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Freshwater science in Africa

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Freshwater science in Africa

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Editorial: Freshwater science in Africa

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Editorial on the Research Topic
Freshwater science in Africa

Introduction

Human activities and global climate change affect ecosystems, species, and human services and resources (Dudgeon et al., 2006; Reid et al., 2019). Freshwater ecosystems are the most fragile habitats, hosting roughly 7% of the world's biodiversity on 1% of land and 0.01% of water (Gleick, 1996; Reid et al., 2019). Human population growth exacerbates climate change, pollution, water abstraction, impoundment of streams and rivers, nutrient and organic matter loading, invasive species, wetland degradation and loss, and other stressors (Dudgeon et al., 2006; Vörösmarty et al., 2010; Cazzolla Gatti, 2016). Knowledge of freshwater ecosystem structure and function helps us understand how they react to different pressures. This knowledge guides freshwater ecosystem conservation, management, and decision-support tools.

Africa's diverse and large freshwater systems include iconic rivers that fostered the earliest civilisations, the Great Lakes that hold 25% of the world's unfrozen fresh water, and extensive wetlands, floodplains, and deltas that support unique species. Fisheries, irrigation, and livestock production from these freshwater ecosystems feed millions of people. Due to rapid population growth, land use change, urbanisation, energy consumption, and water harvesting are threatening freshwater ecosystems (UNEP-WCMC, 2016; Fouchy et al., 2019; Birk et al., 2020). Studies have expressed serious concerns about the continent's freshwater fauna and ecological processes (Darwall et al., 2005; Thieme et al., 2005; Sayer et al., 2018; Masese et al., 2021). Freshwater impacts are reducing the continent's ecological services and human welfare (IPBES, 2018). There is a growing need to investigate the condition of freshwater ecosystems to document their conservation status, and the threats they face, and to devise management and conservation measures.

This Research Topic explores Africa's freshwater science potential and distinct freshwater ecosystems and communities. This Research Topic brings together expert research on African freshwater science by African and non-African scientists to highlight the continent's contribution to the global freshwater community. Articles in this Research Topic help galvanise and harmonize science and information exchange by strengthening the collaborative network of African freshwater scientists. It also leads future freshwater research in the continent and helps researchers develop alliances and explore African freshwater science breakthroughs. The main topics of importance covered by the articles in this Research Topic included.

- Freshwater physical, chemical, and biological characteristics, and human impact.
- Methods to analyse, monitor, conserve, and manage freshwater ecosystems.
- Biomonitoring indices or tools.
- Social/cultural participation in freshwater ecosystem studies, management, and policy.
- Invasive species and their effects
- Multiple stressors, interactions, and management.
- Socio-ecological science, governance, and policy.

Status of African freshwater ecosystems

Africa boosts renowned freshwater ecosystems, including iconic rivers such as the Nile, Niger, Orange, Tana and Zambezi, the African Great Lakes—Albert, Edward, Kivu, Malawi, Tanganyika, Turkana and Victoria, and extensive wetlands such as the Inner Niger Delta, the Okavango Delta, St. Lucia Estuary and the Sudd. Other important freshwater ecosystems in semi-arid and arid areas include the inland oases, wadis and chotts of North Africa. These freshwaters and linked terrestrial and marine ecosystems support a great diversity of plants and animals, and their productivity provides the natural resources essential to the survival of a significant part of the African rural population.

Africa is underdeveloped, but humans nevertheless affect freshwater environments. Many rivers are being dammed for hydropower and water supply (McClain, 2013; O'Brien et al., 2021). Many lakes are stressed by excessive water withdrawal, pollution, invasive species, overfishing, and biomass harvesting, while many wetlands have been reclaimed for forestry, grazing, and settlements (Dalu and Wasserman, 2022). These pressures have weakened freshwater habitats. Land use change and pollution have caused the biggest water quality decline (Jacobs et al., 2017; Fayiga et al., 2018; Wanderi et al.). Threats to indigenous species and their habitats (Weyl et al., 2020) and altered ecological processes such as organic matter processing, nutrient cycling, and ecosystem productivity (Masese et al., 2017; Fugère et al., 2018) have also been noted.

Addressing challenges facing freshwater ecosystems in Africa

Despite the vulnerability of African freshwater ecosystems to human activities, few studies have assessed their status and threats. Lack of data, budget constraints, and inadequate financial and technical capacity hinder research, conservation, and management (Achieng et al., 2023). Multiple stressors threaten freshwater ecosystems, requiring immediate, long-term, and holistic management and conservation methods. One way is using biological indicators to evaluate and monitor freshwater environments.

While efforts have been made to develop ecological indices and other models to assess and monitor the status of inland waters in Africa (Dallas, 2021; Masese et al., 2021; Plisnier et al., 2022), most studies have focused on land use change (e.g., agriculture and urbanisation), industrial discharges, and organic pollution (Masese et al., 2021). Wanderi et al. examined the combined effects of land use change, flow variation, and of organic matter and nutrients by large mammalian herbivores on an Afromontane-savanna Mara River in Kenya to understand how other stressors affect aquatic ecosystems. Dusabe et al. used macroinvertebrates as bioindicators to assess river impacts from dams. Damming rivers for hydropower development in Africa is expanding to meet electricity demands (O'Brien et al., 2021), but little is known about the effects on river flows and biodiversity. To bridge this knowledge gap, Muvundja et al. examined the hydrological regime and water quality of the Ruzizi River. Masese et al. assessed the ecological condition of Afrotropical rivers using the transboundary Mara River, Kenya and Tanzania, as a case study to improve macroinvertebrate-based indices.

Understanding species phylogeny, occurrence, and distribution is crucial to understanding African biodiversity and its threats. Human disease causing vectors must be controlled by knowing their occurrence and distribution. Tabo et al. used a machine learning algorithm and random forest to estimate the distribution of gastropods *Biomphalaria* and *Bulinus*, intermediate hosts of *Schistosoma mansoni* and *S. haematobium*, to help manage Schistosomiasis in western Uganda's crater lakes. Tumwebaze et al. examined pan-African *Bulinus* species from varied habitats across different altitudinal ranges, particularly on the highlands, to determine how contemporary climate and historical geological causes affect species distributions and evolutionary processes. Another biodiversity study by Sands et al. examined the phylogeography of the freshwater limpet genus *Burnupia* in the southern African Highlands to assess its diversity, endemicity, and colonisation date.

Exotic species also threaten African freshwater environments. Many African lakes, rivers, and wetlands have introduced fish. Introduced species have been extensively studied in lakes like Lake Victoria (Taabu-Munyaho et al., 2016), but few studies have examined their effects on rivers and wetlands. Trout are among the most widely introduced fishes, including in African highland streams and rivers (Crawford and Muir, 2008; Weyl et al., 2017). Maimela et al. examined food web interactions between fish communities in invaded and uninvaded river reaches in the upper Blyde River catchment, South Africa, to understand how the exotic *Oncorhynchus mykiss* affects indigenous fish species.

Finally, inland freshwater environments serve millions of Africans through fishing. Overfishing, alien species invasion, pollution, eutrophication, and climate change threaten fisheries resources. Several steps should be taken to maximise the benefits of declining catch fisheries. Nyamweya et al. examined how product diversification, value addition, and sustainable fisheries may maximise Lake Victoria's benefits. This study shows that minimizing post-harvest losses of the silver cyprinid

Rastrineobola argentea will boost revenue and yield higher-quality fish for human consumption. Biowaste (frames, skin, and fish mouth) might triple Nile perch revenues, too. If fishing targeted maximum sustainable yield (MSY), long-term Nile perch and Dagaa landings might increase. In addition, tourism, recreation, and fish cage culture investments can strengthen Lake Victoria's blue economy.

Way forward

This special Topic highlights biodiversity research and human impacts on African freshwater ecosystems. Although many measures are needed, we highlight three areas that should be considered to address the myriad pressures affecting African freshwater ecosystems.

i) Hydropower and excessive water abstractions imperil many freshwater habitats. Environmental flows and integrated water resources management should influence stream and river development plans (Arthington et al., 2018; Dirwai et al., 2021; Meran et al., 2021).
ii) Water-borne diseases continue to be a growing challenge in the African continent. To contribute to the control and/or eradication of water-borne diseases, it is critical to understand the factors influencing the occurrence and distribution of hosts and vectors. Similarly, studies that assess the occurrence and distribution of vectors and hosts of waterborne diseases should be examined to inform management and control measures.
iii) The complex mix of various stressors operating on rivers requires multidisciplinary collaborations to create cost-effective monitoring techniques. Existing indices should be evaluated for performance and improved using indigenous taxa. While developing these tools, emphasis should be placed on stakeholder participation and community engagement.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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ORIGINAL RESEARCH
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Factors Controlling the Distribution of Intermediate Host Snails of *Schistosoma* in Crater Lakes in Uganda: A Machine Learning Approach

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Schistosomiasis affects over 700 million people globally. 90% of the infected live in sub-Saharan Africa, where the trematode species *Schistosoma mansoni* and *S. haematobium* transmitted by intermediate hosts (IH) of the gastropod genera *Biomphalaria* and *Bulinus* are the major cause of the human disease burden. Understanding the factors influencing the distribution of the IH is vital towards the control of human schistosomiasis. We explored the applicability of a machine learning algorithm, random forest, to determine significant predictors of IH distribution and their variation across different geographic scales in crater lakes in western Uganda. We found distinct variation in the potential controls of IH snail distribution among the two snail genera as well as across different geographic scales. On the larger scale, geography, diversity of the associated mollusk fauna and climate are important predictors for the presence of *Biomphalaria*, whereas mollusk diversity, water chemistry and geography mainly control the occurrence of *Bulinus*. Mollusk diversity and geography are relevant for the presence of both genera combined. On the scale of an individual crater lake field, *Biomphalaria* is solely controlled by geography, while mollusk diversity is most relevant for the presence of *Bulinus*. Our study demonstrates the importance of combining a comprehensive set of predictor variables, a method that allows for variable selection and a differentiated assessment of different host genera and geographic scale to reveal relevant predictors of distribution. The results of our study contribute to making realistic predictions of IH snail distribution and schistosomiasis prevalence and can help in supporting strategies towards controlling the disease.

Keywords: schistosomisis, biotic and abiotic predictors, mollusks, random forest, Africa

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INTRODUCTION

Human schistosomiasis (bilharzia) is the second most important tropical parasitic disease after malaria (World Health Organization, 2016) and ranked the most important water-borne disease (Steinmann et al., 2006). It poses a global burden to humankind with over 700 million individuals in 78 countries at risk of infection, claiming over 200,000 lives annually (World Health Organization, 2016). In addition, more than 240 million people are infected worldwide, predominantly in sub-Saharan Africa (World Health Organization, 2016), where the disease burden is up to 90% of the global infections due to poor standards of living (Bergquist et al., 2017). Thus, schistosomiasis is commonly referred to as "the disease of the poor". Countries in sub-Saharan Africa face a challenge of high population growth, and most people live in rural or semi-rural settings associated with poverty, poor sanitation and no access to clean water (Gray et al., 2010; King, 2010; Payne and Fitchett, 2010). In such geographical settings, people might continuously be in contact with water contaminated with schistosome eggs (Stothard et al., 2005), and a large part of the population is at risk of infection.

Despite schistosomiasis being one of the most prevalent tropical diseases (Steinmann et al., 2006), it is also probably the most neglected and was given little priority by the funding bodies compared to HIV/AIDS, malaria and tuberculosis (Hotez et al., 2007; Utzinger et al., 2009). Nevertheless, a recent growing interest in neglected tropical diseases including schistosomiasis has been observed over the last decade (World Health Organization, 2012; Shiff, 2017; King et al., 2020).

So far, strategies to control the spread of the disease *via* the provision of schistosomicides and/or WASH (water, sanitation, hygiene) programmes have shown limited effectiveness, and were consequently leading to disease re-emergences in spite of the interventions (Gryseels and Polderman, 1991; Chitsulo et al., 2000; Fenwick et al., 2009). Schistosomiasis is caused by trematode worms of the genus *Schistosoma* being transmitted through intermediate host (IH) snails. The reproductive cycle of *Schistosoma* trematodes starts with parasitic eggs released into freshwater through faeces and urine by infected humans. Eventually, motile larvae called miracidia hatch from the eggs and swim in search of snails to infect as intermediate host. The parasite then reproduces asexually within the snail, before shedding to the water as cercariae, larvae that penetrate the skin of the human host to complete the cycle and eventually cause the disease (Colley et al., 2014).

Sustainable vector snail control has been suggested as a more reliable approach to the schistosomiasis problem (Gryseels et al., 2006; Steinmann et al., 2006; Wang et al., 2008; Colley et al., 2014). The control aims at interrupting the transmission and stopping the spread of infection (Rollinson et al., 2013; Walz et al., 2015; Sokolow et al., 2016), by interrupting the *Schistosoma* life cycle through eliminating potential host snails from local habitats (King and Bertsch, 2015). Yet, this approach relies on the availability of high-quality snail distribution data, which represents a major knowledge gap in most developing countries in sub-Saharan Africa.

In sub-Saharan Africa, *Schistosoma mansoni* and *S. haematobium* are the major cause of the human disease burden in Africa (Chitsulo et al., 2000; Gryseels et al., 2006). *Schistosoma mansoni* is transmitted by snails of the genus *Biomphalaria* (Planorbidae) and causes human intestinal schistosomiasis. In contrast, *S. haematobium* is transmitted by species of *Bulinus* (Bulinidae) and cause human urogenital schistosomiasis (Wang et al., 2008; Colley et al., 2014). *Schistosoma mansoni* and *S. haematobium* are mainly distributed in and around a variety of freshwater habitats such as dams, lakes and rivers (Brown, 1994; Steinmann et al., 2006; Appleton and Madsen, 2012). *Bulinus* species in particular can live in permanent or seasonal pools, rice fields and ditches. In addition, there are several other species of *Schistosoma* that are of significant veterinary importance causing schistosomiasis in livestock. They are either hosted by *Bulinus* species (*S. bovis*) or selected species of the genus (*S. magrebowiei*) (Standley et al., 2012).

Previous studies dealing with the factors that influence the distribution of *Schistosoma* IH snails included water depth (Prah and James, 1978; Utzinger and Tanner, 2000; Boelee and Laamrani, 2004), altitude (John et al., 2008; Stanton et al., 2017), temperature, precipitation, pH level, forest cover and distance to nearest water bodies (Stensgaard et al., 2013), heat waves, droughts and floods (McCreesh and Booth, 2013; Pedersen et al., 2014), calcium and salinity (Hoverman et al., 2011; Maes et al., 2021). Although the relationships with these potential predictors have been examined and tested individually, they were not quantitatively assessed for their combined importance. The contribution of these factors to the distribution of IH snails and the prevalence of schistosomiasis accordingly across different regions is far from understood.

This study focuses on two main goals: 1) to assess the significance of extrinsic (geographical, hydrological, climatic, faunistic and anthropogenic) factors for the distribution of intermediate IH snails of the genera *Bulinus* and *Biomphalaria*, being the major causes of human infections in Africa, 2) to test for differences of potential predictors and their varying impact across different geographical scales. To do so, we used a model system of 56 crater lakes in western Uganda that variably contain IH species of the two genera, along with a diverse set of geographical, climatic, hydrological, faunistic and anthropogenic parameters. We used a machine learning approach (random forest, RF) to assess the importance of the individual parameters and how they differ across the study area. Considering the geographic variation of population density (Gelorini et al., 2012; Hartter et al., 2012), land use intensity (Hartter et al., 2015), and limnological characteristics of the lakes (De Crop and Verschuren, 2019), we hypothesize that a different set of parameters is relevant in the different regions examined. Moreover, we expect that different parameters control the distributions of the two genera, since they do have different dispersal and drought resistance capacities (Brown, 1994).

MATERIALS AND METHODS

Study Area

The study area is located on the hilly uplands (>1,600 m a.s.l.) of the Edward-George branch of the East African Rift valley in western Uganda (**Figure 1**). It is bordered by the Rwenzori

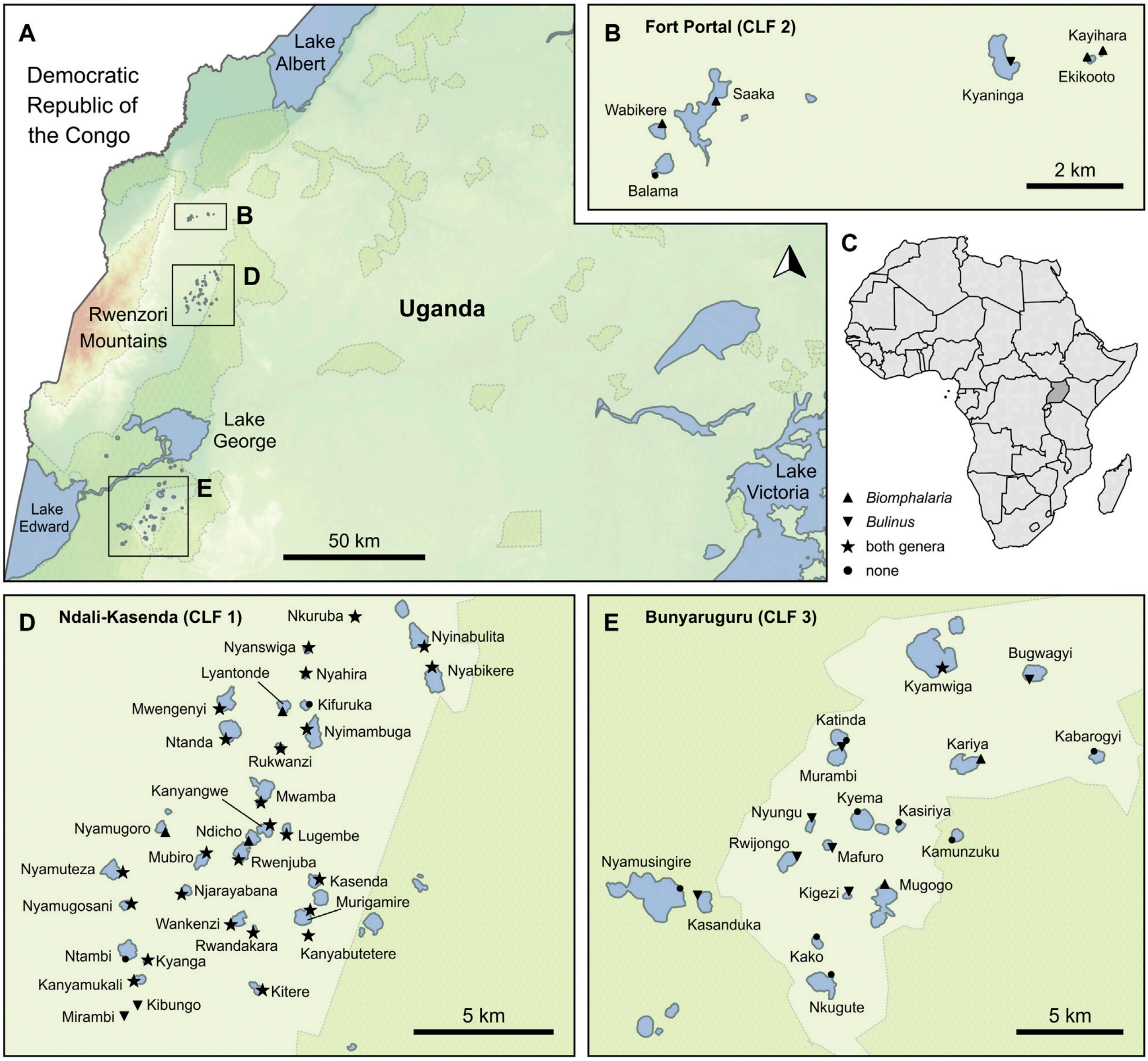

FIGURE 1 | Study area and occurrences of intermediate host (IH) snails. Three crater lake fields (CLF1–3) **(B,D,E)** in western Uganda **(A)** in Africa **(C)** were studied. Symbols at sampled lakes indicate the presence of *Biomphalaria* (triangle), *Bulinus* (inverted triangle), and both genera combined (dark star), whereas lakes marked with a small circle indicate localities where no IH snail was found.

Mountain range in the North-West, Lake Albert in the North and Queen Elizabeth National Park in the South. It contains over 90 small crater lakes, most of which formed between 4,000 and 10,000 years ago as a result of faulting and volcanic eruptions (Vinogradov, 1980; Schumann et al., 2015). Lake Ntambi, which formed c. 50,000 years ago is an exception, (Dirk Verschuren, pers. comm.).

The crater lakes of western Uganda have been repeatedly promoted as an ideal model system for studying large environmental gradients in limnological characteristics in a setting that allows meaningful comparisons due to shared geological history (e.g. Melack, 1978; Mills and Ryves, 2012; Saulnier-Talbot and Lavoie, 2018). The crater lakes region is one of the most densely populated rural areas in sub-Saharan Africa (Hartter et al., 2012), with a population growth rate of 3.3% annually (Gelorini et al., 2012). The population growth is coupled with increasing human impact through settlement, fishing, agriculture on the fertile volcano soils, swimming and water extraction for irrigation and domestic use. As a result of the high population density, the prevalence of (human) schistosomiasis in the region has dramatically increased (Kabatereine et al., 2004; Kabatereine et al., 2006; John et al., 2008; Stanton et al., 2017). In 2014, the crater lakes region covered parts of the 73 out of 112 districts of Uganda with prevalence of schistosomiasis (Loewenberg, 2014). Nationwide, four million people are estimated to be infected and almost 20 million are at risk of infection (Loewenberg, 2014). The only preventative measures in the area are massive drug administration while environmental transmission interruption is rarely emphasized (Loewenberg, 2014).

Melack (1978) classified the region into four crater lake fields (CLFs, **Figure 1**). The Ndali-Kasenda field is locatedin the central part of the region, ~20 km from Fort Portal field in the North and ~65 km from Bunyaruguru field in the South. The fourth field of Katwe-Kikorongo contains lakes with saline waters (Rumes et al., 2011), unsuitable for mollusks (Tumwebaze et al., 2019).

Our dataset combines information from a total of 56 crater lakes in the three fields, including 32 lakes of the Ndali-Kasenda field (CLF 1), six lakes of the Fort Portal field (CLF 2) and 18 lakes of the Bunyaruguru field (CLF three; see **Supplementary Table S1**).

Data Collection

Malacological Field Data

Snails were sampled across the three regions in random months between 2010 and 2019 to account for a range of weather conditions, but mostly in dry seasons at normal or low water levels between 2010 and 2019 (**Figure 1**). Field work periods were aligned to times when highest population densities of the intermediate host snails were expected. Confounding effects by flooding and restricted accessibility during rainy seasons were avoided. Due to the steep and slippery escarpments of the crater lakes, sampling in the wet season was also avoided. Sampling methods involved dredging and/or scoop netting up to a maximum depth of 1.5 m or hand-picking snails found attached to shoreline vegetation and any solid substrates. The sampling time per lake was 40 min, and snails were collected in one to two localities (depending on the size of the respective lake). In some cases, lakes were visited more than once to ensure comprehensive representation of the local fauna. At each locality, we identified and counted the IH snails as well as the associated mollusk fauna on the genus level. The survey also revealed the presence of seven other non-host snail genera (*Radix, Gabbiella, Pila, Melanoides, Segmentorbis, Afrogyrorbis* and *Gyraulus*) as well as the bivalve genus *Sphaerium*.

Climatic and Environmental Data

We included air temperature and precipitation as proxies, because they have previously been shown to influence the distribution of freshwater mollusks (e.g., Hauffe et al., 2016a; Georgopoulou et al., 2016) and particularly those of IH snails (Appleton, 1978; Rowel et al., 2015). Specifically, temperature influences the survival and reproduction rates of snails (Paull and Johnson, 2011; McCreesh et al., 2014; Kalinda et al., 2017). Precipitation is associated with organic matter input and nutrient supply, which affects snail growth and fecundity (Madsen et al., 1987; Camara et al., 2012; Nyström Sandman et al., 2013). We retrieved the climatic data (averaged for the period 1970–2000) from the WorldClim two global database (Fick and Hijmans, 2017). We used mean annual temperature (BIO1), temperature of warmest month (BIO5), temperature of the coldest month (BIO6), annual precipitation (BIO12), precipitation of the wettest month (BIO13) and precipitation of the driest month (BIO14) to account for potential selectivity of the IH snail species to climatic fluctuations. Since the different temperature and precipitation parameters showed a similar range of variation, we calculated principal component analyses and used the first principal component for each of the two sets.

Water chemistry also plays a vital role for the occurrence and abundance of freshwater gastropods. This concerns pH, oxygen, conductivity, surface water temperature, magnesium and calcium (Rumes et al., 2011; Marie et al., 2015; Mahmoud et al., 2019; Alhassan et al., 2020; Olkeba et al., 2020). Surface water temperature, dissolved oxygen, pH and conductivity were measured using a handheld multi-meter probe. Calcium and magnesium data were retrieved from Rumes et al. (2011) and Nankabirwa et al. (2019).

Previous studies have shown the relevance of depth (both absolute lake depth and Secchi depth, i.e., a measure of water transparency) for the occurrence of both *Bulinus* and *Biomphalaria*. Absolute depth was retrieved from De Crop and Verschuren (2019), and water transparency (i.e., Secchi depth) was measured at the sampling points using a Secchi disk.

The crater lakes are characterized by seasonal fluctuations in water levels, and some lakes occasionally dry out (e.g., Lake Mirambi and Lake Kibungo). We included lake surface area and surface area variance over time as parameters in this study. The lake surface area was retrieved from satellite images from Google Earth Pro v. 7.3 taken in 2019. The lake area variance was calculated as the variance of four time slots of lake surface areas traced from satellite images taken in 2003, 2008, 2013 and 2018. Information for a few satellite images was missing, because either some lakes dried out or no records were captured in Google Earth. In such cases, we used the image of the closest time prior or after a given time slot to retrieve lake surface area.

