, and can therefore be used as indicator taxa for headwater pristine conditions, and therefore would be lost in the event of the continued degradation of headwater streams. There is, therefore, a need for inclusion of tributaries in headwater studies and a call for the protection and conservation of high-elevation headwater streams to sustain healthy river ecosystems, and to support biodiversity. The distinctness in the structural and functional composition of macroinvertebrates between the tributaries and mainstems as exemplified in

the study, suggests that the close linkages of streams in a network support and foster biodiversity.

## CRedit authorship contribution statement

**Augustine Sitati:** Conceptualization, Data curation, Formal analysis, Investigation, Visualization, Writing – original draft, Writing – review & editing. **Mourine J. Yegon:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Frank O. Masese:** Conceptualization, Formal analysis, Investigation, Supervision, Writing – review & editing. **Wolfram Graf:** Conceptualization, Funding acquisition, Investigation, Methodology, Supervision, Writing – review & editing.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.indic.2023.100330>.

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