Geographical Data

Longitude and latitude of the sampling sites were included as variables to account for potential variation in the geographical distribution of the IH snails. Altitude has been also proved relevant in the occurrence of snail hosts (John et al., 2008; Stanton et al., 2017) and was therefore considered in our study. Longitude, latitude and altitude were measured with a handheld Garmin GPS eTrex 20 device.

We used two measures for geographical distance to serve as proxies of biogeographical isolation, which might impact colonization and thus the IH presence in the area: 1) distance from a crater lake to the nearest other crater lake, and 2) distance from a crater lake to the nearest larger lake in the surrounding (i.e., lakes Victoria, Edward, Albert and George; **Figure 1**). All distances were measured “as the crow flies” in Google Earth.

Human Impact

To obtain a measure of human impact, we distinguished and quantified the proportion of land use. Since no data are available from online databases, we used the total percentage of cultivatable fields (cropland, fallow land and plantations) as a proxy and visualized a square of 0.0625 km^2 (0.25 × 0.25 km) around each

TABLE 1 | Predictor variables used in this study. Note that temperature and precipitation each represent the first principal component calculated from three climate parameters; see text for details. Sources: 1—Rumes et al. (2011), 2—Nankabirwa et al. (2019), 3—De Crop and Verschuren (2019), 4—Fick and Hijmans (2017).

Category	Predictor variables	Sources
Fauna	Species richness of associated mollusk fauna	This study
Environment/hydrology	Surface water temperature (°C)	This study
	Water pH	This study
	Dissolved oxygen (mg/L)	This study
	Electric conductivity (μS/cm)	This study
	Magnesium concentration (mg/L)	1, 2
	Calcium concentration (mg/L)	1, 2
	Secchi depth (m)	This study
	Lake depth (m)	3
	Lake surface area (km^2)	This study
	Lake area variance	This study
Climate	Temperature (°C)	4
	Precipitation (mm)	4
Geography	Longitude (°E)	This study
	Latitude (°N)	This study
	Altitude (m a.s.l.)	This study
	Distance to the nearest crater lake (km)	This study
	Distance to the nearest large lake (km)	This study
Human impact	Land use (% of area)	This study
	Population density (number of houses)	This study

crater lake in Google Earth (centered around the lake centroid). Final percentages were arcsine/square-root transformed according to Warton and Hui (2011) to limit the influence of outliers.

Additionally, the number of people living in the surroundings of a lake directly relates to the risk of schistosomiasis infection. Due to the lack of population census records in the region, we counted the number of houses in a standardized area of 0.25 km^2 (0.5 × 0.5 km) around each lake using satellite images from Google Earth taken in 2019 as a measure of population density.

A total of 20 predictor variables belonging to five categories were used in our study, 15 of which were retrieved in the course of the present survey, two were obtained from online databases and three were taken from the literature (**Table 1**, **Supplementary Table S1**).

Data Analysis

We applied a machine learning approach, i.e., random forest (RF; Breiman, 2001), to assess the combined impact of the chosen set of predictors on the distribution of IH snails. Machine learning approaches, and particularly RFs, have gained prominence in classification and regression analyses across various fields of science in recent years (e.g., Huang and Boutros, 2016; Pang et al., 2017; Schonlau and Zou, 2020; Collin et al., 2021; Georganos et al., 2021; Ruiz-Álvarez et al., 2021). In classification problems, RFs have been demonstrated to give more accurate predictions than other approaches, such as logistic regression (Boulesteix et al., 2012; Bunyamin and Tunys, 2016; Couronné et al., 2018; Xia et al., 2019; Zhang et al., 2020). Since it is a non-parametric technique, the RF algorithm is not affected by multicollinearity among the predictor variables (Boonprong et al., 2018), which is a common problem in ecology. Also, many RF software packages come with convenient solutions to deal with missing values (Brieuc et al., 2018).

We conducted separate RF analyses to variably predict the presence of *Bulinus*, *Biomphalaria* and both genera combined. To assess variation of the potential predictors across different geographical scales, at a larger geographical extent, we ran analyses for the overall and complete dataset combining data from all the three crater lake fields. On the scale of individual crater lakes regions, we ran analyses for two subsets of CLF 1 and CLF 3. The analysis for Fort Portal field (CLF 2 subset) was not performed because it contains only six lakes. All analyses were done in the R statistical environment v. 4.0.3 (R Core Team, 2020), using the packages randomForest v. 4.6-14 (Liaw and Wiener, 2002), rfUtilities v. 2.1-5 (Evans and Murphy, 2019) and rfPermute v. 2.1.81 (Archer, 2020).

We performed imputation to fill missing data prior to further data analyses, using the function “rfImpute” in the package randomForest, which uses the RF algorithm to obtain weighted averages of the available observations. This was done for the predictors; calcium, magnesium and water depth, for which no data were available for 18, 16 and 16 lakes, respectively. Overall, missing data added up 4.7%. The “rfPermute” algorithm was used to assess variable importance in each RF model *via* permutation. The algorithm creates decision trees from the original dataset by random sampling of rows (i.e., lakes) without replacement. At each node, two-thirds of the rows are taken as training data to create the model, the remaining one-third is taken as so-called out-of-bag (OOB) sample and is used to make predictions and test for the performance of the model (Breiman, 1996; Breiman, 2001). Variables were permuted 100 times over 1,000 decision trees.

Model performance was additionally assessed *via* cross validation. This approach was chosen over the standard train–test data procedure because of the comparably low number of lakes in the dataset. Cross validation is a commonly used resampling method to assess the

TABLE 2 | Error rates and results of cross validation for all runs of the random forest (RF) models. Validation agreement was evaluated in accordance with Viera and Garrett (2005). The co-existence model for CLF three had insufficient data and is not included here. OOB, out-of-bag error.

Dataset	RF model	Error rates			Cross validation		
		OOB error	Error presence	Error absence	Kappa coefficient *K*	Validation error	Validation agreement
Complete dataset	*Biomphalaria*	0.143	0.111	0.200	0.911	0.089	excellent
	Bulinus	0.250	0.111	0.500	0.956	0.044	excellent
	Co-existence	0.179	0.148	0.207	0.919	0.081	excellent
Ndali-Kasenda (CLF 1)	*Biomphalaria*	0.063	0.000	0.500	0.633	0.367	substantial
	Bulinus	0.188	0.037	1.000	0.800	0.200	substantial
	Co-existence	0.188	0.040	0.714	0.633	0.367	substantial
Bunyaruguru (CLF 3)	*Biomphalaria*	0.222	1.000	0.067	0.633	0.367	substantial
	Bulinus	0.556	0.750	0.400	0.800	0.200	substantial

generalization potential of a model and to avoid overfitting (Berrar, 2019). We report here the kappa coefficient *K*, which determines the model's predictive accuracy, i.e., it gives the percentage of the data that is in agreement with the model (and is thus the opposite of the validation error). We adopted the suggestion of Viera and Garrett (2005), who regarded kappa values of 0.81-1 as excellent and 0.61–0.80 as substantial agreement, to evaluate our model performances.

The relevance of individual parameters to the overall RF models was assessed using two importance metrics, i.e., the mean decrease in accuracy (MDA) and the mean decrease Gini (MDG) (Calle and Urrea, 2011; Huang and Boutros, 2016). Due to discrepancies in ranking results between MDA and MDG, the results of a single metric are not completely exhaustive (Strobl et al., 2007; Liu et al., 2011). Therefore, we included both metrics but discussed in detail only those parameters that are found significant by both MDA and MDG. We used partial dependence plots to visualize the relationships and marginal effects of individual predictor variables (Friedman, 2001; Evans et al., 2011).

RESULTS

Models converged and were stable across all datasets, with those for *Biomphalaria* always performing better than those for *Bulinus* or both genera combined (**Supplementary Figure S1**). The RF models resulted in excellent to substantial classification successes for the presences of *Biomphalaria* and *Bulinus* in relation to the chosen set of predictor variables (**Table 2**). Classification errors were generally higher for false negatives compared to false positives, which is probably a result of the low number of absences in all datasets. Nonetheless, the cross validation showed that the classification agreements ranged from substantial (CLF subsets) to excellent (total dataset; **Table 2**).

For the overall and complete dataset, geographical, water chemistry and biotic parameters were the most important predictors, but their relative importance and contributions varied across the three RF models, i.e., *Biomphalaria* vs. *Bulinus* vs. both genera combined (**Figure 2**). The distribution of *Biomphalaria* was mainly controlled by latitude, longitude, diversity of the associated mollusk fauna and distance to large lake, as well as by precipitation to some extent; oxygen was only found significant by MDA. For *Bulinus*, the diversity of the associated mollusk fauna, water conductivity and latitude were most important, whereas only MDG identified altitude as a significant predictor. For the combined model, the diversity of the associated mollusk fauna, latitude, longitude and distance to large lake were found relevant. Distance to the next crater lake, magnesium and calcium played a minor role and were only found significant by MDA. Other predictors such as human impact, water pH, surface area and temperature had very little effect and were not significantly impacting the IH species distribution in the region (**Figure 2**).

A different set of parameters was found to be important for the presence of IH snails in individual crater lake fields. For CLF 1, the distance to the next crater lake was the sole important parameter for the distribution of *Biomphalaria* (**Supplementary Figure S2**). In turn, the diversity of the associated mollusk fauna seemed to be the most relevant factor shaping the distribution of *Bulinus*, in addition to distance to the next large lake and lake surface area (MDA only). The co-existence of both genera was controlled by the diversity of the associated mollusk fauna and distance from the next crater lake (**Supplementary Figure S2**). The model for CLF three yielded comparably high error rates, concerning both overall errors as well as classification errors for presences and absences (**Table 2**). Consequently, any association found for CLF 3 with individual parameters is unreliable and will not be discussed further.

The predicted probabilities for the occurrence of IH snails show non-linear relationships with individual predictor variables, with a generally positive trend being apparent in many cases (**Figure 3**). For *Biomphalaria*, the predicted probabilities of its occurrence increases with a rise in latitude, longitude, distance to the large lake (with a slight increase at the end), diversity of the associated mollusk fauna and precipitation. Increasing mollusk diversity also links with an increased probability to encounter *Bulinus*. The parameter conductivity shows a more complex relationship, featuring a steep probability increase up to ~400 µS/cm followed by a weakly, more or less gradually declining trend towards higher values (**Figure 3**). For both genera combined, similar positive trends are revealed for the relationship with the diversity of the associated mollusk fauna, latitude and longitude. The association with distance to the nearest large lake is more complex but indicates an increasing probability to encounter both genera above a distance of ~35 km.

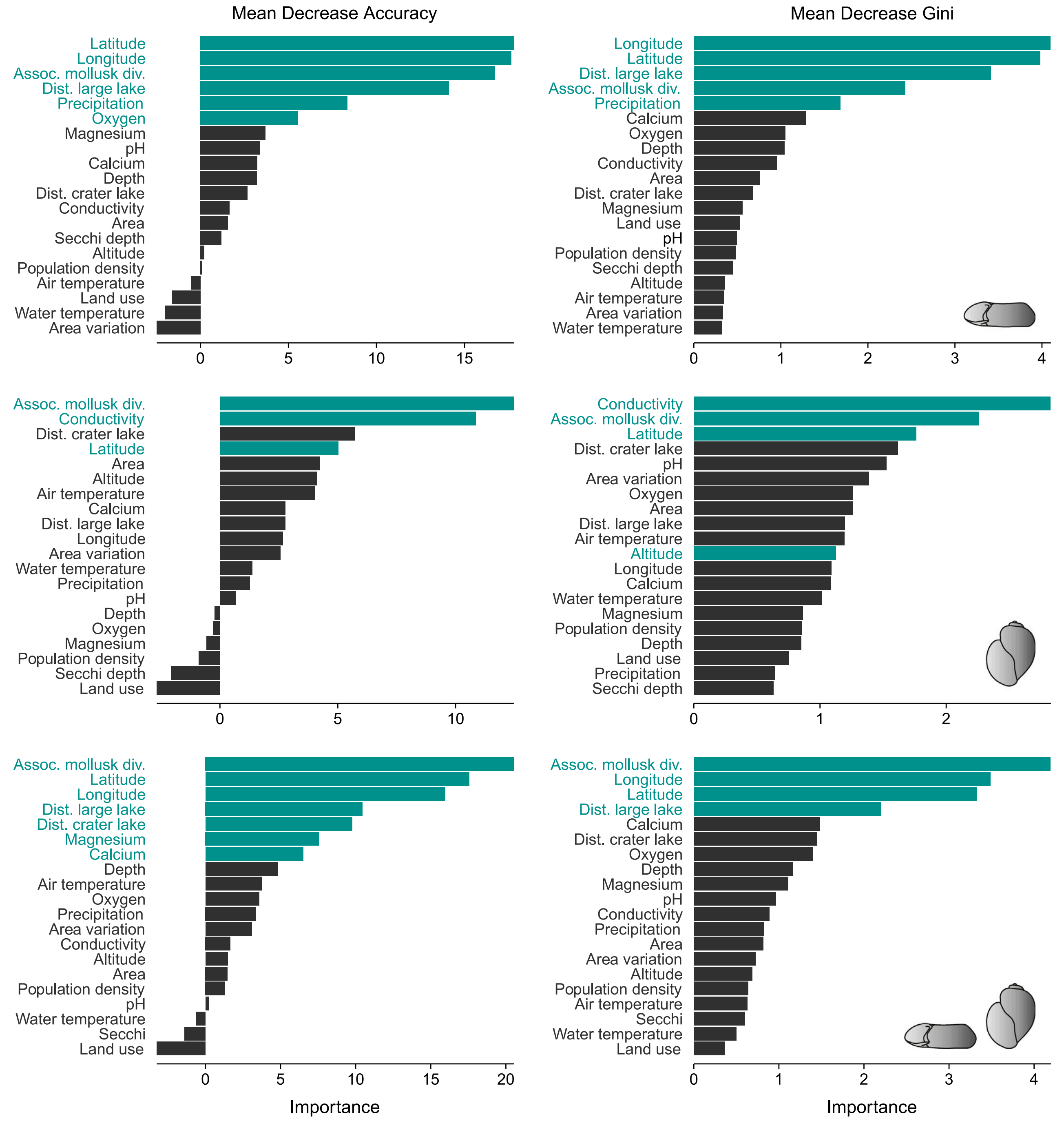

FIGURE 2 | Importance scores of predictor variables for *Biomphalaria* (upper panels), *Bulinus* (middle panels) and both genera combined (lower panels) for the complete dataset. The green bars indicate the significant predictors as determined *via* permutation tests. See **Supplementary Figure S2** for the importance scores of the subset CLF 1.

DISCUSSION

Understanding the factors influencing the distribution of snails acting as IH for *Schistosoma* is crucial for the control of human schistosomiasis. In this study, we explored the applicability of a machine learning algorithm to determine significant predictors of IH distribution and whether they differ among different Ugandan crater lakes. We found distinct variation in the potential controls of IH snail distribution. A different set of parameters is found to influence the distribution of the different genera and across different geographical scales. On the larger scale, geography, diversity of the associated mollusk fauna and

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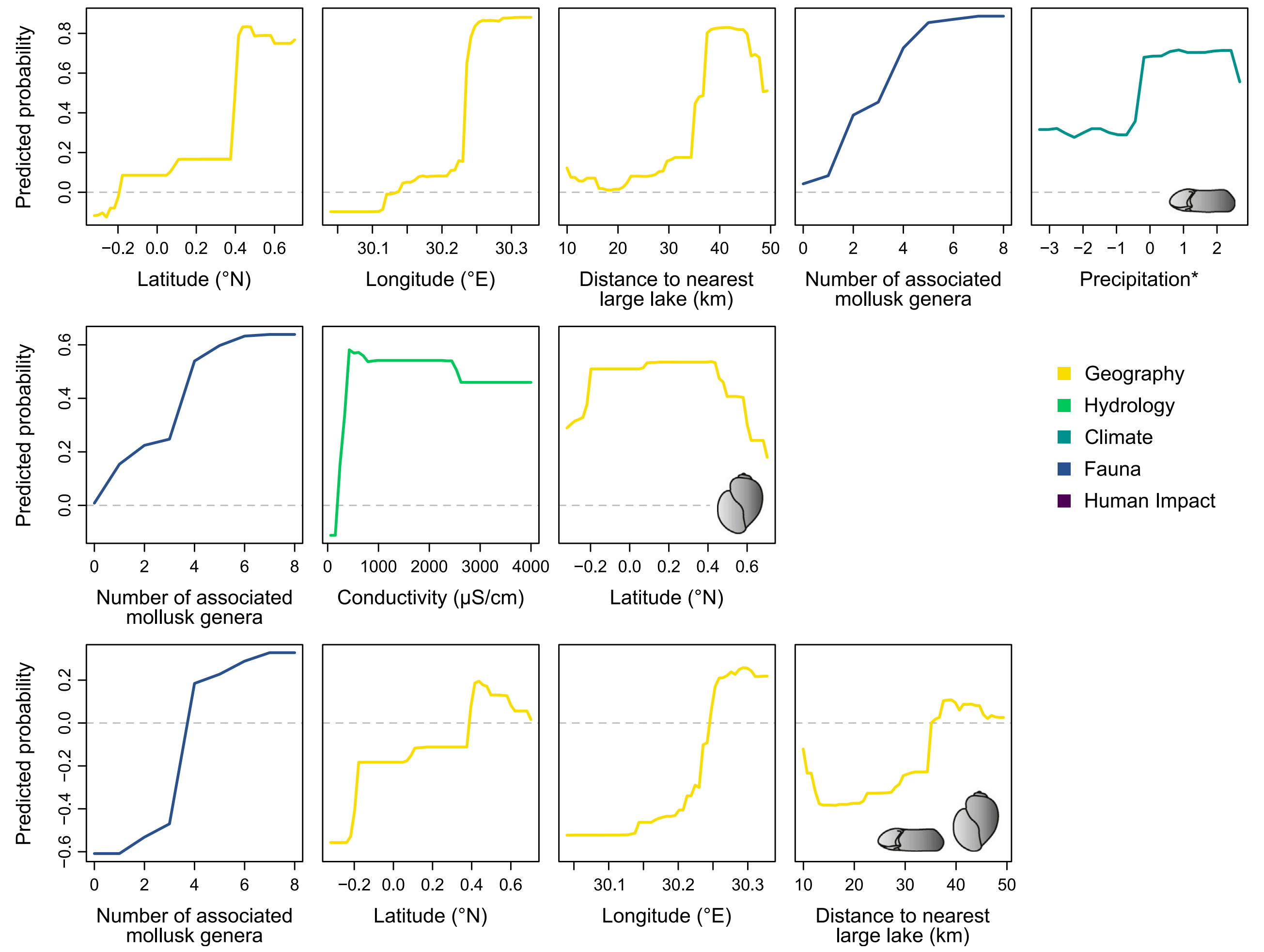

FIGURE 3 | Predicted probabilities for the presence of *Biomphalaria* (upper panels), *Bulinus* (middle panels) and both genera combined (lower panels) in relation to selected predictor variables from each dataset. Only the contributors that were found significant by both importance metrics (MDA and MDG) for the entire dataset are shown (compare **Figure 2**; see **Supplementary Figures S3–S8** for the remaining predictors as well as for the predicted probabilities for CLF 1). The asterisk denotes scaled parameters, i.e., the *x*-axis does not denote the original extent of the variable.

climate are important predictors for the presence of *Biomphalaria*, whereas mollusk diversity and water chemistry control the occurrence of *Bulinus*. Finally, mollusk diversity and geography are relevant for the presence of both genera. On the scale of an individual crater lake field (CLF1) geography (yet with a different variable) and mollusk diversity are relevant for the presence of *Bulinus* and both genera while *Biomphalaria* is solely controlled by geography. These results confirm our hypothesis that a different set of parameters is relevant on different geographical scales.

In the following sections, we discuss the relationships between IH snail occurrence and individual factors and groups of factors (geography, hydrology/water chemistry, climate, biotic and human impact) in an ecological context. Specifically, we discuss the distribution of the two genera with respect to metacommunity assembly processes, i.e., dispersal limitation, environmental filtering and biotic interactions, which limit the successful establishment of species in an ecosystem (see, e.g., Hauffe et al., 2016b for another freshwater gastropod example).

Geography: A Driver on Various Spatial Scales

Geographical variables were found to be among the most important controls for IH snail distribution, but the individual parameters depend on the scale of observation and the taxon in question. Latitude, longitude and distance to the nearest large lake are relevant for the occurrence of *Biomphalaria* as well as both genera combined. In contrast, *Bulinus* is much less influenced by geographical variables. Although latitude and altitude were identified significant, no positive relationship was found for the first (**Figure 3**), whereas the latter was of comparatively low importance (**Figure 2**). A similar trend is found in the Ndali-Kasenda crater lake field (CLF 1), where the distribution of *Biomphalaria* is strongly shaped by the distance to the nearest crater lake, whereas the presence of *Bulinus* is mostly related to the diversity of the associated mollusk fauna (**Supplementary Figure S2**).

The strong positive relationship between the presence of *Biomphalaria* and latitude as well as longitude reflects the uneven occurrence of the genus across the study area (**Figure 1**). Particularly the rarity of the genus in the Bunyaruguru crater lake field (CLF 3) indicates that dispersal limitations and/or environmental filtering (e.g., Hauffe et al., 2016b) might constrain its distribution.

The generally positive association between the occurrence of *Biomphalaria* and distance to the nearest large lake is surprising at first, considering that distance is related to biogeographical isolation and colonization potential (Covich, 2010). Generally, these snails have a high passive dispersal capacity, a high reproduction rate and short generation times (Brown 1994). This combination is pronounced particularly in pulmonate snails such as *Biomphalaria* and makes them prime colonizers (Kappes and Haase, 2012; Kappes et al., 2014). The crater lakes are hydrologically disconnected, the possible longitudinal dispersal means are natural vectors such as birds (Kappes and Haase, 2012) and humans, through the attachment to fishing nets and/or boats.

A possible explanation for the positive association with distance concerns the taxonomic resolution. Here, we investigated the presence of genera, which may overprint patterns of dispersal and colonization of individual species of *Bulinus* and *Biomphalaria*. Also, Euclidean shore-to-shore distances might not reflect real dispersal means or pathways, because hydrological connectivity varies considerably between the large lakes and the crater lake fields. For example, the Fort Portal field (CLF 2) is connected to the Lake George–Lake Edward system *via* the Mpanga River but not to the geographically closer Lake Albert. The pattern may also results from choosing only four major lakes but disregarding smaller ones and river systems. Finally, in colonization processes, stochastic components might play an important role. For instance, regular episodes of shifting mixing regimes in some crater lakes (De Crop and Verschuren, 2021) could lead to low dissolved oxygen and consequently to the demise of most aquatic life. Based on genetic relationships of *Bulinus*, a previous study suggested that the large lakes acted as potential sources for (re)-colonization of the crater lakes (Tumwebaze et al., 2019), a scenario that cannot be tested here given the lack of genetic data. Fast re-appearance or aestivation in fluctuating environments is an intrinsic ecological feature of many *Bulinus* species, including the ones found in the study area (Watson, 1958; Brown, 1994). As such, the colonization and re-colonization patterns might be more complex and faster than previously anticipated.

Altitude was found to have limited influence on the distribution of IH snails. Several previous studies have indicated a shift in recent years of intestinal human schistosomiasis transmission towards higher altitudes (Kabatereine et al., 2004; John et al., 2008; Stanton et al., 2017). This is also supported by our findings showing that IH snails occur up to approx. 1,600 m a.s.l., and thus considerably higher than the previously presumed threshold of 1,400 m a.s.l. (Kabatereine et al., 2004). Future studies will have to establish an upper limit for both forms of schistosomiasis, because both IH snail genera have been found at altitudes above 2,000 m a.s.l. in Uganda (Stanton et al., 2017, unpubl. data).

Associated Mollusk Diversity

A strong control for the distribution of both *Bulinus* and *Biomphalaria* is the diversity of the associated mollusk fauna. The strong positive relationships in all three models (**Figure 3**) indicate that both genera are more likely encountered in generally diverse systems. One may expect the opposite, that a higher number of species (especially in a small crater lake) results in higher competition (e.g. Svanbäck and Bolnick, 2007; Hauffe et al., 2016b) and thus a lower chance of successful establishment. Instead, the positive association with diversity indicates that the IH snail genera are present in environments that provide favorable conditions for mollusks generally.

The associated mollusk fauna consists predominantly of pulmonates (*Radix natalensis* and several species of planorbids other than *Biomphalaria*), which are good colonizers in general and are characterized by a high productivity and shorter generation times (Kappes et al., 2014). Malacological surveys showed the presence of *Biomphalaria sudanica*, *Biomphalaria pfeifferi*, *Bulinus forskalii*, *Bulinus globosus*, and *Bulinus tropicus* (Tumwebaze et al., 2019; Tumwebaze et al., unpubl. data). All *Biomphalaria* species in the region are regarded susceptible to *S. mansoni*. Only *B. forskalii* and *B. globosus* were identified as host species for human schistosomes and the majority of *Bulinus* in the crater lakes region were *B. tropicus*, which is an important IH snail for livestock schistosomes. The non-pulmonate species are common and widespread regional species with no particular ecological requirements (Brown, 1994). More specialized taxa such as *Bellamya* or *Cleopatra* or unionid bivalves were not found in the crater lakes. The mollusk association in the crater lakes could still be seen as depauperate. The most obvious variation in habitat conditions and thus potentially determining mollusk associations is in the limnological characteristics of the crater lakes. Although we gathered a variety of different parameters concerning water chemistry and climatic conditions, the associations with individual parameters do not allow drawing a multifactorial picture. Moreover, the parameters measured for each crater lake do not cover seasonal variability (except for climate and lake area variation).

Hydrology and Water Chemistry

Only selected hydrochemical variables had an influence on the distribution of IH snails in our study region. Water conductivity was a significant driver across the entire study region and in CLF one for *Bulinus*. For *Biomphalaria*, oxygen was found a significant driver by one metric (MDA), but its relative importance was low (**Figure 2**). Similarly, MDA found the presence of both genera linked to magnesium and calcium, but again with low importance values.

Water conductivity determines the ionic strength of the concentration of dissolved solids including calcium and magnesium (Cormier et al., 2013). An expanding distribution with increasing conductivity was also suggested by Camara et al. (2012). This might reflect the presence of dissolved ions (e.g., calcium), which stimulate shell development for snail species

(Dillon, 2000; see also below). The non-linear relationship with a sharp increase around ~400 μS/cm followed by a nearly steady but weak decline indicates that a certain threshold must be met to allow the establishment of *Bulinus* in a lake. Higher electric conductivity is, in turn, less favorable. Conductivity as a complex factor integrating several chemical components has been identified as a determinant for *Bulinus* mortality and thus poses an important constraint on its occurrence (Brown, 1994; Marie et al., 2015).

The predicted probabilities for the presence of both genera in relation to magnesium and calcium concentrations, which were, however, only found significant by one importance metric (MDA), demonstrate that a certain threshold must be surpassed in both factors to promote the establishment of IH snails. Especially the relationship with calcium is not surprising, considering that a low concentration would constrain snail growth, fecundity, survival rate and reproduction, which, in turn, limit snail distribution (Dillon, 2000; Brodersen and Madsen, 2003). The concentration of calcium and magnesium and their ratio in the water both affect the presence and life cycle performances in southern African streams with thresholds at the lower and higher ends of concentrations (Brown, 1994).

Climate

Climatic factors seem to have surprisingly little effect on the distribution of IH snails in Ugandan crater lakes. Precipitation was found a significant but rather weak predictor in the model with *Biomphalaria*, whereas air and water temperature do not appear influential. Generally, climatic conditions and climate change are known to be important predictors for mollusk species distribution on larger geographical scales (Marcogliese, 2008; Stensgaard et al., 2019) and were also found to be relevant for IH snails. Temperature influences the survival and reproduction rates of the snails (Paull and Johnson, 2011; McCreesh et al., 2014; Kalinda et al., 2017). Higher rainfall causes more runoff into freshwater ecosystems increasing the supply of organic matter serving as food for the snails, which, in turn, promotes growth and fecundity (Madsen et al., 1987; Camara et al., 2012; Nyström Sandman et al., 2013; but see also discussion in David et al., 2018 for opposite associations). This may also explain the relationship with precipitation in our case. However, the lack of a (strong) association in our models is likely owed to the constrained geographical scale of our observations. The crater lakes are located in the same climatic zone in western Uganda, which is why precipitation and air temperature vary only little across the study region. Whereas air temperature seems to be irrelevant on that scale, the relationship with precipitation indicates that even small variations might have a significant impact on the IH snail distribution.

Non-Significant Drivers

A series of parameters was found to be non-significant in any of our models. Here, we briefly discuss the potential reasons for these findings, especially in the light of conflicting results reported in the literature. In contrast, previous studies found relationships between the distribution of *Biomphalaria* and water pH, whereas both oxygen and water pH have been suggested to determine the occurrence of *Bulinus* (Yirenya-Tawiah et al., 2011; Stensgaard et al., 2013; Marie et al., 2015; Mahmoud et al., 2019; Alhassan et al., 2020). However, no such influences were noticed despite of the influential factors of high oxygen concentration and water pH of the lakes studied (**Supplementary Table S1**). Possibly, the high carbon dioxide emission from decomposing submerged vegetation and organic matter, together with the presence of other dissolved ions could have indirectly affected such relationships (Tchakonté et al., 2014). Lake area variation, being a measure of ecosystem stability, seems also of little importance. Apparently, most of the lakes show minimal fluctuations through time, and the few that occasionally dry up could not influence the distribution or recolonization, which is facilitated by the short distances between the crater lakes. The mollusk associations of mostly opportunistic species found is also supporting this interpretation (see above).

Human impact, quantified by both land use and population density around the crater lakes, has apparently no impact on snail distribution. This result is rather unexpected, because humans are often involved in introduction of snails into new environments as passive dispersal vectors (Kappes and Haase, 2012). In the Ugandan crater lake fields, extensive anthropogenic activities (e.g., multipurpose water fetching, watering for livestock, littering and pollution) are limited to lower, relatively flat and thus more easily accessible shores of most of the crater lakes. As such, one may have expected an impact on snail occurrences. Moreover, only about 26% of the crater lakes are located in national parks (Queen Elizabeth NP and Kibale NP), thus three-quarter of the lakes are accessible and utilizable by humans. Despite these constraints, some reserves and parks like Kibale NP increasingly face migration of settlers and extensive cultivation (Hartter et al., 2015). Perhaps the prevailing population pressure affects the prevalence of infected snails, but it does not influence their presence in general. Follow-up studies should focus specifically on this aspect for mollusks communities and the prevalence of schistosomiasis. Interestingly, in other non-gastropod taxa such as cladocerans (Rumes et al., 2011) and fungi communities (Gelorini et al., 2012), negative effects of land use change and anthropogenic pressure have already been demonstrated for the crater lakes region. With increasing human activities in the Ugandan crater lakes region (including tourism), we expect further changes in biological communities of the lakes as well as increasing cases of human schistosomiasis among both local communities and visiting travelers (Lachish et al., 2013) in the near future.

Methodological Implications and Limitations

Despite the numerous advantages machine learning approaches like RFs offer, one disadvantage concerns the non-linearity of the approach, resulting in a limited prediction power outside the data range. Therefore, we cannot extrapolate our findings to other datasets or regions. Furthermore, small datasets (e.g. CLF 2, *Biomphalaria* subset with CLF3), decisively limit RF classification due to insufficient data. Optimally, future studies need to include data from a larger geographical scale, involving a greater variation in the ranges of predictor variables, to provide more general predictions for the controls of IH snail distributions. Using machine-learning algorithms like RFs on a comprehensive dataset will eventually facilitate more general conclusions about the importance of individual predictors (or sets of predictors) for the presence of IH snails. Future studies may focus on applying this approach to map infection risk areas, especially in comparison to areas with actual prevalence records and those where preventative measures are in place.

CONCLUSION

The results indicate that *Biomphalaria* is mainly controlled by geography, associated mollusk diversity and climate, while fauna, hydrology and to some extent geography controlled the presence of *Bulinus*. Geography (*Biomphalaria*) and mollusk diversity (*Bulinus*) were the only significant predictors on the scale of an individual crater lake field. The intricate relationship between IH snail distribution and geography likely reflects dispersal limitations and/or environmental filtering on the hand and a complex pattern of (re-)colonization on the other hand. The positive association with the diversity of accompanying mollusks, as well as the relationship with water conductivity, indicates that IH snails are common in ecosystems offering favorable conditions for mollusks in general.

Our machine learning approach helped disentangling relevant factors in IH snail distribution. The results of this study provide baseline data that assist future research towards controlling schistosomiasis.

DATA AVAILABILITY STATEMENT

All data used in the study is available in the paper or the **Supplementary Material**.

AUTHOR CONTRIBUTIONS

CA, ZT, and IT conceived the study. CA and IT conducted field work. ZT and TN performed the analyses. ZT wrote the manuscript with contributions from TN and CA. TN, BS, and CH prepared the figures. LB and CA supervised and critically revised the study. All authors reviewed the manuscript and approved the final version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2022.871735/full#supplementary-material

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Evolution and Biogeography of Freshwater Snails of the Genus *Bulinus* (Gastropoda) in Afromontane Extreme Environments

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Mountains are considered island-like systems often characterized by exceptional biodiversity and endemism. There are many highly isolated mountain ranges in Africa that collectively have been called the Afromontane archipelago. Freshwater snails of the genus *Bulinus* are composed of many veterinary and biomedical important species. These intermediate hosts for schistosomiasis parasites are wide spread and some of the species are considered to be highly adapted to very harsh environmental conditions such as droughts or low temperatures. However, the extent to which the *Bulinus* have adapted to live in high-altitudes and factors influencing these range shifts are not well investigated. In this study, we analyzed pan-African *Bulinus* species from various habitats across different altitudinal ranges, focusing on the high mountains or "sky islands" to examine how the contemporary climate and historical geological factors affect species distributions and evolutionary processes. Using a fossil-calibrated multigene phylogeny composed of two mitochondrial genes (*cox1, 16S*) and two nuclear genes (*ITS2* and *H3*), we tested: 1) how often and when extreme altitudes were colonized, 2) what are the biogeographical affinities and degree of isolation of high-altitude species, 3) which lineages diversified and evolved endemism in the Afromontane environments, and 4) whether the Afromontane regions represent "sky islands". Bayesian phylogenetic inference employing a fossil-calibrated molecular clock resulted in a strongly supported phylogeny resolving the relationships between the four *Bulinus* groups. High-altitude colonization exists exclusively within the *Bulinus truncatus/tropicus* complex. Several independent colonization events occurred in the Pliocene and Pleistocene throughout Africa, mostly from nearby regions of the respective mountain ranges. Most species evolved in low to mid-altitudinal ranges. Endemism is pronounced in the Ethiopian Highlands and those of Kenya and Lesotho. A previously unknown species was found at an extreme altitude (~4,000 m a.s.l.) on Mt. Elgon/Uganda extending the formerly known altitudinal maximum of the genus by roughly 900 m. The endemic species has already diverged in the Pliocene (~4 myr) and is currently characterized by low genetic diversity. There is further cryptic diversity in mountain ranges of Lesotho. Our findings are discussed in a biogeographical, conservation and biomedical context.

Keywords: Afrotropics, endemism, sky islands, biodiversity hotspots, schistosomiasis, climate change, high-altitude, intermediate host snails

INTRODUCTION

Mountains worldwide are well-known cradles of biodiversity additionally characterized by elevated levels of endemism (Perrigo et al., 2020). Mountains are also island-like systems, surrounded by low-lying land masses (Itescu, 2019). A number of mountains are thus termed “sky islands” (e.g., McCormack et al., 2009; Mairal et al., 2017). The complex nature of these sky islands and the isolation between them restrict dispersal, thus affecting species distribution, richness and abundance (Gillespie et al., 2009). Colonisation for sky islands, unlike real islands is through immigration from low lands (altitudinal range niche shifts) or short and long dispersal from the nearby sky islands (latitudinal range shift) (McCormack et al., 2009). These immigration events, followed by *in-situ* speciation may give rise to cryptic species and in some cases species endemism. Consequently, these resulting narrow-range species are often under threat (Mairal et al., 2017; Martín-Queller et al., 2017). Besides historical events such as mountain-building, other factors have been “implicated” in shaping species richness (Georgopoulou et al., 2016). These are primarily temperature, species richness, isolation and area (Steinbauer et al., 2016). Varying climates along steep altitudinal gradients are often seen as prime drivers of isolation of populations, eventually leading to speciation on mountain tops (Rahbek et al., 2019). Mountain species are regularly characterized by pronounced niche conservatism (Antonelli et al., 2018). As isolated geographical features with characteristic species composition and distribution along pronounced altitudinal and thus climatic gradients, they are often used as models in climate change research (e.g., Fischer et al., 2011).

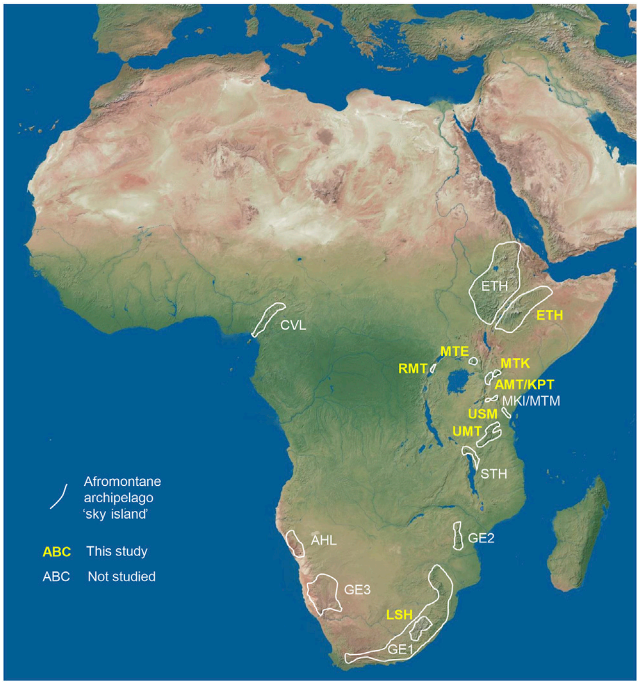

FIGURE 1 | Afromontane “sky islands” studied. The mountain ranges in sub-Saharan Africa covered in the current study are italicized and colored in yellow. Abbreviations: AHL, Angolan Highlands; AMT, Aberdares Mts.; CVL, Cameroon Volcanic Line; ETH, Ethiopian Highlands; GE1–3, Great Escarpment; Kinangop Plateau (KPT)-; LSH, Lesotho Highlands; MKI, Mt. Kilimanjaro; MTK, Mt. Kenya; MTM, Mt. Meru; MTE, Mt. Elgon; STH, Southern Tanzania Highlands; USM, Usambara Mts.; UMT, Udzungwa Mts.

There are many highly isolated mountain ranges in Africa, a topographically diverse continent significantly shaped by the rifting processes involving volcanism. These processes, in turn, produced several prominent volcanic mountains such as iconic Kilimanjaro, Mt. Kenya, Mt. Elgon, or the Cameroon Volcanic line, a long chain of volcanoes. The Ethiopian Highlands, Maloti-Drakensberge, Kenyan-Tanzanian Highlands, Eastern Arc Mts., and the Angolan Highlands represent other important mountain ranges that collectively have been called the Afromontane archipelago, i.e., a collection of widely scattered sky islands (**Figure 1**). They have been studied repeatedly regarding their diversity, endemicity and interconnectivity (e.g., Measey and Tolley, 2011; Mairal et al., 2017). Remarkably, they represent both cradles and refugia for biodiversity (Perrigo et al., 2020). Contrasting hypotheses have been proposed as to corridors connecting these sky islands in close proximity but also across the whole continent, e.g., from the Kenya-Tanzania Highlands to the Cameroon Volcanic line (Allen et al., 2021). Pleistocene corridors of Afromontane forest belts have been proposed (Mairal et al., 2021) but remain to be tested for most taxa, as the results of hitherto conducted studies are equivocal (e.g., Brühl, 1997; Cox et al., 2014). For mountain biota in Africa, montane refugia are often invoked as speciation mechanism, followed by montane gradient speciation and, to a lesser extent, peripatric speciation and rapid adaptive radiation, whereas the role of polyploidization as a genomic mechanism is not well understood (Couvreur et al., 2021).

Many patterns seen in biodiversity of the Afromontane archipelago have been attributed to changing topography and ecology triggered by geological rifting (e.g., Kingdon, 1989; Menegon et al., 2014). In addition, Pleistocene climatic changes produced multiple refugia, including cold-climate areas such as in sky-islands. These often acted as cradles for diversity on the population (Mairal et al., 2017) and species levels (Cox et al., 2014). The African Rift System and associated mountain ranges and beyond are thus an ideal model to study the interacting forces of climatic changes and geographic barriers on genetic biodiversity patterns over evolutionary times (Platts et al., 2013). Such studies are also critical in times of enormous pressure on the remaining montane biodiversity hotspots throughout the continent (White, 1981; Burgess et al., 2007). Even though these outstanding biodiversity hotspots have long been recognized (Mittermeier et al., 2011), most knowledge stems from either plants such as grasses (Mairal et al., 2021) or trees (DeBusk, 1998) and vertebrates such as small mammals, birds and amphibians (e.g., Cox et al., 2014; Loader et al., 2014). Invertebrates are less frequently studied and if so, mostly mobile organisms such as butterflies (but see flightless insects; Brühl, 1997). The overwhelming majority though represent terrestrial taxa, freshwater organisms are exceptionally scarcely studied (e.g., Daniels et al., 2020; Musonge et al., 2020).

Freshwater molluscs are very suitable for biogeographical studies of high-altitude ecosystems since they are less mobile, often highly habitat specific and have a moderate level of diversity but are comparatively well-known taxonomically (Brown, 1994), unlike most other invertebrates groups in tropical regions. African sky islands are only inhabited by a limited number of freshwater mollusc genera, namely pea-clams (Sphaeriidae; e.g., Kuiper, 1966; Clewing et al., 2022) and pulmonate snails (some Bulinidae, Planorbidae, Lymnaeidae; Brown, 1994). Mollusc species living in high-altitudes (roughly above 2,500 m above sea level) may experience extreme environmental conditions. These include, for example, higher radiation, desiccation of habitat, short periods for reproduction, shortage of food or nutrient availability, cold temperatures including freezing and low abundances (Bößneck, 2012).

The pulmonate gastropod genus *Bulinus* is, arguably, the most widespread pulmonate snail species in Afromontane regions. The genus is composed of many veterinary and biomedical important species. Many *Bulinus* species act as intermediate hosts for *Schistosoma* trematodes that cause, especially, human urogenital schistosomiasis, a disease affecting at least 130 million people in Africa alone (Brindley and Hotez, 2013). Factors that affect species distributions in Africa have not been investigated for many of the approximately 35 extant *Bulinus* species (Brown, 1994), especially in high-altitude freshwater habitats. Generally, climate change has been predicted to cause changes in species distribution and consequently changes in endemism, evolutionary processes such as speciation and extinction dynamics (Hua and Wiens, 2013), including molluscs (Böhm et al., 2021). Some species of the genus *Bulinus* are considered to be highly adapted to very harsh environmental conditions such as desiccation (e.g., *B. globosus*) or low temperatures (e.g., *B. africanus*) (Brown, 1994). The role of polyploidization has been discussed in this context (Brown, 1994). However, the extent to which *Bulinus* snails have adapted to high-altitudes in general and extreme altitude above 3,000 m a.s.l., in particular, is not yet accounted for. Here, we explore how and when species of *Bulinus* have evolved altitudinal ranges. This is important in order to understand the climate-related dynamics of host-parasite interactions. Furthermore, it is a prerequisite for potential predictions of how the ongoing climate change throughout sub-Saharan Africa will affect occurrence of intermediate host snails (Stensgaard et al., 2019; De Leo et al., 2020). In turn, this has direct implications for parasite prevalence and infections of both humans and livestock in areas where diseases have previously been absent (Stanton et al., 2017). Predictions for schistosomiasis in Africa foresee disappearance regionally in lowlands due to extended droughts (shortage of water and thus habitat). Contemporarily, however, increase in schistosomiasis in mountainous regions is predicted due to the expansion of suitable conditions, given the warming experienced (Stensgaard et al., 2019). As a consequence, the altitudinal threshold for schistosomiasis is expected to rise, potentially putting more people and an unrecognized part of the populations at risk (Stanton et al., 2017). Currently, there are no empirical studies available on this subject.

In this study, we analyzed pan-African *Bulinus* species from various habitats across different altitudinal ranges, focusing on the high mountains or "sky islands" to examine how the contemporary climate and historical geological factors affect species distributions and evolutionary processes. High-altitude samples from most Afromontane regions were collected and altitudinal ranges compiled from our own continent-wide sampling, the literature and databases.

Using a multigene phylogeny of three mitochondrial and two nuclear markers, molecular clock and character evolution analyses, we tested several evolutionary and biogeographical hypotheses for the evolution of the altitudinal niches for all species groups of *Bulinus*.

Specifically, we tested:

1) how often and when extreme altitudes were colonized,
2) what are the biogeographical affinities and degree of isolation of high-altitude species,
3) which lineages diversified and evolved endemism in the Afromontane environments, and
4) whether the Afromontane regions represent "sky islands".

Our findings are discussed in the context of biogeographical and diversification history and the dominant mode of speciation. Potential future developments are evaluated given changing climate predictions as well as biomedical and conservation implications.

MATERIALS AND METHODS

Sampling

Specimens were collected from mountains and highland regions throughout Africa between 2010 and 2018. These areas included the Lesotho Highlands (Maloti-Drakensberge), Ethiopian Highlands (Abyssinian Massif), Kenyan Highlands (Aberdares range including Kinangop Plateau, Mt. Kenya/Laikipia), Tanzanian Eastern Arc Mts. (Udzungwa, East Usambara), and Rwenzori Foothills (**Supplementary Table S1**; **Figure 1**). We also obtained samples from lower altitudes, primarily from Uganda, Malawi, Tanzania, the Democratic Republic of Congo, and Cameroon, for comparative purposes and maximized taxonomic representation. Sampling was done using mainly a scoop net, and in some occasions, handpicking from pools, wetlands marshes, lotic and lentic waterbodies. The material was fixed and preserved in 70%–80% ethanol. Coordinates and the altitude records were obtained using a hand-held Garmin etrex V global position system (GPS) device, and were later verified with Google Earth Pro version 7.3.4.

DNA Extraction, Amplification and Sequencing

At least two snails of each of the *Bulinus* population were selected for DNA isolation, resulting in a total of 104 *Bulinus* specimens.

Prior to DNA isolation, each of the selected snails was photographed using a Keyence digital microscope system (KEYENCE VHX-2000; Keyence Deutschland GmbH, Neu-Isenburg, Germany). Genomic DNA was then extracted from a small piece of the foot muscle (~2 mg) using a CTAB method (Wilke et al., 2006) or a DNeasy Blood and Tissue Kit (Qiagen, Mississauga, ON, Canada) following the manufacturer's protocol. The specimen vouchers (shells and DNA) are stored in the University of Giessen Systematics and Biodiversity collection (UGSB; Diehl et al., 2018). DNA amount and quality was checked using a NanoDrop 2000 (Thermo Fisher Scientific Inc., Waltham, MA, United States). Mitochondrial cytochrome *c* oxidase subunit I (*cox1*; "Folmer" and "Asmit" regions) and large subunit ribosomal RNA (*16S*) gene fragments were amplified using primers LCO1490 and HCO2198 (Folmer et al., 1994) or COR722b (Wilke and Davis, 2000) for the "Folmer" region, Asmit1, Asmit2 for the "Asmit" region (Bowles et al., 1992), and 16Sar and 16Sbr for *16S* (Palumbi et al., 1991). The internal transcribed spacer 2 (*ITS2*) and histone 3 (*H3*) were amplified using primers LT1 (Bargues et al., 2001), ITS2-RIXO (Almeyda-Artigas et al., 2000) and H3F and H3R (Colgan et al., 2000), respectively.

PCR conditions and primer details are described in the **Supplementary Table S4**. Sanger sequencing was performed on an ABI 3730xl DNA analyzer using the BigDye Terminator Kit (Life Technologies, LGC Genomics GmbH, Berlin, Germany).

Dataset Compilation, Alignment, and Substitution Models

DNA sequences were edited and aligned using BioEdit version 7.0.5 (Hall, 1999). For phylogenetic analyses, additional sequences for all five genetic markers were downloaded from the NCBI database, resulting in a dataset composed of 145 ingroup taxa. After the sequences were reduced to unique haplotypes (**Supplementary Figure S1** and **Supplementary Table S1**), the final dataset consisted of 96 taxa and was used for subsequent analyses. *Indoplanorbis exustus*, the sister species to *Bulinus* spp. (Albrecht et al., 2007; Albrecht et al., 2019), was used as an outgroup. Multiple sequence alignment for *cox1* (both the Folmer and Asmit regions) and *H3* was conducted using ClustalW tool implemented in BioEdit software program while the gap forming partitions *16S* and *ITS2* were aligned using MAFFT an online alignment tool (Katoh et al., 2019). Gblocks program version 0.91b (http://molevol.cmima.csic.es/castresana/Gblocks_server.html), was used to remove poorly aligned regions, applying less stringent settings. The final alignments contained 655 bp, 390 bp, 432 bp, 452 bp and 328 bp for *cox1* (Folmer region), *cox1* ("Asmit" region), *16S*, *ITS2*, and *H3*, respectively. All new sequences have been deposited in NCBI GenBank (**Supplementary Table S1**).

The concatenated sequence dataset was created using SequenceMatrix version 1.7.8 (Vaidya et al., 2011). The software jModelTest version 2.1.10 (Darriba et al., 2012) was used to select the best-fit substitution models for each partition, with the number of substitution schemes set to 3.

Fossil-Calibrated Molecular Phylogeny

The estimated substitution models were used for the subsequent Bayesian inference analysis employing a molecular-clock approach for estimating species divergence using BEAST version 1.8.4 (Drummond et al., 2012). The analyses were run on the CIPRES Science Gateway V 3.3 (Miller et al., 2010). A fossil calibration was used to estimate divergence times in *Bulinus* using BEAST version 1.8.4 (Drummond et al., 2012). We used the oldest *Bulinus* fossil (c. 20 myr; Pickford, 2008) to date the most recent common ancestor (MRCA) of the ingroup.

Based on the Akaike information criterion (AIC), the best-fit substitution models for the concatenated dataset were: HKY + I + Γ, HKY + I + Γ, GTR + I+Γ, GTR + I + Γ and SYM + I for *cox1* (Folmer region), *cox1* ("Asmit" region), *16S*, *ITS2*, and *H3*, respectively.

The Markov chain Monte Carlo (MCMC) in BEAST analysis was run for 40 million generations, sampling every 1000th tree, using both a strict-clock and a relaxed-clock model with a birth–death tree prior (Gernhard, 2008) and a gamma prior for the fossil calibration point with the settings: offset = 19, shape = 1.0, and scale = 2.0. The log file was checked in Tracer version 1.5 (http://tree.bio.ed.ac.uk/software/tracer) to examine the parameter convergence. The maximum clade credibility (MCC) tree was generated using TreeAnnotator version 1.8.4 (BEAST package) with a burnin of 50%.

Whereas most of the parameters revealed effective sampling size (ESS) values for the strict-clock model slightly better (ESS of most parameters >300) than with the relaxed-clock model (ESS of most parameters <150), convergence for some of the strict clock model parameters was not reached (i.e., cases in which ESS values were <200). Therefore, the maximum clade credibility (MCC) tree obtained from the strict-clock analyses was used for subsequent analyses, with adjusted substitution models as follows: HKY + I + Γ, HKY + I + Γ, HKY + I + Γ, HKY + I + Γ and HKY (all base frequencies equal), for *cox1* (Folmer region), *cox1* ("Asmit" region), *16S*, *ITS2*, and *H3* respectively, resulting in sufficient ESS values (>200). We also tested a general invertebrate molecular substitution rate (mitochondrial) clock (Wilke et al., 2009) for the HKY + I + Γ, model of sequence evolution (1.57% My^{-1}) in the preliminary analyses (hence data not shown), to check the suitability of the fossil in estimating the divergence time of the *Bulinus* phylogeny., and this did not result in much difference.

Species Delimitation

Specimens were preliminarily determined based on their shell morphology and ecological setting information such as habitat and geographical position. However, we also used approaches for molecular species delimitation recently conducted (Chibwana et al., 2020; Clewing et al., 2020; Mahulu et al., 2021). Molecular operational taxonomic units (MOTUs) were identified in the BEAST MCC tree using this approach and computational methods for species delimitation; specifically the Generalized Mixed Yule Coalescent (GMYC) for multiple thresholds (Fujisawa and Barraclough, 2013; Pons et al., 2006) and Poisson Tree Process (PTP; both maximum likelihood and highest Bayesian supported solutions; Zhang et al., 2013). The

MOTUs were finally assessed in an integrated approach considering the preliminary identifications and named by integrating as many nominal taxa as possible. Doubtful cases or apparently new lineages were simply labelled as *Bulinus* sp., sometimes with a qualifier (e.g., *Bulinus* sp. 8 MTE for Mt. Elgon).

Reconstruction of Ancestral Altitudinal Ranges

Two sets of altitudinal information were compiled. Each specimen had a specific sampling altitude, either measured by ourselves in the field or taken from the literature for the GenBank sequences. Google Earth Pro was used to determine the altitudes in cases when only locality names were available in the original literature. In the cases of BtroAY18 and BtruAY19 (**Supplementary Table S1**), the altitude ranges of their countries of origin were used since no more specific locality information was available.

The second set of altitudinal information consisted of published altitudinal occurrences for all nominal species of *Bulinus*, which we screened for minima and maxima (**Supplementary Table S2**). This information was compiled through literature research in comprehensive publications (Mandahl-Barth, 1957; Mandahl-Barth, 1965; Brown, 1994) or species-specific accounts (e.g., De Kock and Wolmarans, 2005a; De Kock and Wolmarans, 2005b). In addition, online databases such as Global Biodiversity Information Facility (GBIF), International Union for Conservation of Nature (IUCN) Redlist, NHM London Zoological Specimen database were checked. A character matrix with six categories was created: A) 0–500 m, B) 501–1,000 m, C) 1,001–1,500, D) 1,501–2,000 m, E) 2,001–2,500, and F) > 2,500 m. The altitude ranges of all the MOTUs were coded as present (1) or absent (0) in these categories. In very few cases, our own sampling served for character coding. Character evolution of the altitudinal range distribution was traced along the phylogenetic tree using stochastic character mapping (Huelsenbeck et al., 2003) as implemented in phytools 1.0-1 (Revell, 2012) for the R statistical environment 4.1.2 (R Core Team, 2021). Three models of character evolution, ARD (all rates different), SYM (symmetric rates), and ER (equal rates), were tested by calculating 100 stochastic character maps and finally compared with AIC.

RESULTS

Species Diversity, Phylogenetic Relationships, and Divergence Times

The automated species delimitation methods yielded 24 and 38 MOTUs for bPTP and 33 for GMYC (**Supplementary Figure S1**). Our integrative approach resulted in a final set of 30 MOTUs. *Bulinus wrighti* is the sole representative of the *B. reticulatus* group. The *B. forskalii* group comprises *B. forskalii*, *B. bavayi*, *B. cernicus*, *B. barthi,* and *Bulinus* sp. 6. The *B. africanus* group primarily consists of *B. globosus* and *B. nasutus*, but also *B. africanus* and *B. obtusispira*. In addition, it contains *Bulinus* sp. 2 (of Chibwana et al., 2020) and a MOTU which could not be assigned to nominal names (*Bulinus* sp. 3). Most species belong to the *B. truncatus/tropicus* complex, of which a total of 18 MOTUs correspond to species-level taxa. Names cannot be applied to all of them, but instead nine nominal taxa could be assigned. *Bulinus* sp. 1 was previously reported in Chibwana et al. (2020). A further two have been labelled *Bulinus* sp. 4 and *Bulinus* sp. 5 for convenience. *Bulinus* sp. 7 LSH (= Lesotho) and *Bulinus* sp. 8 MTE (= Mt. Elgon) were previously unknown species. The MOTU called *Bulinus* sp. 9 MTK (= Mt. Kenya) might represent a small species complex of closely related taxa (*B. rumrutiensis/laikipiensis*). Both *B. truncatus* and *B. tropicus* were not found monophyletic, instead there where other MOTUs resembling them morphologically (*B.* cf. *truncatus*, *B.* cf. *tropicus*).

The phylogenetic reconstruction (**Figure 2**) revealed four major clades, which are strongly supported. These clades correspond to the four traditional species groups of *Bulinus*, i.e., the *B. forskalii*, *B. africanus*, *B. reticulatus* groups, and the *B. truncatus/tropicus* complex. *Bulinus reticulatus* and the *B. truncatus/tropicus* complex are well supported sister-groups (PP 1.0) and *B. africanus* is the sister group to them (PP 1.0). The *B. forskalii* group is ancestral to the other three groups (PP 1.0).

High-mountain lineages from the Aberdares Mts. and the Mt. Kenya region are sister groups in *B. tropicus*, whereas the Udzungwa Mts. lineage is not closely related to it. It clusters with regional lineages (**Figure 2**; **Supplementary Figure S1**). *Bulinus* sp. 9 MTK is sister to a specimen from Malawi (BtruAY19), but this relationship is not supported. This is also the case for *Bulinus* sp. 4 the sister of which is BtroAY18, a specimen from Zimbabwe. *Bulinus permembranaceus* from the Kinangop Plateau is closest to *Bulinus* sp. 5, represented by specimens from Cameroon and the D.R. Congo. *Bulinus hexaploidus* from the Ethiopian Highlands is sister to *B. guernei*.

According to our molecular-clock analysis, *Bulinus* started to diversify in the Early Miocene, which is also when the *B. forskalii* group diverged from the remaining groups. In the mid-Miocene (~16 Ma), the *B. africanus* group diverged from the *B. reticulatus* group and the *B. truncatus/tropicus* complex. The latter two diverged from each other around 11 Ma. Within the *B. truncatus/tropicus* complex, *Bulinus* sp. 8 MTE from Mt. Elgon originated in the Pliocene (4.71 Ma) (**Figure 2**). A series of divergence events leading to all other lineages in the complex happened near the Plio-Pleistocene border at 2.62 Ma (**Figure 2**). This marks the split of *Bulinus* sp. 7 LSH from the remaining *B. truncatus/tropicus* complex. All other divergence events for the *B. truncatus/tropicus* complex are dated to the Pleistocene, concerning all high mountain linages and also *B. liratus* from Madagascar. Much more lineages were accumulated in the Pliocene in both the *B. africanus* and *B. forskalii* groups. Here, the *B. obtusispira* from Madagascar is sister to the remaining members of the *B. africanus* group from which it split 8.05 Ma (**Figure 2**; **Supplementary Figure S1**).

Biogeographical Patterns

Extreme altitudes (>2,500 m) have been colonized by *Bulinus* at least three times independently and exclusively in the *B. truncatus/tropicus* complex: 1) The highlands of Lesotho are

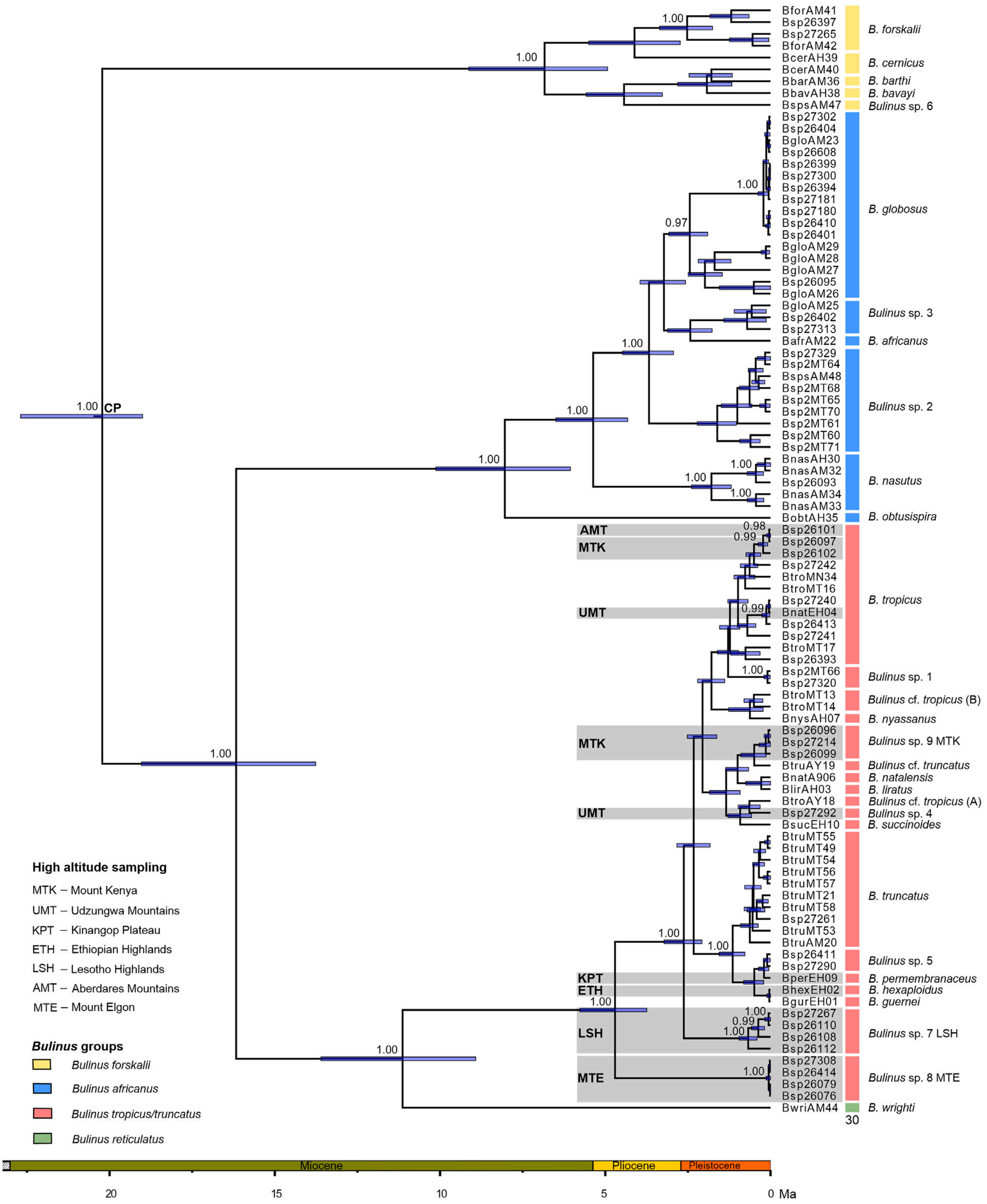

FIGURE 2 | BEAST MCC tree with the posterior probabilities above 0.95 given. 95% highest probability densities (blue bars) and the calibration point (CP) are provided. The outgroup was removed *a posteriori*. Specimen details can be seen in **Supplementary Table S1**.

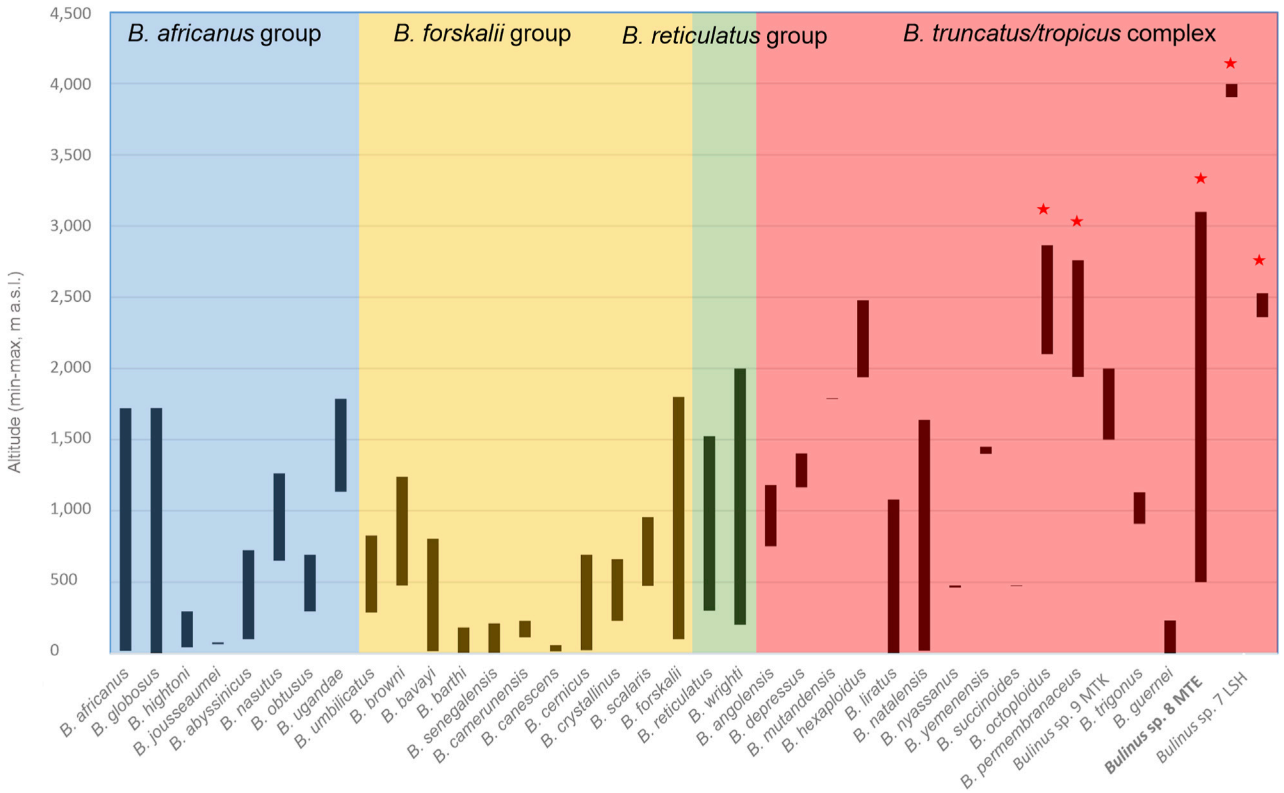

FIGURE 3 | Altitudinal distribution of members of the *Bulinus* groups (highlighted with colors) throughout their entire range compiled from various literature and collection sources. Red stars mark species occurring in extreme altitudes above 2,500 m a.s.l. Note the extraordinary high occurrence of *Bulinus* sp. 8 MTE on Mt. Elgon, Uganda. This species is new to science and is only distantly related to the *B. truncatus/tropicus* complex. Further details on altitudinal ranges can be found in the **Supplementary Tables S2, S3**.

inhabited by an apparently endemic lineage (*Bulinus* sp. 7 LSH, up to 2,528 m a.s.l.); 2) *Bulinus* sp. 8 MTE (up to ~4,000 m a.s.l.) represents an absolute extreme for *Bulinus* (**Figure 3**); 3) The Ethiopian Highlands are represented by a specimen of *B. hexaploidus* from 2,592 m (note that *B. octoploidus* is missing in the phylogeny).

Distinct colonizations occurred on Mt. Elgon and the Lesotho Highlands, spatially and temporally independent from one another and the other Afromontane regions. Diversification on Mt. Elgon is very recent though (0.06 Ma), whereas populations in Lesotho are roughly 0.67 myr old. The colonization patterns of the Aberdares Mts. and other Kenyan Highlands as well as the Udzungwa Mts. (Eastern Arc Mts.) are more complex with the respective species and lineages being widely scattered among the *B. truncatus/tropicus* complex. *Bulinus hexaploidus* is closely related to *B. permembranaceus* from the Kinangop Plateau in Kenya, though they are not sister species. There are two distinct MOTUs from the Mt. Kenya region, representing independent colonizations of the region (*B. tropicus* and *Bulinus* sp. 9 MTK). The Udzungwa Mts. as part of the old Eastern Arc Mts. complex are inhabited by at least two MOTUs (*B. tropicus* and *Bulinus* sp. 4) (**Figure 2**). All Ethiopian, Kenyan, and Tanzania MOTUs occurring in higher altitudes represent comparatively young lineages, having diversified in the late Pleistocene (**Figure 2**). No species of either the *B. forskalii*, nor in the *B. africanus* group occur in the second highest altitudinal band from 2,001 to 2,500 m. *Bulinus wrighti* has been described from low altitudes up to 2,000 m.

Altitudinal Range Evolution

The MOTUs in this study range in altitudinal distribution from sea level to almost 4,000 m (**Figure 3**; **Supplementary Table S2**). However, the majority occurs from sea level to 1,500 m with 22 MOTUs in 0–500 m, 15 in 501–1,000 m band and 17 MOTUs found in band 1,001–1,500 m. Much fewer occur in band 1,501–2,000 m (10 MOTUs) and 2,001–2,500 m (six MOTUs), whereas only four MOTUs are found at altitudes above 2,500 m (**Supplementary Table S3**). Considering altitudinal ranges of all *Bulinus* spp. including the new MOTUs of this study, the average range is 639 m (5–2,600 m). The average minimum altitude is 678 m (0–3,905 m) and the average maximum is 1,317 m (57–3,997 m) (**Figure 3** and **Supplementary Table S2**).

Most species evolved in low to mid-altitudinal ranges and only approximately nine times evolved adaptations to Afromontane regions. The Equal rates model (ER) was the best-fit model, assuming equal rates for the transition between altitudinal range bands (AIC: ARD: 148.99, ER: 109.51; SYM: 136.27). As many species of *Bulinus* occur in more than a single altitudinal range

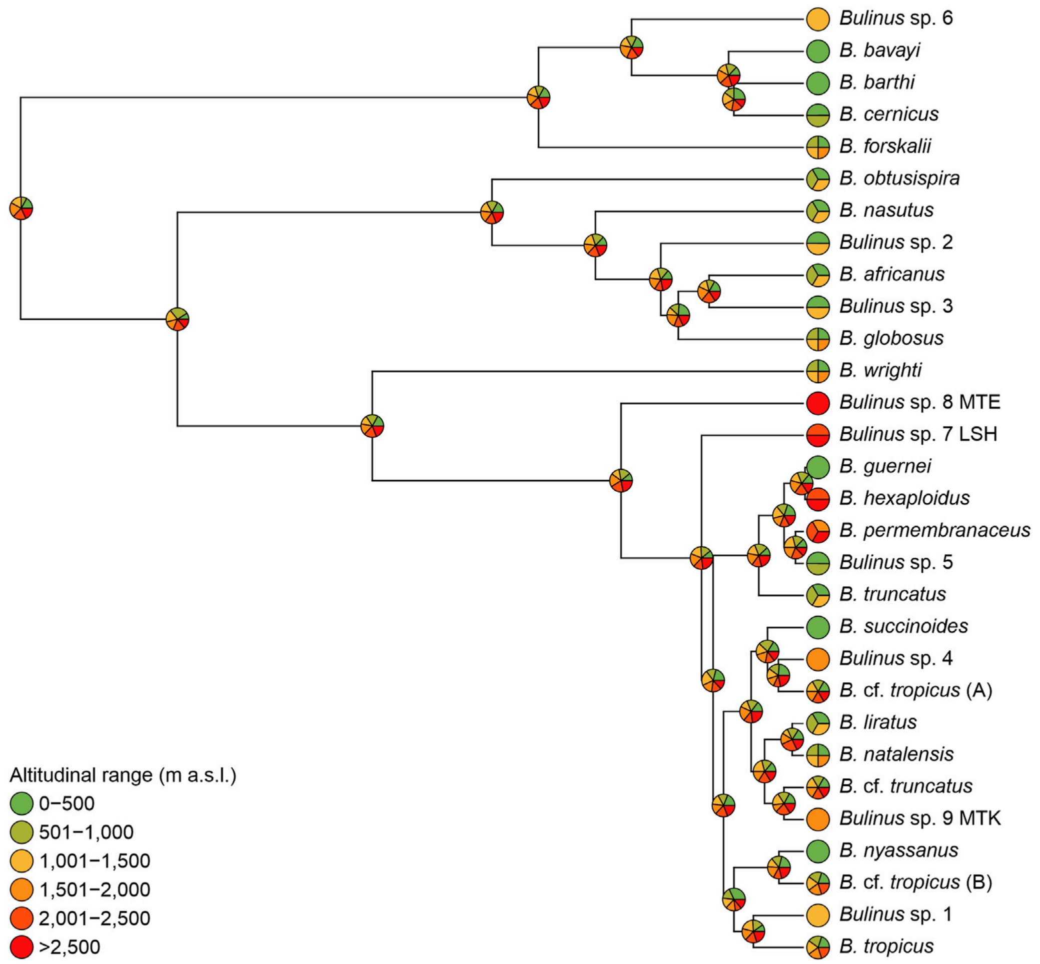

FIGURE 4 | Character evolution of altitudinal bands along the BEAST MCC MOTU-like tree. Six classes of altitudinal occurrences are used and pie charts represent probabilities of ancestral states at the respective nodes (see also **Supplementary Tables S2, S3**).

band, estimation of ancestral ranges is equivocal for each node in the phylogeny (**Figure 4**). The MRCA of the *B. forskalii* group most likely lived in low to intermediate altitudes (0–2,000 m) whereas the MRCA of the *B. africanus* group most likely lived in altitudes from 0 to 1,500 m, except for *B. globosus* which extends its distribution up to 2,000 m. *Bulinus reticulatus* group was only represented by *B. wright*, and the MRCA of this group has already lived in altitudes covering four bands from low- to mid-altitudes (0–2,000 m). The MRCA of *B. truncatus/tropicus* complex is shown to have lived in all the six altitudinal bands, and is from which the *Bulinus* sp. 8 MTE in the extreme altitude at Mt. Elgon has evolved (**Figure 4**).

DISCUSSION

Phylogenetic Relationships and Species Delimitation

Based on the increased taxon and character sampling of the new multi-gene phylogeny, all previously defined subgroups of *Bulinus* spp. were recovered. Unlike in previous phylogenies based on single markers and less extensive sampling (Kane et al., 2008; Jørgensen et al., 2007; Jørgensen et al., 2011; Jørgensen et al., 2013), intergroup relationships were also well supported in our phylogeny. This is important since the relationship among the *Bulinus* groups has been extensively debated in the past (e.g., Jørgensen et al., 2011; Jørgensen et al., 2013). Our findings strongly support that the *B. reticulatus* and the *B. truncatus/tropicus* complex are sister-groups and *B. africanus* forms the sister group to the first two whereas the *B. forskalii* group is ancestral to the other three groups. A sister relationship of the *B. reticulatus* group and the *B. truncatus/tropicus* complex has been well supported before (Kane et al., 2008; Jørgensen et al., 2011; Jørgensen et al., 2013). Various constellations of this group relative to the *B. africanus* and *B. forskalii* groups had been proposed and none conforms with the one found here (Stothard et al., 1996; Stothard et al., 2001; Jørgensen et al., 2007; Kane et al., 2008; Nalugwa et al., 2010; Jørgensen et al., 2011; Jørgensen et al., 2013; Zein-Eddine et al., 2014; Tumwebaze et al., 2019; Clewing et al., 2020).

The species delimitation overall found a match of morphologically identified species and MOTUs in many cases, however, there were also several cases of non-monophyly and mismatches. Most often this occurred in the *B. tropicus/truncatus* complex, which has been known for notorious difficulties in species assignments (e.g., Brown, 1994; Kane et al., 2008). *Bulinus globosus* was another case, known to be an "umbrella" name for various lineages of similar shell morphology (Pennance, 2020). In our conservative approach, we preferred to label unclear specimens as "sp." rather than assigning names to these lineages in order to not further complicate the situation of potentially wrongly labelled sequence information in GenBank. A major obstacle in *Bulinus* systematics is to disentangle wrongly identified specimens from cases where indeed cryptic species are involved in potentially rapidly diverging lineages (Tumwebaze et al., 2019). The complexity of the species level systematics stems from different taxonomic values of character used and even species concepts applied by the various authors. Here, either shell characters or anatomy, chromosome numbers, parasite susceptibility and the pre-condition of hermaphroditic reproduction mode have been implemented to various extents over time (Mandahl-Barth, 1957; Mandahl-Barth, 1965; Brown, 1994). Given these circumstances, a comparatively consistent level of MOTUs that correspond to nominal taxa has been found in this study. It should be further substantiated in future studies, which should strictly focus on type or topotypic material of all recognized species. In order to disentangle the real diversity of *Bulinus* and also the roles that certain processes play in diversification (such as polyploidization), genomic approaches should be used, especially since mitochondrial and nuclear genomes are already available for *B. truncatus* providing the much needed baseline (Young et al., 2021; Young et al., 2022; Zhang et al., 2022).

The sparse fossil record of Bulinidae has been discussed elsewhere (Jørgensen et al., 2013; Albrecht et al., 2019). The fossil-calibrated tree might potentially underestimate the real age of the group. Given that external substitution rates are often taxon- and gene-specific and may be saturated over time (see, e.g., Wilke et al., 2009), it was still worthwhile to explore it in the case of *Bulinus*. It should be noted though that the fossil-independent dating strategy using a universal invertebrate molecular clock rate did not yield substantially different age estimates for the major splits in the phylogeny (data not shown). As such, the ages of the endemics on Madagascar (*B. liratus* and *B. obtusispira, B. bavayi*) are interesting. Although *B. bavayi* and *B. obtusipira* represent comparatively old lineages in their respective *Bulinus* groups, their splitting from other lineages by far postdates the separation of Madagascar from East Africa and the Indian subcontinent (e.g., Masters et al., 2021). The *Bulinus* spp. on Madagascar thus do likely not represent vicariant forms (Wright, 1971; Stothard et al., 2001; Jørgensen et al., 2011). Interestingly, a similar pattern has been found for another gastropod species of the genus *Lanistes* endemic to Madagascar (Mahulu et al., 2021). The now available temporal framework of *Bulinus* evolution also allows specific colonization patterns and evolutionary patterns of endemism to be evaluated.

Colonization History and Evolution of Endemism

Members of the genus *Bulinus* have colonized altitudes from sea level to real alpine altitudes in some Afromontane regions. This study has extended the known altitudinal range of the genus by 900 m to around 4,000 m on the top of Mt. Elgon, where a new species of *Bulinus* has been characterized for the first time from such an extreme environment. Interestingly only four species (14% of the currently recognized biodiversity), all belonging to the *B. truncatus/tropicus* complex, evolved very high altitude occurrences (>2,500 m). All except *B. tropicus* are "true" high-altitude species whereas *B. tropicus* has an exceptional wide altitudinal range (500–3,100 m), the widest of all *Bulinus* species. To which extent the high-altitude populations of this species indeed belong to *B. tropicus* or also represent independent lineages needs to be shown in future studies. *Bulinus* sp. 7 LSH, previously considered to belong to *B. tropicus*, is a good example of how cryptic morphology can mask true phylogenetic and biogeographical patterns. The Bokong bogs (Lesotho) at 3,100 m, was recorded as the highest place for *B. tropicus* so far (Brown, 1994). It remains to be seen whether this indeed represents *B. tropicus* or rather *Bulinus* sp. 7 LSH as identified in this study. Although the sampling was certainly limited, it is noteworthy that no *B. tropicus* lineage was found in the highlands of Lesotho. Colonization might have occurred via a continuous upwards dispersal since *Bulinus* populations are usually found in all intermediate altitudes if suitable habitats are present and the slopes are not too steep. A notable exception it's the conically shaped Mt. Elgon, where intermediate altitudes are free of *Bulinus* (Howell et al., 2012).

Altitudinal endemism is pronounced in the case of the Ethiopian species as well as the Kenyan Highlands. Here, phylogeographical sampling within these mountain ranges has to further disentangle the species status of several nominal taxa such as *B. octoploidus* in Ethiopia and *B. permembranaceus* in Kenya. This specifically applies to the *Bulinus* sp. 9 MTK which potentially includes the taxa *B. laikipiensis, alluaudi, rumrutiensis* (currently fallen in synonymy). It would also be interesting to test the hypothesis that polyploidisation might be linked to the ability to adapt to extreme altitudes (Brown, 1994). A striking finding of this study is the fact that high altitude *Bulinus* sp. 8 MTE from Mt. Elgon and *Bulinus* sp. 7 LSH from Lesotho are representing old lineages (= long branches), very isolated in the *B. truncatus/tropicus* complex with no immediate sister species currently known. The genetic distinctness of *Bulinus* sp. 8 MTE is very pronounced and might even warrant separation from the *B. truncatus/tropicus* complex. Even though this "stand alone" situation could potentially be a sampling artefact, it is the East African and Southern African regions that are comparatively well represented in previous phylogenetic studies of *Bulinus* spp. (Kane et al., 2008; Jørgensen et al., 2011; Jørgensen et al., 2013; Pennance, 2020), which render missing major lineages less likely. Given the phylogenetic pattern and acknowledging the sampling available to date, it appears as if living in high-altitudes is a condition already present in the MRCA of the *B. truncatus/tropicus* complex. It was an equally likely ancestral condition in the MRCA of the *B. reticulatus* group and the *B. truncatus/tropicus* complex. The character tracing revealed an equally likely colonization from altitudes from 0 to 2,000 m for *Bulinus* sp. 8 MTE. The Lesotho Highlands in contrast were colonized from either low (0–500 m) or high (>2,500 m) altitudes. Given the wide altitudinal ranges many *Bulinus* species occupy and their easy colonization capabilities, it is challenging to reconstruct ancestral conditions using phylogenetic approaches. Here, phylogeographical studies of species or species-groups might help to further elucidate the evolution of rare adaptations to extreme altitudes.

Role of Paleogeography and Paleoclimate

The Afromontane mountains are recognized as the most isolated mountain ranges in the world (see Mairal et al., 2021). This isolation has led to speciation and endemism in floras and faunas (Levin et al., 2020; Cuypers et al., 2022) and is also seen in *Bulinus* spp. It is noteworthy that isolation is apparently more pronounced in the Lesotho Mts. and on Mt. Elgon, where the oldest lineages of the *B. truncatus/tropicus* complex are found. There is no indication of hopping dispersal from “sky island” to “sky island” in these areas. Similarity in floras and faunas of Afromontane regions has been attributed to the existence of habitat corridors in the Holocene potentially facilitating dispersal between the various montane regions (Cooper, 2021). In *Bulinus*, this has likely not happened between the southern mountains and East Africa. To what extent such biotic exchange occurred between the Ethiopian Highlands and the Kenyan and Eastern Arc Mts. cannot be answered conclusively with the data at hand. Judging from the branching patterns and the molecular clock estimates it is less likely that such exchange happened as recently as after the last glacial maximum. Inter-mountain long distance dispersal has been shown for various taxa, the majority of which are terrestrial (Mairal et al., 2017; Mairal et al., 2021). This is also less likely in the case of *Bulinus* since no direct sister-group relationships were found. However, it is interesting since in a similar case of a lymnaeid gastropod (*Galba mweruensis*) such a pattern connecting widely isolated Afromontane populations was demonstrated, though the actual dispersal mechanism remained unknown (Mahulu et al., 2019). In the case of *Bulinus* it is likely that adaptation to cold climates (= high-altitudes) represents a form of niche conservatism which makes these lineages or species trapped in their specific environment with subsequent *in situ* speciation. This has been shown for pulmonate snails in other high-altitude regions (Albrecht et al., 2022). It is again noteworthy that such adaptations happened considerably earlier in the southern African Mts. and Mt. Elgon, i.e., in the Pliocene or Plio-Pleistocene transition times. Interestingly, this older or longer separation of populations from Lesotho and Mt. Elgon has also been found in *Galba* (Mahulu et al., 2019), with those populations from Mt. Elgon to be the most distinct ones as well. A striking feature remains regarding the roles of volcanic activities and glaciation of Mt. Elgon. Extensive icefields and glaciers existed on Mt. Elgon in the Late Pleistocene (Osmaston, 2004). Earlier (around 3 Ma), volcanic eruptions occurred on Mt. Elgon (Scott, 1998). Both conditions argue against a Pliocene colonization and uninterrupted existence of *Bulinus* populations on Mt. Elgon. Alternatively, a post-glacial long dispersal from a yet unknown source must be assumed, possibly located in East Africa. The current study clearly demonstrated that Afromontane regions represent “sky islands” for freshwater organism, here *Bulinus* gastropods. Rifting processes and associated climatic fluctuations have impacted the distribution patterns of *Bulinus* lineages at least since mid-Pliocene times. It is likely that recurrent climatic changes (Kohler et al., 2014) might lead to range shifts, reduction or even extinctions in high mountain populations. These populations have been postulated to be particularly sensitive to changing climates (Trew and Maclean, 2021). Such range shifts might also have significant implications for species of *Bulinus* that act as intermediate host for trematode diseases.

Implications for Schistosomiasis

Whereas *Bulinus* snails adapt to cold conditions, this is not necessarily the case for their parasites. Though our knowledge is still scanty for most trematode parasites, species of the genus *Schistosoma* received considerable attention due to their role in causing, e.g., cancerogenic urogenital schistosomiasis in humans (e.g., Rollinson, 2009). With warming climates, regions in altitudes where schistosomiasis was previously unknown, might become suitable for thermally restricted schistosomes. Warming means that known intermediate hosts might occur in high places in the future and that species not yet susceptible to schistosomes might become susceptible or get into contact with the parasites. A multiplex-PCR based screening of the specimens of *Bulinus* sp. 8 MTE did not detect infections (data not shown). These populations at altitudes of around 4,000 m are less likely to be visited by final hosts. Lower altitudes between 2,000 and 3,000 m are much more frequented in Afromontane regions. *Bulinus permembranaceus* and *B. hexaploidus* are currently not known to be intermediate hosts for human schistosomes (Brown, 1994). *Bulinus octoploidus* on the other hand could be experimentally infected with *S. haematobium* (Lo et al., 1970) and is naturally infected with *S. bovis* a major parasite causing livestock schistosomiasis (Brown, 1994). *Schistosoma bovis* is also known from *B. tropicus* and thus potentially also from *Bulinus* sp. 7 LSH. Future assessments of high-altitude species should use the newly developed xenomonitoring approaches (Pennance et al., 2020; Hammoud et al., 2022) to enhance our knowledge of snail-trematode communities in these particular species. This is relevant since the above mentioned shift of prevalence towards higher altitudes have already been documented for the *Biomphalaria-S. mansoni* system (John et al., 2008; Stanton et al., 2017). It is also desirable to carry out transect assessments along altitudinal gradients in all Afromontane regions (Howell et al., 2012; Stanton et al., 2017) as a baseline for tracing future shift of both snail and parasite communities. It would also help to more precisely define the actual and potential altitudinal limits of the disease that is so pertinent for both humans and livestock throughout Africa.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/, GenBank accession numbers of newly sequenced samples are provided in the **Supplementary Table S1**.

AUTHOR CONTRIBUTIONS

IT and CA designed the study, CA and IT collected in the field and compiled data and wrote the draft manuscript. IT conducted lab work. IT and CC performed the analyses. FC organized field work in Tanzania and helped in the lab. JK facilitated work in Kenya. CA secured funding and did project administration. All authors read, contributed to the initial drafts, and approved the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2022.902900/full#supplementary-material

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Family-Level Bio-Indication Does not Detect the Impacts of Dams on Macroinvertebrate Communities in a Low-Diversity Tropical River

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The Ruzizi River, the outlet of Lake Kivu in the Albertine Rift, flows into Lake Tanganyika and is important for hydropower generation and irrigation. The impacts of 2 dams in the Ruzizi River on macroinvertebrate community composition and diversity were surveyed every 3 months from December 2015 to October 2017. Macroinvertebrate samples were collected at sites upstream and downstream and additionally at two sites further downstream of the dams, in both comparatively pristine and highly disturbed areas. Several indices (Shannon-Wiener index, Simpson index, Pielou's evenness, Rare Family Prevalence, and Average Score Per Taxa) were used to determine the alpha diversity and evenness of macroinvertebrates at the family level. Our results showed little to no immediate effect of the dams on macroinvertebrate diversity. Macroinvertebrate composition differed slightly below the dams compared to upstream. Communities near Dam II had slightly higher diversity compared to Dam I, probably because the vicinity to Lake Kivu has an immediate effect on diversity upstream of the first dam and likely because Dam II is 30 years younger than Dam I. This study suggests the importance of using species-level indices to better understand the ecological impacts of dams on macroinvertebrate diversity of tropical rivers with low species diversity.

Keywords: Ruzizi River dams, macroinvertebrates, biodiversity indices, pollution, environmental flows, hydropower

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INTRODUCTION

Increasing demand for electricity creates an urgent need to build hydropower plants for renewable energy and as a valuable source of revenue (IEA: International Energy Agency, 2019), especially in tropical regions where the world's largest rivers are located (Latrubesse et al., 2005; Sinha et al., 2012). The demand for hydropower in such regions has even led to a global "megadam mania" (Gross, 2016). In addition to hydropower, dams provide many benefits to societies such as flood control, irrigation, and water level regulation (Altinbilek, 2002). Despite the great importance of dams, they alter rivers and their ecosystems significantly (Poff and Matthews, 2013; Zarfl et al., 2015; Couto and Olden, 2018; Grill et al., 2019) by changing the flow

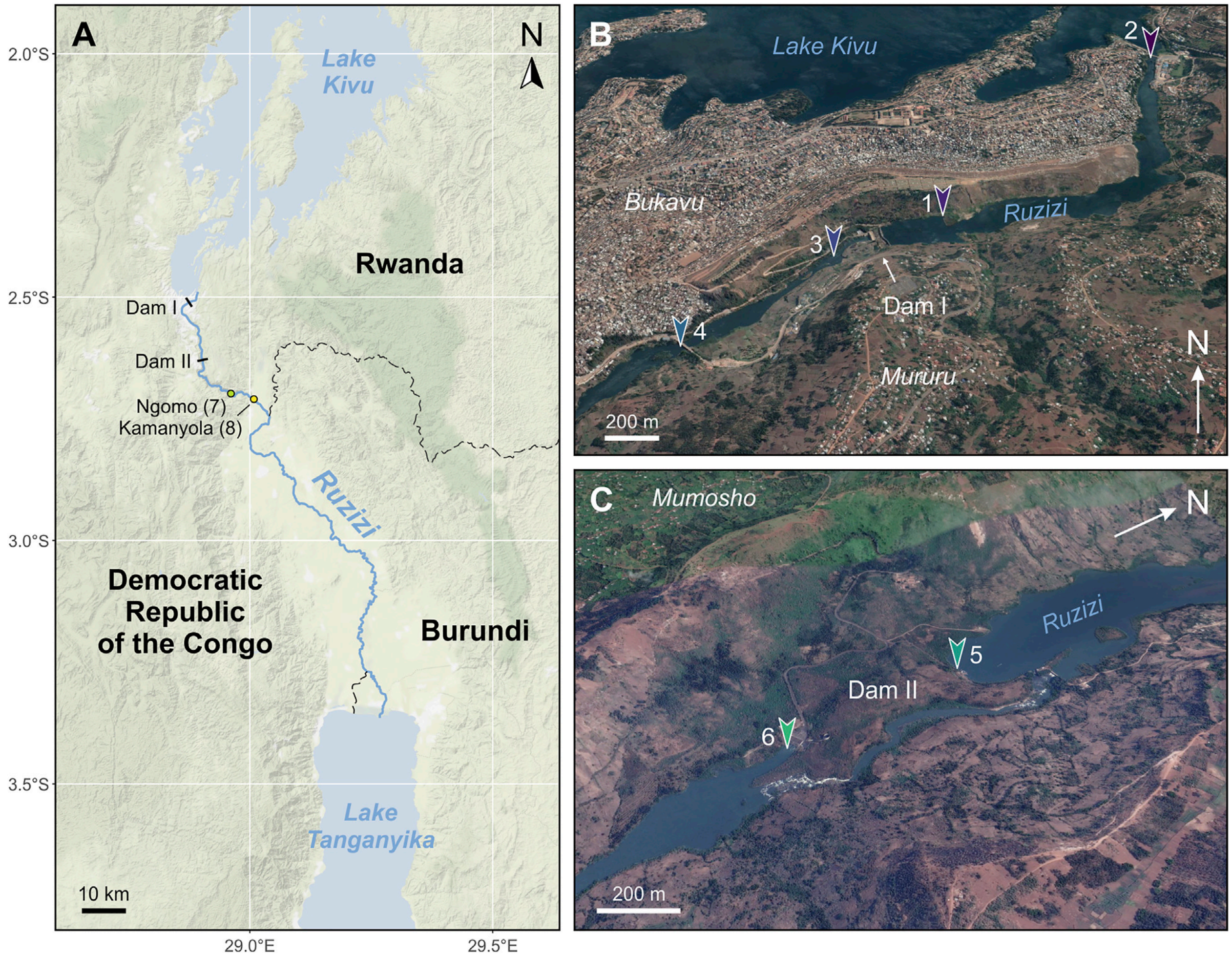

FIGURE 1 | Location of the Ruzizi River, the dams, and collecting sites. See **Table 1** for detailed information on the localities. **(A)** Ruzizi River with Dam I and Dam II and further downstream sites. **(B, C)** Sampling site locations downstream and upstream of Dam I (between Mururu and Bukavu) and Dam II (in Mumosho). The map in **(A)** was created with the R package ggmap 3.0.0 (Kahle et al., 2019). **(B, C)** were created in Google Earth Pro 7.3.4.8248, map data © 2022 Google/Maxar Technologies.

regime, e.g., reducing river connectivity, altering temperature and nutrient status (Bunn and Arthington, 2002; Renöfält et al., 2010; Reid et al., 2019; Kuriqi et al., 2019, 2020). These hydrological changes and habitat fragmentation are mainly related to loss of sediment connectivity, resulting in significant changes in downstream sections and affecting macroinvertebrate communities (Mueller et al., 2011; Martínez et al., 2013). In addition to the mentioned threats, dams can facilitate the establishment of invasive species that could further drive the loss of other aquatic organisms (Dick et al., 2002; Johnson et al., 2008).

Aquatic macroinvertebrates are the most popular organisms used to assess freshwater biological quality (Wright, 2010; Kaaya et al., 2015; Wronski et al., 2015; Dusabe et al., 2019), especially in the stream and river assessments (Dallas, 2021). Macroinvertebrates are recognized as good indicators for monitoring habitat quality (Greenwood et al., 1999; Carter et al., 2017) as well as many different anthropogenic stressors, including changes in flow regime, pollution, eutrophication, and biological invasions (Bonada et al., 2006; Fornaroli et al., 2018; Guareschi and Wood, 2019; Mellado-Díaz et al., 2019).

The effects of dams on downstream macroinvertebrate diversity and abundance have been documented repeatedly (Cortes et al., 1998; Santucci et al., 2005; Sharma et al., 2005; Xiaocheng et al., 2008; Bredenhand and Samways, 2009; Vaikasas et al., 2013; Serrana et al., 2018; Wang et al., 2019). Species richness and abundance of sensitive and tolerant taxa have been the most commonly used metrics to determine the ecological impacts of macroinvertebrates below dams (Martínez et al., 2013). Using biotic indicators to detect the effects of dams on the macroinvertebrate community requires a profound knowledge of the species identities because even closely related species may have different tolerances to environmental stressors (Macher et al., 2016; Mezgebu, 2022). However, accurate identification of freshwater macroinvertebrates to the species level can be difficult, especially for larval or subadult specimens, and often results in low taxonomic resolution or misidentification (Haase et al., 2010; Sweeney et al., 2011).

This in turn reduces the accuracy of the approach and can lead to inaccurate biological assessments and eventually even misguided management (Stein et al., 2014).

A promising alternative to morphological identification is DNA-based identification (Elbrecht et al., 2017), which has been shown to be reliable in non-tropical regions (Stein et al., 2013; Elbrecht and Leese, 2015). Nevertheless, most available bio-indication systems are nowadays based on the family taxonomic level (Dallas, 2021; Mezgebu, 2022). The objective of this research is to evaluate whether a family-level bio-indication can determine the impact of dams on macroinvertebrates in a low-diversity tropical river.

MATERIALS AND METHODS

Study Area

The Ruzizi River, also known as Rusizi, flows from Lake Kivu into Lake Tanganyika (**Figure 1**). It is the only outlet of Lake Kivu and one of the most important tributaries of Lake Tanganyika in the Congo Basin and lies between the Democratic Republic of Congo (DRC) and Rwanda on the one hand and DRC and Burundi on the other (Descy et al., 2012). The Ruzizi River has an average long-term annual flow of about 86 m^3/s (Muvundja et al., 2014; ABAKIR, 2020). For the first 50 km from Lake Kivu to the village of Kamanyola (headwaters), the river lies between the steep, heavily deforested, and barren watersheds (upper Ruzizi) of South Kivu in the D.R. Congo and Rusizi District in Rwanda. After crossing the escarpment, the river drops from an elevation of 1,450 m to 962 m (ABAKIR, 2020). Numerous waterfalls make it a potential hydropower source. After the escarpments (Ngomo in DRC and Nzahaha in Rwanda), the Ruzizi River extends into a vast plain and gradually drops from an elevation of 962–770 m with a low average gradient before entering Lake Tanganyika. The river provides important habitat for a variety of aquatic species (Hughes and Hughes, 1992). The Ruzizi water at the first dam has a high salt concentration (~1.1 g/L or 1,200 μS/cm electrical conductivity), being as is as salty as the surface waters of Lake Kivu due to dissolution of volcanic ashes in most rivers of North Kivu and subaquatic discharge of underground hydrothermal springs. Towards the Lower Ruzizi in Kiliba, the river water freshens considerably (~0.5 g/L or 650 μS/cm) due to further freshwater inputs from the watershed (Muvundja et al., 2022). The Ruzizi River is of outstanding importance to the African Great Lakes Region (DRC, Rwanda, and Burundi) because of hydroelectric power generation. There are two active hydropower dams on the river: the first dam built in 1959 is located 3 km downstream of the outlet of Lake Kivu at Mururu at an altitude of 1,460 m a.s.l. It has an installed capacity of 28 MW (TRACTIONEL and RRI, 1980; Fichtner Gmbh und co, 2008). The second dam was built in 1989 and is located about 16 km from Bukavu in Mumosho at an altitude of 1,393 m a.s.l, with a capacity of 44 MW (**Figure 1**) (TRACTIONEL and RRI, 1980; Fichtner Gmbh und co, 2008; ABAKIR, 2020). Two more dams are planned: Dam III (147 MW) and Dam IV, to be built downstream of Dam II and between dams II and III, respectively (Fichtner Gmbh und co, 2008; Dombrowsky et al., 2014; ONEC-BAD, 2015; ABAKIR, 2020).

Macroinvertebrates Sampling

We sampled the macroinvertebrate community at eight stations from December 2015 to August 2017 (**Supplementary Table S1**). Samples were collected every 3 months to cover both wet and dry seasons. Each collection site was sampled eight times to account for a potential variation in community composition over time. Samples were collected at sites upstream and downstream of Dam I and Dam II. Additional samples were collected further downstream of Dam II at Manda/Ngomo and Kamanyola as reference sites that are unlikely to be impacted by the dams. We collected macroinvertebrates in various habitats by kicking, hand picking, or hand scooping samples for leaf litter and sapropel (Dusabe et al., 2019). We collected the samples on the banks of the Ruzizi River in different substrates (sand, stones, rocks, and macrophytes) by using hand tweezers on stones and rocks and scoop nets (diameter: 20 cm, mesh size: 1 mm) in sands and macrophytes and on the water surface as well as in the water column. Organisms were separated by taxonomic groups, sorted using featherweight tweezers.

At each sampling site, the samples were collected within 50 m along the river shoreline. Some sites were accessible by foot, others by boat. Latitude, longitude, and elevation were recorded at each sampling site using a Garmin GPS IV (**Table 1**). Sampling lasted 60 min and was conducted by three individuals. Samples were preserved with 70% ethanol. All macroinvertebrates were identified to family level using predominantly keys developed for the southern African sub-region (Cape Province to northern Zambia; Day et al., 1999, 2001a, 2001b, 2002; Day and de Moor, 2002; de Moor and Day, 2002; Stals and de Moor, 2007; de Moor et al., 2003). Oligochaeta and Polychaeta were determined to order level due to the lack of identification keys for the region.

Data Analyses

We calculated five different diversity indices: Shannon-Wiener index, Simpson index, Pielou's Evenness, Rare Family Prevalence (RFP) index, which indicates the proportion of families at each station represented by single individuals (Emberton et al., 1997), and Average Score Per Taxa (ASPT) based on TARISS (Tanzania Rivers Scoring System; Kaaya et al., 2015) to categorize the sensitive and tolerant taxa (Kaaya et al., 2015). The ASPT scores of macroinvertebrate groups were categorized as follows: Low sensitivity (1–5), moderate sensitivity (6–10), and high sensitivity (11–15) (Gerber and Gabriel, 2002).

In order to test for an effect of the sampling position with respect to dams (upstream vs. downstream) on each of the five diversity indices we ran linear mixed effects models. We divided the analyses into two batches, one including only the samples upstream and downstream of Dams I and II, respectively, the second one including the samples taken further downstream at Ngomo and Kamanyola (**Figure 1**) as a separate category. The rationale behind the second approach was to compare upstream/downstream samples to communities less impacted by dam

TABLE 1 | Locality information with the villages where Dam I and Dam II are located and coordinates of sampling sites upstream and downstream of the dams.

Village	Dams	Loc. #	Localities	Latitude N	Longitude E
Mururu/Bukavu	Dam I	1	Ruzizi I upstream site 1	−2.507755	28.878461
		2	Ruzizi I upstream site 2	−2.491257	28.892775
		3	Ruzizi I downstream site 1	−2.510530	28.873312
		4	Ruzizi I downstream site 2	−2.515492	28.867921
Mumosho	Dam II	5	Ruzizi II upstream	−2.628099	28.901870
		6	Ruzizi II downstream	−2.633562	28.902669
Ngomo	Planned Dam III	7	Planned Ruzizi III upstream	−2.700285	28.964059
Kamanyola		8	Planned Ruzizi III downstream	−2.709655	29.009136

building. The communities at Ngomo and Kamanyola are located several kilometers downstream of Dam II and are distinct from the communities downstream and upstream of Dam I and Dam II. This could be due to the streams that join the Ruzizi River upstream of the Ngomo site and therefore bring new macroinvertebrate communities. Kamanyola is disturbed by several anthropogenic stressors because the site is located on the Congo-Rwanda border in an area with a high population density, causing the river to be used heavily for domestic purposes and agriculture.

We tested for the influence of two different types of random effect structures, one including the sampling period (month/year), the other including sampling period and sampling area (Dam I, Dam II, and Ngomo/Kamanyola). In all cases we applied random intercept models. Preliminary analyses with more complex random effect designs yielded distinctly less supported or singular models.

We used Akaike Information Criterion (AIC) to select the best model, in each case comparing a candidate model with the corresponding null model lacking fixed effects but having the same random structure. Following Harrison et al. (2018) we used maximum likelihood estimation to compare models with the same random structure, but restricted maximum likelihood to estimate variance components of random effects and model parameters as well as to compare models with different random structure. We applied the classical ΔAIC cutoff of 2 after Burnham and Anderson (2004) to evaluate significant difference among models, but we are aware of the problems associated with such an assumption (compare Harrison et al., 2018) and critically discuss this issue below.

Models that were found significantly better than the corresponding null models were further examined for model adequacy and model fit. We assessed model residuals through simulations that transform them to a standardized scale, tested for potential dispersion issues, and inspected autocorrelation function plots to detect a possible influence of temporal autocorrelation. Marginal R^2 and adjusted intraclass correlation coefficient (ICC) were calculated to assess the variances explained by the fixed and random effects, respectively (Nakagawa et al., 2017; Harrison et al., 2018).

Finally, to compare communities among sampling sites and periods, we ran a nonmetric multidimensional scaling based on a Bray-Curtis dissimilarity matrix for each sampling period. To account for strong variation in abundances, both Wisconsin double standardization and square-root transformation were applied.

All analyses were carried out in R vs. 4.0.3 (R Core Team, 2020) using the packages DHARMa 0.3.3.0 (Hartig and Lohse, 2020), ggeffects 1.0.1 (Lüdecke et al., 2020a), lme4 1.1–26 (Bates et al., 2020), performance 0.6.1 (Lüdecke et al., 2020b), and vegan 2.5–7 (Oksanen et al., 2020).

RESULTS

A total of 4,439 individuals from 13 macroinvertebrate orders were detected across all sampling sites and periods (**Supplementary Table S1**). Mollusca formed the dominant group (40.9%), followed by Odonata (17.8%) and Diptera (13.4%). Other groups found in this study were Trichoptera (7.7%), Ephemeroptera (5.9%), Heteroptera (5.5%), Decapoda (3.9%), Coleoptera (2.2%), Hirudinea (1.2%), Plecoptera (0.72%), and Oligochaeta (0.2%). The composition of the macroinvertebrate community slightly varied across sites. Almost all groups were represented at all sites, but Trichoptera, Ephemeroptera, and Plecoptera dominated at the control site (Ngomo) of the Ruzizi River.

In general, the Shannon-Wiener index was very low, ranging from 0.63 to 2.39. Most families at the sites were represented by more than one individual. The Rare Family Prevalence (RFP) score was low at most sites before and after the dams. At one site from Dam I downstream (loc. 4), a very low RFP value of 0.8 was recorded in December 2015. The highest RFP value (60) was recorded in October 2016 at Kamanyola, where most of the taxa collected were represented by single individuals. The evenness was high when RFP increased, meaning that the number of individuals in a community was fairly constant when many families were represented by single individuals. The ASPT ranged from 2.5 to 6.8, and almost all sites were represented by tolerant and moderately tolerant taxa, except for the Ngomo site, which had more sensitive taxa.

The nonmetric multidimensional scaling analyses for the eight sampling periods yielded stress values between 0.016 and 0.097, suggesting a good fit for each of the ordinations. The plots indicate variation among communities as well as through time (**Supplementary Figure S1**). The plots showed that the least sensitive taxa were represented both upstream and downstream of the 2 dams. Belostomatidae, Libellulidae, Lymnaeidae, and

TABLE 2 | Linear mixed effects models that are better than their corresponding null models, with indication of random effects structure (random intercept) and model fit. See **Supplementary Table S2** for the complete list and **Supplementary Results S1** for model diagnostics. AIC, Akaike Information Criterion; ASPT, average score per taxon; ICC, intraclass correlation coefficient; RFP, rare family prevalence.

Index	Ngomo/Kamanyola	ΔAIC to Corresponding Null Model	Random Effects Structure		Model Fit	
			Sampling area	Sampling period	Marginal R^2	Adjusted ICC
Pielou's Evenness	excluded	4.225	x	x	0.068	0.478
RFP	excluded	2.936	-	x	0.096	0.005
RFP	excluded	3.195	x	x	0.089	0.160
ASPT	included	16.675	-	x	0.268	0.032
ASPT	included	3.191	x	x	0.241	0.120

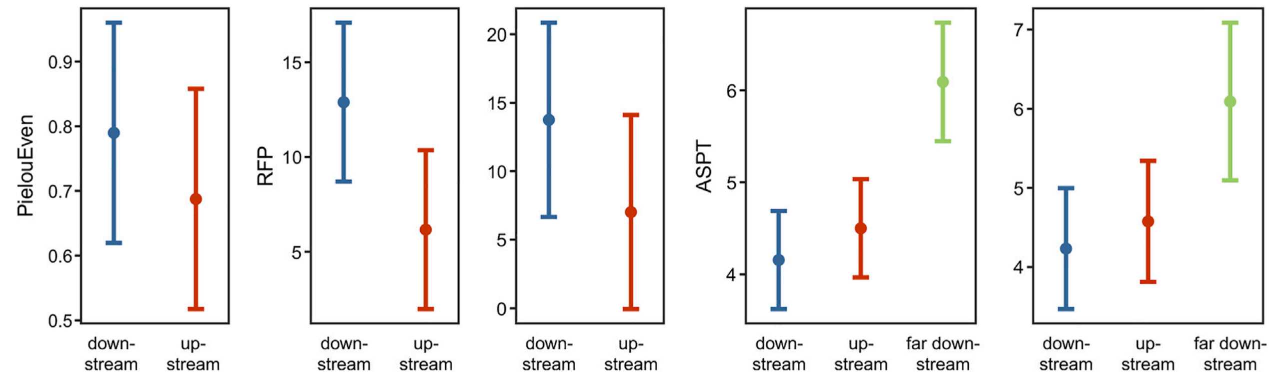

FIGURE 2 | Predicted values of the fixed effects of the five significant linear mixed effects models. The fixed effects of the ASPT models show that the variance in both models is mostly contributed by the communities sampled further downstream of Dam II. ASPT, average score per taxon; RFP, rare family prevalence.

Bithyniidae dominated upstream of Dam I, while Planorbidae, Lymnaeidae, and Atyidae dominated downstream (**Supplementary Figure S1**). Sites at Dam II were more represented with taxa moderately sensitive to pollution and disturbance. Corduliidae and Chlorocyphidae dominated upstream of Dam II, while Hydraenidae and Belostomatidae dominated downstream of Dam II (**Supplementary Figure S1**). Community composition differed at sites further downstream. The Ngomo site was dominated by sensitive taxa of Trichoptera (Hydropsychidae), Plecoptera (Perlidae), and Ephemeroptera (Baetidae). The Kamanyola site, further downstream, was dominated by taxa that are both tolerant and moderately tolerant to pollution and disturbance, such as Nepidae, Libellulidae, and Gomphidae.

For most of the linear mixed effects models between each of the five diversity indices and sampling position (upstream vs. downstream), a null model yielded a better or equally good (ΔAIC <2) fit (**Supplementary Table S2**). Moreover, despite the generally simple structures of the models, several of them had a singular fit (**Supplementary Table S2**), suggesting an overfitting of the model or insufficient data. Since the structure of the models cannot be further reduced reasonably, these models will not be further considered. Only five models were found better than their corresponding null models (**Table 2**). However, ΔAIC values for four out of the five model are below 5, suggesting only a weakly better fit than a null model. Upstream/downstream position had significant but weak effects on Pielou's Evenness and Rare Family Prevalence (RFP) when including only Dams I and II. However, most of the variation detected by the models is due to the random effects structure, particularly the sampling area. Conversely, a moderate effect of upstream/downstream position on ASPT was found when including the communities further downstream of Dam II (**Table 2**; **Figure 2**). As in the other models, sampling area contributed more to the variation of the models than sampling period. Residual and dispersion checks found no violation of model assumptions in any of the five models (**Supplementary Results S1**). Only minor signs of temporal autocorrelation were detected for the two models with ASPT (**Supplementary Results S1**).

DISCUSSION

The studied river system provided an opportunity to examine whether family-level biotic indices should be used to assess the ecological impacts of dams, particularly in a low-diversity river. We found low-diverse macroinvertebrate communities both upstream and downstream of the dams. Our analyses indicated general differences in the community compositions through space and time (**Supplementary Figure S1**), but upstream/downstream communities were found significantly different only for few selected indices (**Table 2**; **Figure 2**).

There are several potential causes for the low macroinvertebrate diversity in the Ruzizi River. Generally, factors contributing to low taxon richness can be low habitat diversity, unstable water levels, altered thermal regime, and altered food supply (Munn and Brusven, 1991). Moreover, macroinvertebrate assemblages below dams often have lower taxon richness and are typically dominated by certain species (Bona et al., 2008; Takao et al., 2008). In the specific case of the Ruzizi River, the hydrological and geographical setting of the dams is also relevant. The water predominantly comes from Lake Kivu, which is a species-poor lake because of its geological history and catastrophic volcanic events that affect also its current limnology (Jones, 2021). The lake's environmental condition affects the Ruzizi River, especially at Dam I close to the outlet. We found that the habitat is disturbed and degraded by high water release and retention downstream of dams, leading to hydropeaking events (Tonolla et al., 2017; Muvundja et al., 2022). Sometimes water is retained upstream, dramatically altering the river's habitat to the point of complete dry-up downstream. This kills many small organisms such as macroinvertebrates due to their low mobility (Bruder et al., 2016). Conversely, habitat alteration due to flooding is the proposed cause of low macroinvertebrate diversity in the Ruzizi River just upstream of the dams (Hyangya et al., 2014).

The slight difference in macroinvertebrate diversity between sites near dams I and II could be due to the proximity of Dam I to Lake Kivu, which carries the same saline water and therefore has lower diversity than communities around Dam II, whose water is diluted by tributaries (Muvundja et al., 2022). The age difference in their existence could be another factor contributing to the lower diversity at Dam I compared to Dam II, which was constructed 30 years later. For the benthic macroinvertebrates, the impacts may be more noticeable in the short and medium-term due to their reduced capacity for movement and their affinity for the bottom substrates that constitute their living environment (Bhandari et al., 2018; Min and kong, 2020). Our result, however, is consistent with a study that showed that dams of different ages can affect downstream organisms differently, with older dams having a greater impact than those recently built along the same river (Wang et al., 2020).

Additionally to richness differences, we found differences in the community compositions. This especially concerns the relative abundance of sensitive and tolerant taxa. In general, at the sites near Dam I and Dam II, between December 2015 and October 2017, we observed an increase of families with low TARISS scores (representing species that tolerate pollution and disturbance), particularly Mollusca, Diptera (strongly represented by Chironomidae), and Heteroptera (compare Kaaya et al., 2015), while species that are highly sensitive to pollution (such as Trichoptera, Ephemeroptera, and Plecoptera; Kaaya et al., 2015) were rare both upstream and downstream of the dams. We observed considerable variation in flow rate and water level over the studied time interval. Such hydropeaking and drying events usually cause changes in macroinvertebrate communities and decrease the number of sensitive taxa in impacted areas (Wang et al., 2013).

Another factor causing the rarity of highly sensitive taxa could be river bank disturbance and domestic pollution observed upstream and downstream of the dams. Macroinvertebrates are known to be differentially sensitive to water quality degradation (Bonada et al., 2006; Arimoro and Muller, 2010; Fouche and Vlok, 2010). Their presence is therefore considered an indicator of the state of water quality and aquatic health of the environment in which they live. The disturbance and domestic utilization of water at the shores of sites of Dam II were minimal compared to the sites of Dam I. Dam II sites contained more taxa moderately sensitive to pollution and disturbance. Taxa tolerant or moderately tolerant to disturbance dominated at sites further downstream in Kamanyola on the Rwanda-Congo border. The low abundance of sensitive taxa at this site, which is not directly impacted by dams and related flow fluctuations, may have been caused by further anthropogenic activities (such as swimming, washing), agriculture, nearby irrigation, and pollution of the on-site bank. In contrast, the Ngomo site is isolated, and we witnessed little anthropogenic activities and no disturbance, making it a suitable habitat for taxa that do not tolerate pollution.

The surprisingly little effect of the dams on upstream vs. downstream community composition contrasts several previous studies (Cortes et al., 1998; Santucci et al., 2005; Xiaocheng et al., 2008; Bredenhand and Samways, 2009; Serrana et al., 2018; Wang et al., 2019). Recently, a global analysis of the ecological impacts of small hydropower dams generally showed negative ecological effects (Kuriqi et al., 2021) affecting macroinvertebrate communities downstream due to changes in flow velocity (Mcintosh et al., 2002; Sharma et al., 2005; Martínez et al., 2013). However, there are a number of studies that found similarly weak or no immediate impact of dams on macroinvertebrate communities (Ambers, 2007; Xiaocheng et al., 2008; Vaikasas et al., 2013). A global review (Mbaka and Wanjiru, 2015) reports that more than 70% of small dams have either a positive or negative impact on macroinvertebrates by causing either a decrease or an increase in macroinvertebrate abundance and richness downstream of the dams (Mueller et al., 2011; Martínez et al., 2013; Wang et al., 2013).

The reason for this contrast could be the different methods used (Wang et al., 2020). Some studies look at specific taxonomic groups of macroinvertebrates, while others include the entire community. Other factors include differences in the climatic and geomorphological conditions at dam sites (Carr et al., 2019; Turgeon et al., 2019), downstream distance from the dam (Ruhi et al., 2018), and dam size (Poff and Hart, 2002). Additionally, using family-level identifications could potentially obscure relevant information. Most rapid assessment systems for impacts on rivers are based on family-level data (Dallas, 2021; Mezgebu, 2022). However, different macroinvertebrate species within the same family may have very different pollution tolerances (Arimoro and Ikomi, 2008) or ecosystem functions (Baulechner et al., 2020). Lumping them in a single unit can severely bias assessments and eventually be the result of the apparently lacking impact of dams on biodiversity in the Ruzizi River.

Africa has a very high demand for energy supply and hydropower facilities will play an important role in the near future, especially in the Congo River system (Winemiller et al., 2016). At the same time, there is still very little knowledge about the impact of dams on environmental flows and biodiversity in these drainage systems. The outlook for freshwater biodiversity near dams requires a more organized assessment for predicting, restoring, and managing the resulting changes in river ecosystems (Rolls et al., 2018; Turgeon et al., 2019). Changes in the management of environmental flow regimes can assist the protection and restoration of the aquatic fauna and maintaining river ecosystems downstream of dams in order to maintain ecosystem functioning (Poff and Schmidt, 2016; Kuriqi et al., 2019). This could also help increase the poor macroinvertebrate fauna of the Ruzizi River, and suggestions for altering flow practices have already been made (Muvundja et al., 2022; see also Bruder et al., 2016). Our study examining the macroinvertebrate communities in the low-diversity tropical Ruzizi River shows a weak impact of dams on downstream macroinvertebrates when using family-level bio-indications. We recommend that future studies focus on species level identifications to deliver more precise and ecologically relevant assessment. For such an approach to work, we urgently require more profound baseline studies on the species compositions of freshwater macroinvertebrate communities in Africa.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**; further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

MD collected data, performed laboratory identification, and wrote the draft article; TN performed data analyses and contributed to writing the manuscript; FM contributed to the conceptualization of the topic, data collection and writing the manuscript; BH contributed to data collection; CA contributed to the conceptualization of the study and writing the manuscript. All authors read and approved the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2022.902246/full#supplementary-material

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Against the Flow: The Colonisation of the Lesotho Highlands by Freshwater Limpets

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To date, limited research has taken place on the evolutionary history of biodiversity in the high-altitude zones of southern Africa, particularly the Lesotho Highlands. The few studies that do exist point to similar high-altitude zones being either prolific museums (i.e., refugia and cradles) for cold-evolved species or sinks for temperate species post the Last Glacial Maximum, yet the role this zone has played for freshwater biodiversity is unknown for almost all freshwater taxa. In this study, we address this lack of knowledge by looking at the phylogeography of the freshwater limpet genus, *Burnupia*, across its southern and eastern African range, but particularly focusing on the Lesotho Highlands. We used COI data to reconstruct the evolutionary history of the genus, quantify phylogenetic species diversity, test both isolation by distance (IBD) and by elevation (IBE) and model ancestral area estimation "in" and "out" of the Highlands to determine: 1) The diversity and endemicity of *Burnupia* spp. in the Highlands in comparison to the broader southern African region and 2) when did the colonisation of the Highlands happen. Our results showed that at least two of the nine southern African phylogenetic species delimited occur in the Highlands (which appears average for the geographical extent of this area in comparison to the broader southern African region) and that the genus has been present in the Lesotho Highlands for somewhere between 1.38–0.23 million years. However, we found the endemicity of at least one of the two Highland species, supported by weak but significant IBD and IBE in *Burnupia*. Therefore we favour the notion that the Highlands are likely an important haven for cold-evolved species. As our results also generated a lot of data useful for *Burnupia* systematics, we discuss some taxonomic implications of our findings.

Keywords: biogeography, *Burnupia*, freshwater ecology, high-altitude, molluscs, phylogeography, southern Africa

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INTRODUCTION

The Lesotho Highlands, primarily located in Lesotho and marginal parts of South Africa (and which includes a large portion of the World Heritage, transfrontier Maloti-Drakensberg Park), are some of the highest elevated land in Africa (maximum elevation = 3,482 m.a.s.l.) which maintain a particularly cooler climate against the backdrop of the broader, temperate southern African region (Norström et al., 2018; Hoogendoorn et al., 2020; **Figure 1A**). Although it is part of the Drakensberg mountain range and the Great Escarpment, which stretches from the Eastern Cape to

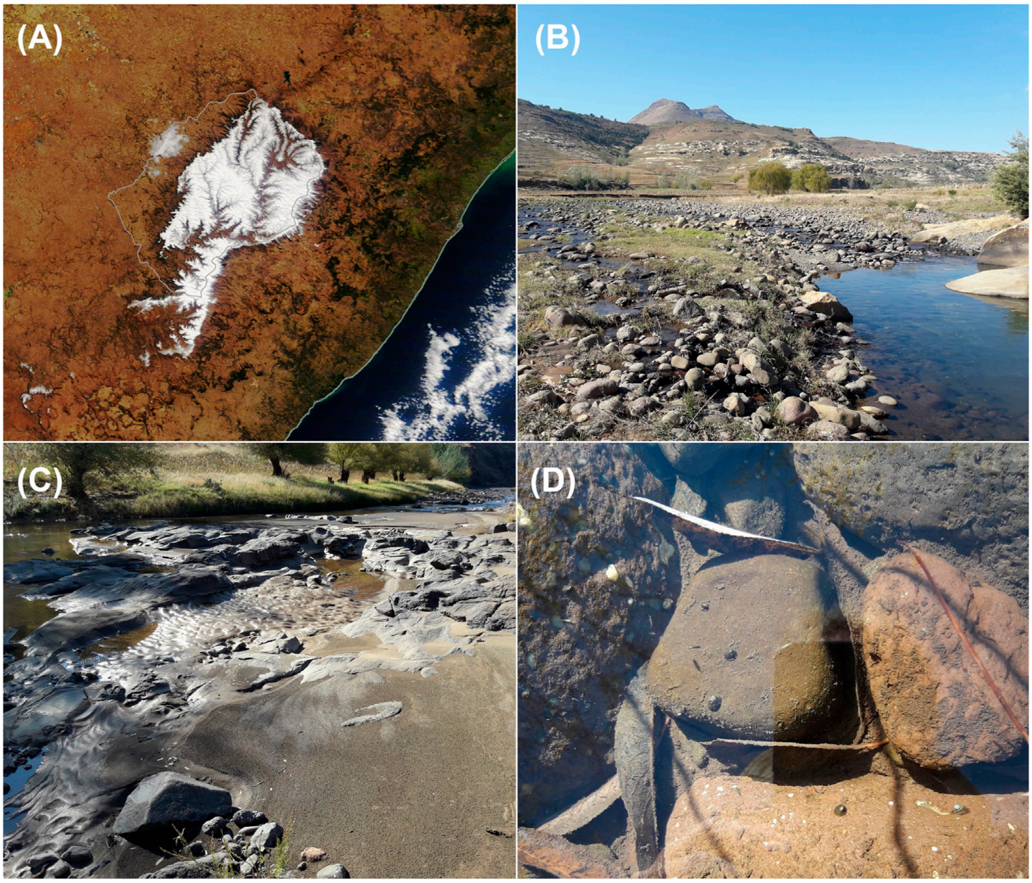

FIGURE 1 | (A) Lesotho Highlands covered in winter snow from space (NASA Earth Observatory images by Lauren Dauphin, using MODIS data from LANCE/EOSDIS Rapid Response), **(B,C)** Typical freshwater streams in the Lesotho Highlands and **(D)** *Burnupia* attached to rocks in a Lesotho Highland stream.

Limpopo, the Lesotho Highlands are isolated by being ~1,500 m higher due to Jurassic vulcanism which led to the extra basaltic cover giving the highland its pronounced elevation (see Eriksson, 1981; Smith et al., 1993). The Highlands act as the catchment and water tower for several large drainages that filter off in different directions across the southern African landscape. As such, the region holds a diverse array of freshwater environments from rivers and streams to ponds and wetlands (Norström et al., 2018; **Figures 1B,C**), but species richness is virtually unknown for most aquatic groups. Similar cooler, high-altitude regions in temperate landscapes have been shown to support high species richness due to acting as a melting pot of museums (i.e., refugia) for freshwater fauna that evolved under the Pleistocene glacial periods (and require cooler conditions to survive—i.e.,"cold-evolved" species), as well as sinks for opportunistic, temperate species that are expanding their ranges post the Last Glacial Maximum (Füreder et al., 2006; Rahbek et al., 2019; Hågvar et al., 2020; Brighenti et al., 2021; Clewing et al., 2022). As such, similar has been predicted for the Lesotho Highlands aquatic environments (Skelton, 2000) and the region could therefore be important for the conservation of freshwater biota (certainly elevated diversity and/or high endemism has been shown in several terrestrial groups, including land molluscs; Hamer and Slotow, 2009; Kopij, 2015; Perera et al., 2021), but it is equally possible that the generally reduced dispersal capabilities of aquatic fauna in conjunction with glacial cycles, if these were dramatic enough, could have caused several turnover events—where the current species assemblages are all the result of very recent colonisations, thus being relatively species poor.

In the case of freshwater species conservation in the Lesotho Highlands, knowing if there is indeed high relative species richness and cold-evolved species is paramount given growing anthropogenic activity (e.g., waste pollution and agriculture; see Pullanikkatil and Urama, 2011; Chatanga and Seleteng-Kose, 2021; Turpie et al., 2021) and global warming—this is particularly relevant for southern Africa and cold-evolved species, where climatic change is likely to increase substantially over the next century (Dallas and Rivers-Moore, 2014; Serdeczny et al., 2016; Archer et al., 2018; Bentley et al., 2018; Weber et al., 2018). However, conservation relies on accurately being able to quantify relative biodiversity richness in the Highlands as compared with the surroundings and, in this case, decipher cold-evolved species from not (i.e., through determining the evolutionary and spatial origins of species). Molecular phylogenetic techniques can provide a suitable way of establishing the above when dated with molecular clocks and can circumvent some of the pitfalls quantifying species richness through traditional morphology-based methods

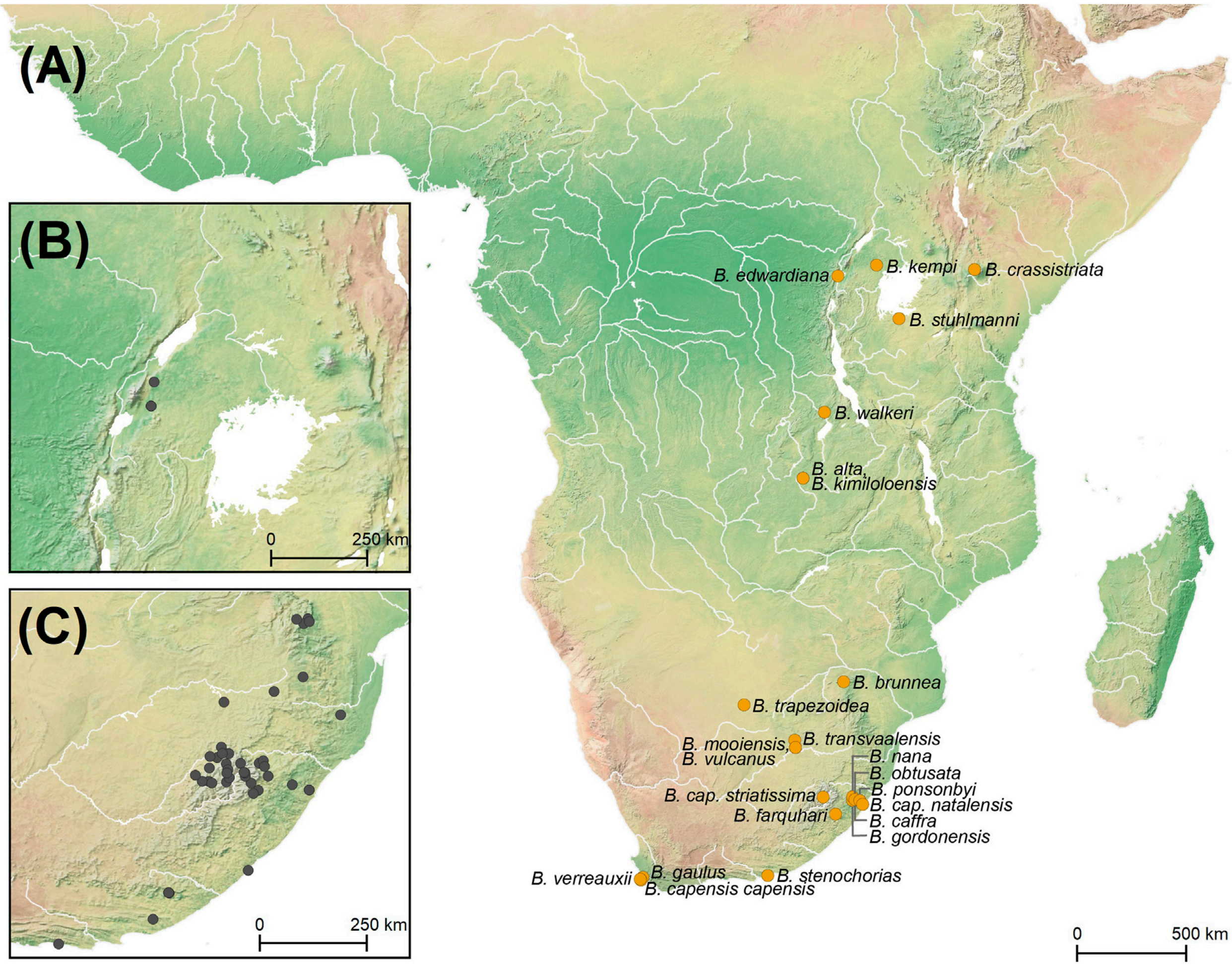

FIGURE 2 | (A) Map of Africa with the type localities of 22 *Burnupia* species and three subspecies of *Burnupia capensis* marked in gold dots, with sub maps showing **(B)** the sampling localities of *Burnupia* incorporated into the current study from eastern Africa and **(C)** southern Africa (inclusive of the Lesotho Highlands) in grey dots.

(e.g., which often have the inability to identify cryptic species). Sadly, there has been a neglect of modern molecular evolutionary aquatic research in the Lesotho Highlands and few studies exist that can even attempt to address these questions in freshwater biota (e.g., Daniels et al., 2003; Swartz, 2005; Swartz et al., 2009; Tolley et al., 2010; Phiri et al., 2016).

In the absence of suitable literature and having molecular evolutionary data for a multitude of species, reconstructing the evolutionary history of a model group may help provide some indications on the relative levels, origins and types of diversity in the Lesotho Highlands. A model taxon needs to be well distributed across southern Africa, have several species, lack a free-swimming larval stage to avoid dispersal biases associated with currents or flow and be a running-water species to avoid dispersal and temporal biases that may be caused by species that occur in transient or short-lived aquatic environments. In this regard, the freshwater limpet genus *Burnupia* meets all the criteria (**Figure 1D**): They are found in high-oxygenated, moving waters from the Western Cape in South Africa right up, through Lesotho, into Botswana, Zimbabwe, Mozambique, Eswatini and even further into the burbling littoral zones of the great lakes of eastern Africa (Brown, 1994; de Kock and Wolmarans, 2009, 2016, 2017; Albrecht and Clewing, 2019). Moreover, there are 22 morphospecies described from across Africa (with 15 occurring specifically within the southern African region; Connolly, 1939; Brown, 1994; **Figure 2A**) which lay a stationary egg mass from which fully formed juvenile limpets emerge (Davies-Coleman, 2001).

In this study, we sampled the genus *Burnupia* across southern and eastern Africa to gain evolutionary insights into the relative freshwater biodiversity of the Lesotho Highlands using molecular phylo- and population-genetic approaches. Specifically, we aimed to; 1) determine if molecular species diversity is rich in the Highlands in comparison to the broader region, 2) assess the evolutionary history of the genus to determine their origin in/colonisation history of the Highlands and the degree of endemism occurring there, as well as, 3) establish if cold-evolved species exist for the genus in the Highlands. To address these aims, we first reconstruct the evolutionary history of the genus using multiple phylogenetic approaches and place a temporal perspective on divergence events using an established

TABLE 1 | Preparation numbers, GPS coordinates (Longitude and Latitude), altitude and GenBank accession numbers of all *Burnupia* specimens used in the current study and the *Protancylus* outgroup (Prep. number: 20024).

Prep. number	GPS Lat.	GPS Long.	Altitude (m a.s.l.)	GenBank accession number	Prep. number	GPS Lat.	GPS Long.	Altitude (m a.s.l.)	GenBank accession number
27789	−26.61000	30.67777	1,260	ON256428	**27816**	−28.73638	28.61833	2,615	ON256537
27790	−27.66222	31.72083	273	ON256429	27817	−28.81527	28.09556	1,578	ON256538
27791	−29.75667	29.43444	1,488	ON256515	27818	−24.99333	30.81444	1,229	ON256539
27792	−29.84527	29.29944	1,632	ON256516	27819	−25.13083	30.69472	1,125	ON256540
27795	−29.00250	29.46138	1,178	ON256517	27820	−25.07388	30.85083	890	ON256541
27796	−28.93611	29.57638	1,064	ON256518	27821	−25.00861	30.49972	1,337	ON256542
27797	−29.09583	29.60861	1,554	ON256519	**27822**	−28.55694	28.41250	1701	ON256543
27798	−29.36361	29.70972	1,655	ON256520	27823	−27.30833	28.48361	1,501	ON256544
27799	−29.55269	29.23520	2,789	ON256521	27826	−31.97861	29.15583	27	ON256545
27800	−29.00805	28.94527	2,342	ON256522	20936	−34.02175	23.89737	34	ON256546
27801	−29.52750	28.55333	2,301	ON256523	20937	−32.60727	26.96279	1,080	ON256547
27802	−29.50805	28.06333	2,207	ON256524	20938	−32.59489	26.94708	1,149	ON256548
27803	−29.50472	27.88056	1,837	ON256525	20939	−32.59489	26.94708	1,149	ON256549
27804	−29.33611	27.69166	1,549	ON256526	20943	−27.01371	29.87412	1,624	ON256550
27805	−29.55298	28.14870	1823	ON256527	**20944**	−28.73313	28.43822	1,673	ON256551
27806	−29.40777	28.63222	1,863	ON256528	20946	−29.74703	30.84878	435	ON256552
27807	−29.36805	29.07444	2,200	ON256529	20947	−29.74703	30.84878	435	ON256553
27809	−29.27111	29.06639	2,073	ON256530	24770	0.64320	30.39399	1,431	ON256557
27810	−29.25472	29.03972	2,059	ON256531	24771	0.06782	30.32354	1,039	ON256554
27811	−29.02027	28.54861	2,102	ON256532	24772	0.06782	30.32354	1,039	ON256555
27812	−29.18722	28.59416	2,141	ON256533	24752	0.06782	30.32354	1,039	ON256556
27813	−29.27611	28.56361	2,061	ON256534	AY282584	−29.60000	30.38000	635	AY282584
27814	−28.84916	28.29166	1,639	ON256535	AY282585	−33.33330	26.51660	639	AY282585
27815	−29.13694	28.07944	1,628	ON256536	20024	−1.78883	120.63465	536	MT925561

Preparation numbers in bold denote those from the Lesotho Highlands.

molecular clock rate. Secondly, we delimit phylospecies to determine the diversity in the Highlands and surroundings. Thirdly, we determine the temporal and spatial origin and/or colonisation history of Highland species by modelling ancestral area estimations and, finally, assess for cold-evolved species therein. As these outcomes and methodological approaches generate a lot of data useful for the *Burnupia* systematics, and several authors have encouraged a molecular review of the genus (Brown, 1994; Albrecht et al., 2004; de Kock and Wolmarans, 2016, 2017; Albrecht and Clewing, 2019), we also discuss the taxonomic implications of our work.

It is hoped that the outcomes of this research will provide us with a preliminary indication of when and where the Highland's freshwater diversity originated and if it is an important area for conservation consideration in respect to relative diversity and/or cold-evolved species. Moreover, we hope the results will encourage and provide guidance and direction for freshwater biogeographic research in the Highlands going forward.

METHODS

Sampling Design, Laboratory Protocols and Sequence Alignment

Forty-five *Burnupia* specimens were hand-collected from hard substrate in shallow water streams and river pools and dams (e.g., **Figures 1D**, **2B,C**). *Protancylus pileolus* was used as an outgroup to root downstream phylogenetic analyses (**Table 1**). Specimens were preserved in 80% ethanol, before being utilised for DNA extraction. Emphasis was placed on collecting *Burnupia* from as many locations across the Lesotho Highlands and surrounding regions as possible (**Figures 2B,C**; **Table 1**). Genomic DNA was extracted from the foot tissue of each specimen using a DNeasy Blood and Tissue kit (QIAGEN) or *via* the CTAB extraction method (Winnepenninckx, 1993). Amplification occurred for a 655 bp region of the mitochondrial DNA cytochrome *c* oxidase subunit 1 (COI) gene through PCR with the primers LCO1490 (Folmer et al., 1994) and COR722b (Wilke and Davis, 2000) and under the following cyclic conditions: 95°C—5 min; 30 cycles of 95°C—10 s, 40°C—30 s, 70°C—1 min; final elongation at 72°C—10 min. The success of PCR amplifications was confirmed by 1% agarose gel electrophoresis, before purification of gene fragments and bidirectional Sanger sequencing were carried out by LGC Ltd. (Berlin, Germany). Sequence ends were trimmed in Geneious 10.1.2 (Biomatters Ltd., 2017). To expand our dataset, all additional sequences from *Burnupia* specimens with corresponding COI data published on GenBank (www.ncbi.nlm.nih.gov/genbank) where incorporated (i.e., two sequences; **Table 1**). Alignments of the 48 gene fragments were performed using Geneious 10.1.2 and the Geneious alignment algorithm.

Phylogenetic Analyses and Molecular Dating

To reconstruct the evolutionary history of *Burnupia*, phylogenetic relationships were established following the methods of Sands et al. (2019a) with only minor differences. In summary, PAUP 4.0b (Swofford, 2002) and MrBayes 3.2.6 (Ronquist et al., 2012) were used to generate unweighted maximum parsimony (MP) and model-informed Bayesian inference (BI) based phylogenies respectively conducted through the CIPRES Science Gateway (Miller et al., 2010). Firstly, the MP analysis was constructed using the heuristic search function, with TBR branch swapping and 100 random taxon additions. Only the best tree was saved during each replicate and the robustness of nodes were assessed by 10,000 bootstrap (BS) replicates (where values ≥70% were considered supported; Felsenstein, 1985). Secondly, the BI analysis was performed to determine the posterior probabilities (PP) of associations. The HKY+I+Γ was selected as the best-fit model of sequence evolution (determined using jModelTest 2.1.10; Darriba et al., 2012) using the Akaike Information Criterion (AIC; Akaike, 1973) and two parallel Markov Chain Monte Carlo (MCMC) simulations used five chains for 100,000,000 generations, saving one tree in every 2,000 generations. The BI trees were summarised after the first 20% of trees were discarded as burn-in, as assessed by parameter convergence in Tracer 1.6 (Rambaut et al., 2014). Nodes with PP ≥ 0.95 where considered supported. Finally, as the BI and MP topologies were highly comparable and no conflict was found between supported nodes of these methods, BS values ≥70% from the MP phylogeny (phylogeny not shown) where transferred onto the BI phylogeny.

To place a temporal perspective on divergence events, to observe when the Lesotho Highlands were colonised and if these overlap with major climatic or geological changes, a dated phylogeny was constructed with the BEAST package (Bouckaert et al., 2014). Four independent runs of 100,000,000 MCMC generations, saving one tree in every 10,000 generations, were constructed in BEAUti 2.6.5 and implemented in BEAST 2.6.3 through the CIPRES Science Gateway (Miller et al., 2010). For each run, a lognormal relaxed clock and the birth–death tree prior were selected. bModelTest 1.1.2 (Bouckaert and Drummond, 2017), as implemented in BEAST, was used to determine the best-fit model for the COI dataset which was subsequently determined to be a variant of the HKY (with an additional group for the rates rct and rgt; 121,323). As fossil dating is challenging for *Burnupia*, the phylogeny was calibrated using published molecular clock rate for the COI gene in Wilke et al. (2009). The clock rate was set to the 95% confidence intervals found therein and linearly distributed [COI = 1.695% per million years; standard deviation (SD) = 1.33%–2.06%]. Thereafter, LogCombiner 2.5.2 (Bouckaert et al., 2014) was used to combine trees and log files of each run with 75% burn-in removed. Validation of the convergence and mixing of the combined log file was assessed in Tracer 1.7.1 (Rambaut et al., 2014) to ensure that all effective sample size (ESS) values were ≥200 and TreeAnnotator 2.5.2 (Bouckaert et al., 2014) was used to summarise trees, with no further burn-in removed.

Species Delimitation

Three species delimitation methods were followed to get an idea on the species richness and population structure in *Burnupia* in the Lesotho Highlands and surroundings: These included the General Mixed Yule-coalescent (GMYC), bayesian Poisson Tree Processes (bPTP) and Discriminant Analysis of Principal Components (DAPC). In all species delimitation instances, the outgroup (*P. pileolus*) was removed from sequence alignment files or input topologies (using TreeGraph 2; Stöver and Müller, 2010) to limit impacting *Burnupia* spp. delimitation results. The GMYC species delimitation method made use of the amended time-calibrated, ultrametric tree (generated in BEAST) and run using single and multiple thresholds, while the bPTP species delimitation method made use of the amended Bayesian topology (generated through MrBayes). All settings were otherwise kept as default for these approaches and the analyses were run through the online server at https://species.h-its.org/ptp/. The DAPC approach was performed using the adegenet 1.4-1 (Jombart and Bateman, 2008) package as run in the R statistical environment 4.0.2 (R Core Team, 2020) using a fasta alignment of all *Burnupia* haplotypes. Herein, Bayesian Information Criterion (BIC; Schwarz, 1978) was used to determine the optimal number of genetic clusters (k) before the DAPC analysis defined the specimens in each cluster.

Ancestral Distribution Analyses

To gain better perspectives of the evolutionary origins or colonisations of the established *Burnupia* phylospecies in the Lesotho Highlands ancestral area estimation was assessed across the dated phylogeny. BioGeoBEARS (Matzke, 2013) was implemented in RASP 4.2 (Yu et al., 2015) and used to estimate ancestral areas under six different biogeographical models for the entire dated phylogeny. This included the DEC, DIVA-like and BayArea-like models, including the +J parameter for each. Here, a simplified analysis with default settings in which only two areas were predefined (namely "within the Lesotho Highlands" and "outside the Lesotho Highlands") was run and the best-fit model was determined by using the AIC—as implemented in BioGeoBEARS.

Isolation by Elevation and Distance

To assess the level of genetic diversity that can be attributed to Isolation by Distance (IBD) and Elevation (IBE), and thus help identify if the genus may contain cold-evolved species (i.e., those restricted by elevation and/or geographic distance at high altitudes), analyses of IBD and IBE were calculated within the genus as a whole following the methods of Sands et al. (2019b). In summary IBE and IBD was calculated using genetic, elevation and geographic distance matrices between samples (as compiled in GenAlEx 6.5; Peakall and Smouse, 2006) and following the distance-based redundancy analysis method (db-RDA; Legendre and Anderson, 1999; Legendre and Fortin, 2010) with the package vegan 2.5-4 (Oksanen et al., 2019) in the R statistical environment 4.0.2 (R Core Team, 2020).

RESULTS

Phylogenetics and Species Diversity

Between the BI and MP topologies generated in MrBayes and Paup respectively there are 19 supported nodes among the

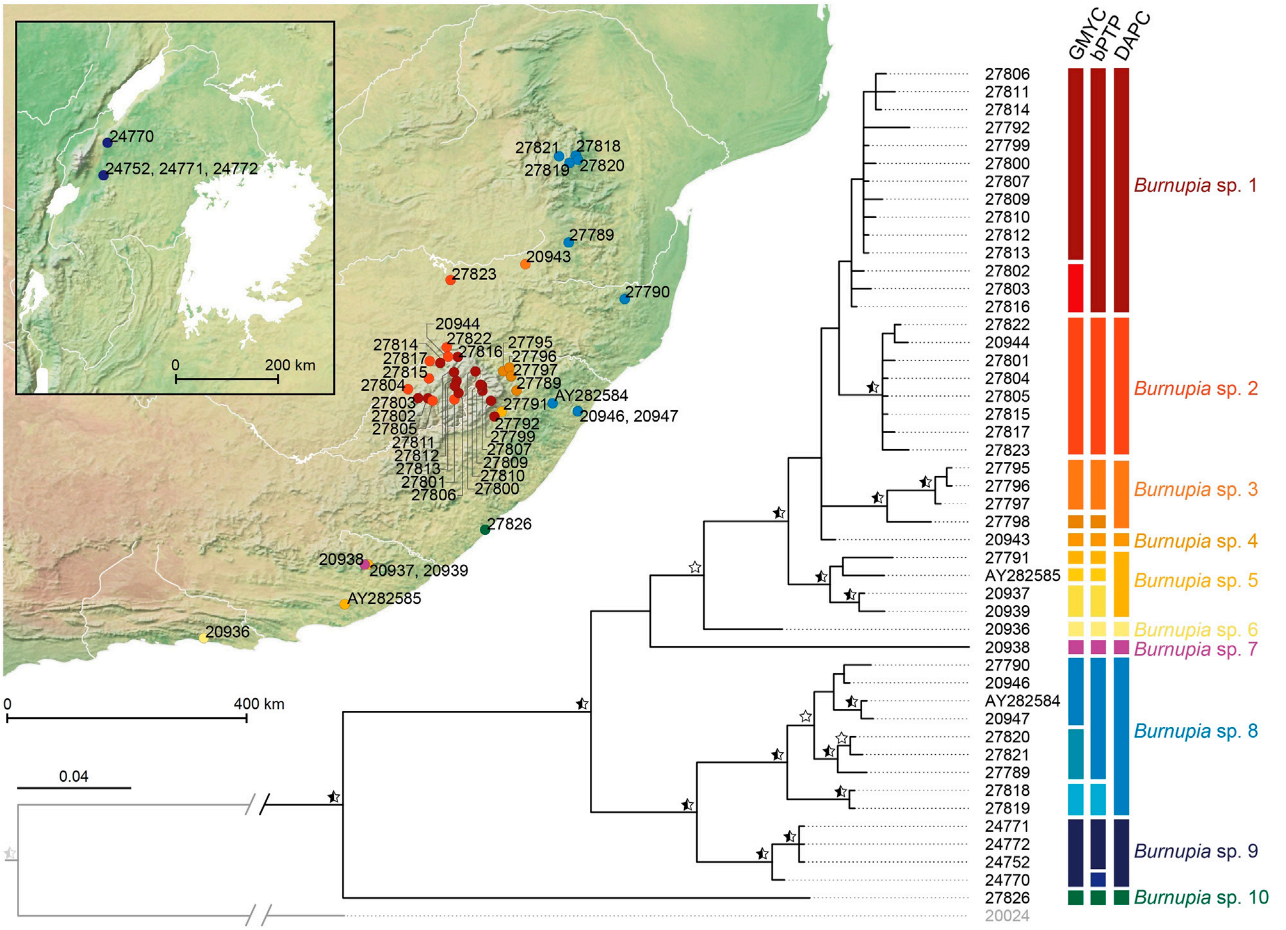

FIGURE 3 | Bayesian phylogeny of *Burnupia* generated in MrBayes. Supported nodes (PP ≥ 0.95) are marked with black stars. Nodes additionally supported by parsimony analyses (Paup; BS ≥ 70) are marked with white stars or half black/white stars where PP support already exists. Results of the species delimitation methods (i.e., GMYC, bPTP and DAPC) are given alongside the tips in the form of coloured bars where different colours within each method denote different species and shared colours across methods indicate shared species. A distribution map with dots coloured and following the DAPC derived species are also presented. Further details on the samples and localities can be found in **Table 1** following the specimen alphanumeric labels.

relationships of the 47 *Burnupia* specimens (PP ≥ 0.95 and/or BS ≥ 70; **Figure 3**). Species delimitation results range from between 10–16 *Burnupia* spp. among the dataset (GMYC = 16, bPTP = 15, DAPC = 10; **Figure 3**). The DAPC approach is the most conservative and, although not always reflective of phylogenetic support, best corresponds to the supported clade structures of the phylogenies. The other methods (i.e., GMYC and bPTP) tend to define species across multiple unsupported nodes creating increased complications with phylogenetic support. The ten species classified by the DAPC approach and regions where specimens of each were found are as follows: *Burnupia* sp. 1 (Lesotho Highlands), *Burnupia* sp. 2 (northern and western Lesotho Highlands and the South African Highveld), *Burnupia* sp. 3 (South African Highveld) and *Burnupia* sp. 4 (lower slopes of the eastern Drakensberg, KwaZulu-Natal), *Burnupia* sp. 5 (lower slopes of the south-eastern Drakensberg, KwaZulu-Natal down into the Eastern Cape), *Burnupia* sp. 6 (Tsitsikamma), *Burnupia* sp. 7 (Katberg Mountains), *Burnupia* sp. 8 (South African Lowveld and KwaZulu-Natal), *Burnupia* sp. 9 (Great Lake drainages of western Uganda) and *Burnupia* sp. 10 (Wild Coast) (**Figure 3**).

Dated Phylogeny and Ancestral Distributions

The genus *Burnupia* plausibly arouse somewhere between 25.95–5.64 Ma (data not displayed in **Figure 4**). While this study lacks several species of *Burnupia* (e.g., those from South America—which possibly represent a separate genus), the species occupying southern Africa likely began interspecific diversification from a common ancestor around 9.26 Ma [95% highest posterior density (HPD): 13.45–5.64 Ma; **Figure 4A**]. Following the DAPC species delamination results, by 0.60 Ma (95% HPD: 1.00–0.29 Ma; for *Burnupia* sp. 1 and *Burnupia* sp. 2) all species have diverged and the remaining genetic diversity is held intraspecifically (**Figure 4A**).

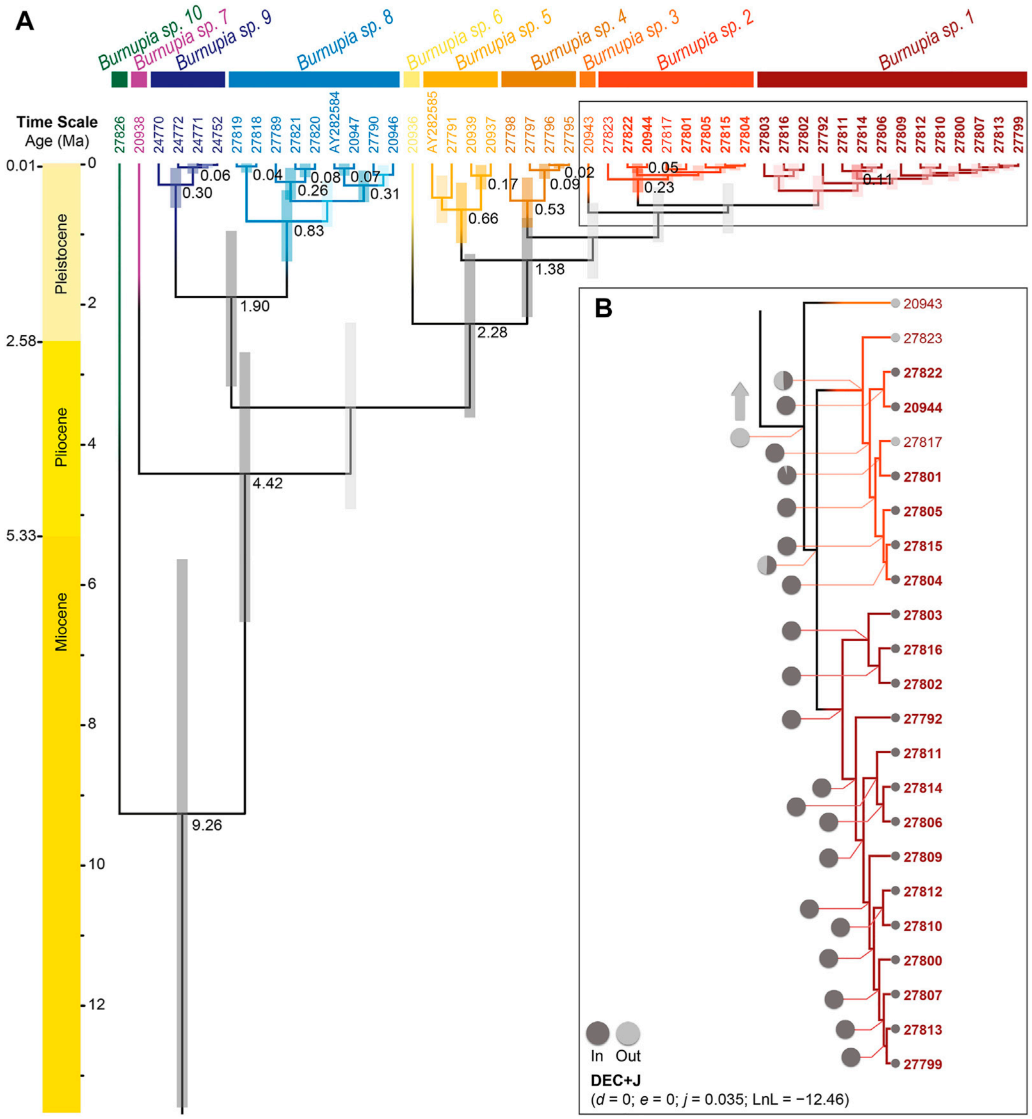

FIGURE 4 | (A) The dated phylogeny of *Burnupia* spp. sampled across southern and eastern Africa constructed in BEAST based on COI sequence data. Supported divergence events (PP ≥ 0.95) are indicated by written dates at the nodes (in millions of years ago; Ma) and brighter shaded 95% credibility interval (i.e., HPD) bars. Species or clades are partitioned and coloured according to the DAPC species delimitation results (see **Figure 2**). Tip labels correspond to the preparation numbers of specimens noted in **Table 1** and, therein, those in bold correspond to specimens occurring in the Lesotho Highlands. **(B)** The portion of the DEC+J results of the ancestral area estimation for *Burnupia* spp. on the dated phylogeny that correspond to Lesotho Highland occurrences. Ancestral area estimations were obtained from BioGeoBEARS run through RASP. Nodal pie charts represent the relative likelihood of the lineage occurring within the Lesotho Highlands (dark grey) and outside of the Lesotho Highlands (light grey). Smaller pies at the tips represent the current occurrence of each specimen. All other nodes across the phylogeny (data not shown; indicated by the light grey arrow) are equivalent to ancestral areas outside of the Lesotho Highlands.

Ancestral distribution estimations suggest that the common ancestors of all *Burnupia* species included in this study is not likely to have occurred in the Lesotho Highlands (**Figure 4B**). The analysis demonstrates only *Burnupia* sp. 1 and *Burnupia* sp. 2 have had historical presences in the Lesotho Highlands. The results point to the common ancestor of these two species likely colonising the Lesotho Highlands between 1.12–0.29 Ma, however significant PP and BS support for nodes surrounding this clade could not be found across our phylogenetic topologies (**Figure 4A**). Nevertheless, given species of *Burnupia* from the Lesotho Highlands seem to be closely related and *Burnupia* sp. 1, *Burnupia* sp. 2 and *Burnupia* sp. 3 diverged from *Burnupia* sp. 4 around 1.38 Ma (95% HPD: 2.19–0.78 Ma; PP ≥ 0.95 and BS ≥ 70; **Figures 3**, **4**), this would indicate colonisation only occurred after this point (**Figure 4**).

Isolation by Distance and Elevation

Within the genus *Burnupia* as a whole, both IBD and IBE were significant yet both very weak in accounting for genetic diversity between species within the genus. We found

$R^2 = 0.097$ ($p < 0.001$) for IBD and $R^2 = 0.102$ ($p < 0.001$) for IBE, respectively (also see **Supplementary Material**).

DISCUSSION

Diversity and Biogeography in the Highlands

Although not particularly species rich in *Burnupia*, our results point to the Lesotho Highlands as being an important area for freshwater diversity.

Our results support between 10–16 phylogenetic species established through the molecular delimitation methods across, primarily southern Africa but also Uganda, albeit limited sampling the latter (**Figures 2B**, **3**). Following the most conservative delimitation method, the DAPC approach, that best corresponds to our phylogenetic support and clade structure in our trees, two species can be found in the Lesotho Highlands, six can be found in the lower laying areas of KwaZulu-Natal and the Eastern Cape, three in the inland areas of South Africa (i.e., in the Highveld and Lowveld) and a single species was identified in the Western Region of Uganda (**Figure 3**). Diversity in the Lesotho Highlands is therefore to be seen as relatively average in comparison to at least the broader southern African region. However, given the temporal persistence of *Burnupia* in the Highlands and the endemicity of at least one of these species our results do suggest the region is important for cold-evolved freshwater biodiversity. Similar patterns have been observed for other aquatic organisms in the region (Daniels et al., 2003; Tolley et al., 2010). Our study is based on mitochondrial DNA variation. Studies in the European freshwater limpet genus *Ancylus* has demonstrated that diversity of young species can be underestimated—genome-wide approaches can help detecting hidden diversity in such cases (Weiss et al., 2018).

It is interesting that *Burnupia* partly overlaps with two other cold-evolved endemic species in the Highlands (Prinsloo and van Eeden, 1973). *Bulinus* sp. occurs up to 3,100 m.a.s.l. in Lesotho (Tumwebaze et al., 2022) and *Galba mweruensis* reaches similar altitudes (Mahulu et al., 2019). These two species are primarily found in stagnant and even temporary waters, whereas *Burnupia* species predominate on hard substrates as found in the permanent streams in the Highlands. A high oxygen demand and less drought tolerance has been shown for *Burnupia* (but see de Kock and Wolmarans, 2017) and some species have been used in lowland regions for ecotoxicological monitoring (e.g., Gerhardt and Palmer, 1998; Davies-Coleman and Palmer, 2004; Vellemu et al., 2018). Temperature sensitivity of *Burnupia* could potentially be established as a proxy for ongoing climatic changes, also in other Afromontane regions. In eastern Africa *Burnupia* also coincides with *Bulinus* and *Galba*, for example in the Ethiopian Highlands and the Aberdares Mountains, the Mau region and the Mount Kenya Massif (Brown, 1994; Mahulu et al., 2019; Tumwebaze et al., 2022). Comparative phylogeographical studies should be conducted to establish general patterns for freshwater benthic organisms in the Afromontane archipelago of sky-islands. Such studies could also help understanding better the means of colonisation across various taxa (Daniels et al., 2003; Tolley et al., 2010; Taylor et al., 2020). For *Burnupia*, the intrinsic characteristics of this limpet group, restrictive active dispersal and the narrow ecological tolerances question the actual dispersal and thus colonisation mechanism. Given that populations occur along basically whole drainage systems from lowland to sometimes up to the spring regions, jump dispersal is less likely. On the other hand, the nature of the habitat, high currents and the mobility of the (gravel) substrate restricts the ability of active dispersal upstream tremendously in aquatic mollusc (Kappes and Haase, 2012), more even for limpets (Albrecht et al., 2006). Birds as dispersal vector are probably not important in this case of upstream movement. Fish are often invoked as biological passive vector (Kappes and Haase, 2012). In fact, there are candidate species of fish including *Labeobarbus* yellow fishes (see Plug et al., 2010; Schrijvershof, 2015). Their role in the dispersal context of *Burnupia* should be studied in a comparative population-genetics framework.

The common ancestor of *Burnupia* sp. 1 and *Burnupia* sp. 2 was likely the first to colonise the region (**Figure 4B**). Our temporal reconstruction of the evolution of *Burnupia* suggests this may have occurred around 1.12–0.29 Ma, however phylogenetic support for the divergence events between *Burnupia* sp. 1, *Burnupia* sp. 2 and *Burnupia* sp. 3 are not well supported. Nevertheless, given the closest supported nodes, colonisation certainly happened post 1.38 Ma (95% HPD: 2.19–0.78 Ma; **Figure 4**) and prior to 0.23 Ma (95% HPD: 0.41–0.08 Ma; **Figure 4**). This Pleistocene period means *Burnupia*'s persistence in the Highlands likely overlapped with possibly several glacial maxima (Elderfield et al., 2012). Glacial cycles probably had two effects: firstly, glacial maxima caused the freezing of suitable freshwater environments in the Highlands (Mills et al., 2009, 2012; Hall and Meiklejohn, 2011)—this likely drove the several allopatric speciation events that can reasonably explain the speciation of *Burnupia* sp. 1 and *Burnupia* sp. 2 and the population structures therein. Secondly, glacial cycles probably drove several retreats and recolonisations (i.e., sinking) in *Burnupia* sp. 2 (**Figure 4**) and likely forced *Burnupia* sp. 1 to become cold-evolved given the endemicity of this species in the Highlands (**Figures 3**, **4**), which likely accounts for at least some signals of IBD and IBE within *Burnupia*. Given our results, the *Burnupia* might serve as an ideal model group for testing the existence and extent of refugia and generally the role of climatic changes in rapidly changing Afromontane environments and colonisation patterns and processes, including co-evolution with dispersal vectors. As such, *Burnupia* could well become an Austral pendant to freshwater limpets in the northern hemisphere such as *Ancylus* (Albrecht et al., 2006) or *Acroloxus* (Stelbrink et al., 2016). An important asset in such studies would be an enhanced and better resolved taxonomic framework for all African *Burnupia* spp.

Taxonomic Implications

Freshwater scientists operating in Africa have long been advocating for a molecular based review of the genus *Burnupia* given the distribution of type localities among described species and shallow morphological differences among certain groups (Brown, 1994; Albrecht and Clewing, 2019), yet our molecular results only partly elucidate the complex picture. Brown (1994) presumed a very high level of synonymies among the available names but still listed no less than 21 species of *Burnupia* in his seminal book.

Our sampling regime largely overlapped the type localities of 14 of the 22 *Burnupia* species described from Africa as well as two subspecies of *B. capensis* (**Figure 2**). It was therefore interesting to find between 10–16 phylogenetic species established through the molecular delimitation methods (**Figure 2**). While the DAPC approach showed the best correspondence between the phylogenetically supported clade structure and the expected morphological diversity, the distribution of phylogenetic species did not entirely always correspond well with the distribution of described species and their type localities (**Figures 2**, **3**). For example, at least six species have been described or noted in the KwaZulu-Natal Midlands (**Figure 2A**), yet through our sampling we could only find a single species in this area (i.e., *Burnupia* sp. 8; **Figure 3**). This species corresponds to *B. caffra* (Albrecht et al., 2004). While our sampling regime did not specifically target type localities, it is not unreasonable to suggest careful review and possible synonymisation of *B. caffra*, *B. capensis natalensis*, *B. gordonensis*, *B. nana*, *B. obtusala* and *B. ponsonbyi* may need to be looked at, especially given the broad distribution range of *Burnupia* sp. 8 (i.e., *B. caffra*) and that some of these described species share type localities in very close proximity to one another—sometimes even in the same drainage systems (Brown, 1994). Additionally, only a single species has been described (i.e., having a type locality) from the Eastern Cape region of South Africa (i.e., *B. stenochorias*; **Figure 2A**), yet we found three seemingly endemic lineages in this area (i.e., *Burnupia* sp. 6, *Burnupia* sp. 7 and *Burnupia* sp. 10; **Figure 3**). These could possibly represent the range extremities of Western Cape species whose type localities and systems were sampled but specimens failed to be amplified for COI (i.e., *B. capensis capensis*, *B. gaulus* and *B. verreauxii*), however, *B. c. capensis* and *B. gaulus* have been synonymised (see Brown, 1994) and it seems more realistic that they are as yet undescribed lineages given the lack of drainage collection between the southern Western Cape type localities and the large environmental and climatic shifts up the east coast of southern Africa (Werger, 1978; Abell et al., 2008; Linder et al., 2012). Placing names to phylogenetic species is therefore still a major challenge.

These naming uncertainties also extend to the *Burnupia* diversity in the Lesotho Highlands. For example, the distribution of *Burnupia* sp. 1 largely conforms with that attributed to *B. capensis striatissima*, while *Burnupia* sp. 2 conforms with that noted for *B. trapezoidea* (Brown, 1994; de Kock and Wolmarans, 2009, 2017). However, it seems unlikely that *B. capensis striatissima* is a subspecies of *B. capensis* given the endemicity in the Highlands and very close relationship with *Burnupia* sp. 2—noted as *B. trapezoidea* (see de Kock and Wolmarans, 2017; **Figures 3**, **4**). The name *B. trapezoidea* (described from modern day Botswana) for *Burnupia* sp. 2 itself also requires careful review given three species (i.e., *B. mooiensis*, *B. transvaalensis* and *B. vulcanus*) have been described across the Highveld, particularly in the Vaal River catchments (Brown, 1994; **Figures 2A**, **3**).

The taxonomic complications and conflicts in *Burnupia* are not unexpected. Other freshwater limpets have a similar history of splitting and lumping, which often masks real biodiversity (e.g., in *Ancylus*; Albrecht et al., 2006). Increasingly in aquatic malacology, when traditional shell-based taxonomic approaches are reviewed with molecular data and modern morphological methods, differences in shell shapes and patterns are noted to sometimes be poor tools for distinguishing species and likely to be influenced by environmental changes in some groups (Schultheiss et al., 2009; Sands et al., 2020; van Bocxlaer et al., 2021). Such changes have even been documented in a variety of molluscs in laboratory settings by small alterations in their environments (Neumann, 1959; Kistner and Dybdahl, 2013). If the same is corroborated for *Burnupia*, research involving type material is vitally needed to properly update the systematics of the genus and attribute the correct names to the phylogenetic species discovered.

CONCLUSION AND FUTURE OUTLOOKS

Our study revealed that the Lesotho Highlands are an important region for freshwater diversity and likely holds cold-evolved species. These Highlands were colonised roughly during Calabrian–Chibanian stages of the Pleistocene by *Burnupia* species and may be a useful model to study the evolution of the Highlands and likewise its impact on freshwater organisms given indications of population structure. It requires NGS approaches to be used which are more sensitive to reconstructing population-level history. The study has improved our knowledge of *Burnupia* systematics, but it still remains mostly unresolved. Further research is desperately needed using type material and establishing if morphological features such as shell shape and patterning is affected by environmental conditions.

DATA AVAILABILITY STATEMENT

The DNA sequence data presented in this study can be found in the NCBI's GenBank repository online (www.ncbi.nlm.nih.gov/genbank). The accession numbers can be found in **Table 1**.

ETHICS STATEMENT

Ethical review and approval was not required for the study. All material was sampled through bilateral agreements among collaborating institutes or with local permits where required.

AUTHOR CONTRIBUTIONS

The team of authors has a strong research focus on freshwater biogeography, phylogenetics and geology—especially in Africa. All authors contributed to the general framework of the article: CA, VSG, and FR performed fieldwork and collected samples; CA generated the data; AFS performed the analyses; AFS and CA led the writing.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2022.914272/full#supplementary-material

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The Land–Water–Energy Nexus of Ruzizi River Dams (Lake Kivu Outflow, African Great Lakes Region): Status, Challenges, and Perspectives

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Hydroelectric power (HP) represents the main source of electricity in Africa, including the Democratic Republic of Congo. The demand for new dam construction is high, and major projects are currently progressing through planning and implementation stages. New HP dams should comply with both past and emerging environmental requirements. River systems need water to maintain hydraulic and ecological functions. Flow regime disturbance can prevent rivers from providing their ecosystem services and disrupt riparian communities. Most dammed rivers in Africa are understudied, however, in terms of their environmental flow requirements. This study analysed the hydrological regime and water quality of the Ruzizi River. The research investigated conditions of minimum water flow and hydropeaking at the Ruzizi I HP dam in terms of land management constraints and ecological impacts. According to Gumbel's hydrological model, a discharge of ~130 m^3/s showed the longest return period (12 years) among the most recurrent flows. By contrast, the maximum recorded discharge of 143 m^3/s showed a return time of 76 years. Any discharge between 46 and 120 m^3/s could occur at any time within three years. The discharge–hydropower production relationship for the power plant provided a possible minimum environmental flow of 28 m^3/s (i.e., 25%). Drinking water

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Abbreviations: APHA, American Public Health Association; BOD, biological oxygen demand [mg/L]; COD, chemical oxygen demand [mg/L]; DO, dissolved oxygen [mg/L]; DRC, Democratic Republic of Congo; EC, electrical conductivity of water [µS/cm]; E-Flow, environmental flow [m^3/s]; EGL, Energie des Grands Lacs; EWi, effective weight of *i*th species; HP, hydropower; IHA, International Hydropower Association; MAF, monthly annual flow [m^3/s]; MMF, monthly mean flow [m^3/s]; MPCA, Minnesota Pollution Control Agency; NTU, nephelometric turbidity unit; ONEC-BAD, Energy, Environment and Climate Change Department, African Development Bank; Q_{25}, Q_{50}, Q_{75}, and Q_{90}, percentile exceedances of flow duration curve [m^3/s]; RRI, Rhein Ruhr International; SINELAC, Société Internationale d'Electricité des Pays des Grands Lacs; SNEL, Société Nationale d'Electricité; SRP, soluble reactive phosphorus [mg/L]; TSI-266, Turkish Standards Institution; TSS, total suspended solids [mg/L]; VMF, variable monthly flow [mg/L]; WCD, World Commission on Dams; WHO, World Health Organization; and WQI, water quality index.

quality was assessed according to WHO water quality index (WQI) standards. Turbidity (i.e., total suspended solids) upstream and downstream of dams correlated strongly with rainfall (r = 0.8; n = 12) and land use. WQI values observed in excess of WHO drinking water standards indicate that the Ruzizi River is currently unsuitable for drinking water purposes.

Keywords: hydropeaking, reservoir siltation, reservoir pollution, water quality index, minimum hydrological flow

INTRODUCTION

Human communities will experience increasing demand for freshwater and energy in the coming decades (Holland et al., 2015). Freshwater represents only ~3% of global water reservoirs (Du Plessis, 2017) with much less of this accessible for human use. Dams provide irrigation, urban water supplies, navigation, hydropower, and flood control services (WCD, 2000). In 2020, hydroelectric power (HP) capacity accounted for ~16% of the global electricity supply (Mekonnen and Hoekstra, 2011). This included a worldwide power production capacity of 1,330 Gigawatts and a total of 4,370 Terawatt-hours annual HP energy production (IHA, 2019). Global estimates indicate the number of large dams (over 15 m high or with a volume larger than three million m^3) to be around ~45,000 and the number of small dams and reservoirs to be in millions (Moore et al., 2010). In the Democratic Republic of Congo (DRC), 85% of domestic energy use derives from fuel wood (Seyler et al., 2010) and only ~6% of the population has access to electricity (Kahindo, 2012). Government initiatives seek to use large rivers for HP production. The country currently hosts 51 HP dams, which are operational or under construction, and plans to build 13 more (Winemiller et al., 2016). Unfortunately, almost all of these dams have been built without environmental impact assessments, which could reconcile environmental imperatives with electricity production (Wang et al., 2012). Dombrowsky et al. (2014) describe the limited address of social and environmental impacts before and during the construction of the Ruzizi II HP dam. In their cost assessment of 245 dam projects in 65 countries between 1934 and 2007, Ansar et al. (2014) found that many of these dams did not meet expected economic returns. Poor planning and management thus may cause significant economic and environmental losses. Economic and political instability in developing countries often prevents effective HP planning and implementation (Jadoon et al., 2020; Kiriqi et al., 2021).

Research on environmental impacts of HP projects has occurred in an unequally distributed manner leaving African countries understudied. Truffer et al. (2003) considered impacts of water abstraction/diversion on water quality and biodiversity (fishes, macroinvertebrates, macrophytes, etc.). In Switzerland, national-level HP plant regulations require operators to reduce the negative ecological impacts of hydropeaking (Bruder et al., 2016; Tonolla et al., 2017). Hydropeaking usually entails upstream (reservoir) inundation and intensive, unnatural downstream discharges that can drastically impact the channel and sediment transport regimes over short timescales (Bruder et al., 2016). Jones (2014) describes river hydropeaking as creating two rivers, one of low flow and one of peak flow.

The concept of environmental flows (E-Flows) appeared in Europe and the United States in the mid-20th century as a response to rapid water resource infrastructure development and the impact of severe flow regulation and diversion of natural waters on biodiversity (Matthews et al., 2014; Winemiller et al., 2016). Before, little or no consideration had been given to ecosystem services or the water needs of river ecosystems. Since the 1980s, E-Flow research assessing ecological impacts of HP plants has proliferated exponentially in the scientific records (Kuriqi et al., 2021). E-Flow regime implementation has become a crucial framework for conserving fluvial ecosystems and mitigating ecological impacts of HP plants. The E-Flow for a river ecosystem includes estimates of not only the amount of water to be released downstream of diversions and abstractions but also the frequency, duration, timing, and rate of discharge changes (Matthews et al., 2014; Kuriqi et al., 2021). E-Flows must guarantee permanent connectivity of the river continuum for fish and other aquatic organisms, as well as habitat integrity (e.g., water quality, substrate, riverbank, and bed morphology) upstream and downstream of the diversions. In less well-studied river ecosystems, hydraulics-based methods using tables or other basic data to estimate E-Flows include the Tennant (1976) and Tessman (1980) methods. These specifically use percentages of the mean annual flow of the duration curve (% MAF; Tennant, 1976; Tessmann, 1980; Smakhtin et al., 2004; 2006). Other methods include hydraulic and habitat modelling, holistic methods (King and Louw, 1998; Hughes et al., 2001), and recently developed “dynamic approaches” (Kuriqi et al., 2021). Although criticized for not addressing ecological factors, hydrology-based methods require fewer resources and have provided effective results in several cases (King and Louw, 1998; Hughes 2001; Smakhtin et al., 2006; Pastor et al., 2014).

Tennant (1976) recommends a minimum instantaneous flow representing 10% of the average flow of a river necessary to sustain habitat and survival for most aquatic life on short timescales. The study also recommended a flow average of 30% to sustain the biological integrity of the river ecosystem as a whole. Tessmann (1980) described monthly time steps for determining flow thresholds and suggested that monthly mean flows (MMFs) may act as E-Flows if they equal ~40% of the river’s mean annual flows (MAFs). According to Pastor et al. (2014), 60% of the MMF can be allocated during the low-flow season, 45% during the intermediate-flow season, and 30% during the high-flow season. Low-, intermediate-, and high-flow seasons are respectively defined as periods with MMF <40% of MAF, MMF between 40 and 80% of MAF, and MMF >80% of MAF. Smakhtin et al. (2004) used annual river discharge to define E-Flow

TABLE 1 | Characteristics of the Ruzizi hydropower dams$^{(*)}$.

HP dam	Ruzizi I	Ruzizi II	Ruzizi III	Ruzizi IV (Sisi V)
Year of construction	1959	1989	Planned	Planned
Current status	Operational	Operational	Preliminary work started	Planned
Altitude (m)	1,462	1,391	968	-
Catchment (km^2)	32	90	224	-
Reservoir volume (10^6 m^3)	1.46	1.75	1.9	Not yet defined
Hydraulic dam height (m)	23	11	110	Not yet defined
Potential power (MW)	28	44	147	287
Available power (MW)	16	25	Not applicable	Not applicable

(*)Catchment area (not counting the Lake Kivu catchment) calculations by Eisenberg (2018).

requirements for global applications by determining pristine, good, fair, and degrading hydrological conditions for river ecosystems. This method used Q_{50}, Q_{75}, and Q_{90} percentiles to respectively designate good, moderate, and fair or degrading ecological status for the river. Q_{90} was then used as a base flow for the E-Flow requirements.

This study describes the Ruzizi River, which forms the border between DRC and the Republic of Rwanda to the north, and DRC and the Republic of Burundi to the south. The HP dams Ruzizi I and Ruzizi II (28 MW and 44 MW installed capacity, respectively; **Table 1**) have operated along the river since 1959 and 1989. Ruzizi I rests 3 km downstream of Lake Kivu outflow (Mururu site), while Ruzizi II rests 16 km downstream (Mumosho site; TRACTIONEL and RRI, 1980; Fichtner, 2008; ONEC-BAD, 2015). The Ruzizi River links Lake Kivu with Lake Tanganyika, which receives 30% of its total riverine inputs from the Ruzizi (Vandelannoote et al., 1999). No EIAs were conducted prior to the construction of either dam. According to ONEC-BAD (2015), two more dams are planned, including Ruzizi III (147 MW; Dombrowsky et al., 2014) and Ruzizi IV (also known as "Sisi V"; 287 MW). These or other forthcoming projects demonstrate the urgent need for hydrological and ecological analysis of the system (SHER and ARTELIA, 2017).

Ruzizi I and Ruzizi II have been subject to technical problems and poor management leading to dam malfunction and major flow disturbances. Most of the time, these dams operate in a way that causes habitat fragmentation for migrating fish. Field campaigns from 2015 to 2018 revealed that the dam operators were not aware of E-Flow requirements. Water levels were not maintained in fish ladders installed during dam construction (1959, 1989) for migrating fish including cyprinids.

Geochemical studies of river basins have helped constrain exogenic cycles of elements in the continent–river–lake–ocean system (Giridharan et al., 2010; Khan et al., 2022). The hydrogeochemical properties of water are important factors determining its suitability for domestic use, irrigation and industrial purposes. Interactions of water with lithologic units control water chemistry and quality (Subramani et al., 2009; Khan et al., 2022). Khan et al. (2022) reported that discharge and human activities within the basin were among the major factors responsible for temporal and spatial variation in sediment discharge of the Ramganga River (India). Several approaches have been used to assess the chemical status of water quality in rivers (Tsegaye et al., 2006; Möller et al., 2007). Ramakrishnaiah et al. (2009) evaluated the water quality of the Tumkur and Karnataka (India) and Aksu (SW Turkey) rivers using Turkish water quality indexes. Yidana and Yidana (2010) used conventional graphical methods with multivariate statistical methods and GIS to interpret controlling hydrochemical factors at different locations in a river system. These researchers also used water quality index (WQI) methods to assess the suitability of groundwater for human consumption. Kannel et al. (2007) used WQIs to evaluate spatial and seasonal changes in the water quality of the Bagmati River Basin. Debels et al. (2005) reported WQI estimates based on nine physicochemical parameters periodically measured from 18 sites to characterize spatial and temporal variability of surface water quality in the Chill'an River basin.

The present study differs from previous studies in that it uses empirical data to estimate E-Flow for power production and ecological integrity in an existing river basin. Compliance with E-Flow standards can render energy produced by Ruzizi dams safer and more sustainable. This study also analysed environmental challenges related to HP production along the Ruzizi River. The research specifically sought to 1) analyse the hydrological regime, 2) evaluate the sustainability of HP operations, 3) quantify the minimum hydrological flow required to cope simultaneously with electricity production and river ecosystem functioning, and 4) evaluate the current water quality status and sediment transport within the river.

Study Site

The Ruzizi River formed during the Quaternary period in the eastern part of the Congo basin's Albertine Rift Region (**Figure 1A**). Beginning with its outflow from Lake Kivu, the Ruzizi drains a 5,800-km^2 catchment (Eisenberg, 2018). The drainage density map shows five Strahler's orders (**Figure 1B**; Stahler, 1952). The Upper Ruzizi (where Ruzizi I and Ruzizi II are located) follows a V-shaped valley in a mountainous and high altitude region (1,460 m a.s.l. at Bukavu–Cyangugu; **Figure 1A**&B). The Lower Ruzizi (below Ruzizi III) meanders across a wide floodplain before reaching Lake Tanganyika after 120 km of total river length (770 m a.s.l. at Uvira–Bujumbura; Asselberghs, 1939). Eruptions of the Virunga volcanoes reversed the drainage of Lake Kivu from the Nile into the Congo basin (Célérier, 1931). The Upper Ruzizi catchment experiences a humid climate with a bimodal annual precipitation regime in the Lake Kivu basin of ~1,200 mm/yr and evapotranspiration of

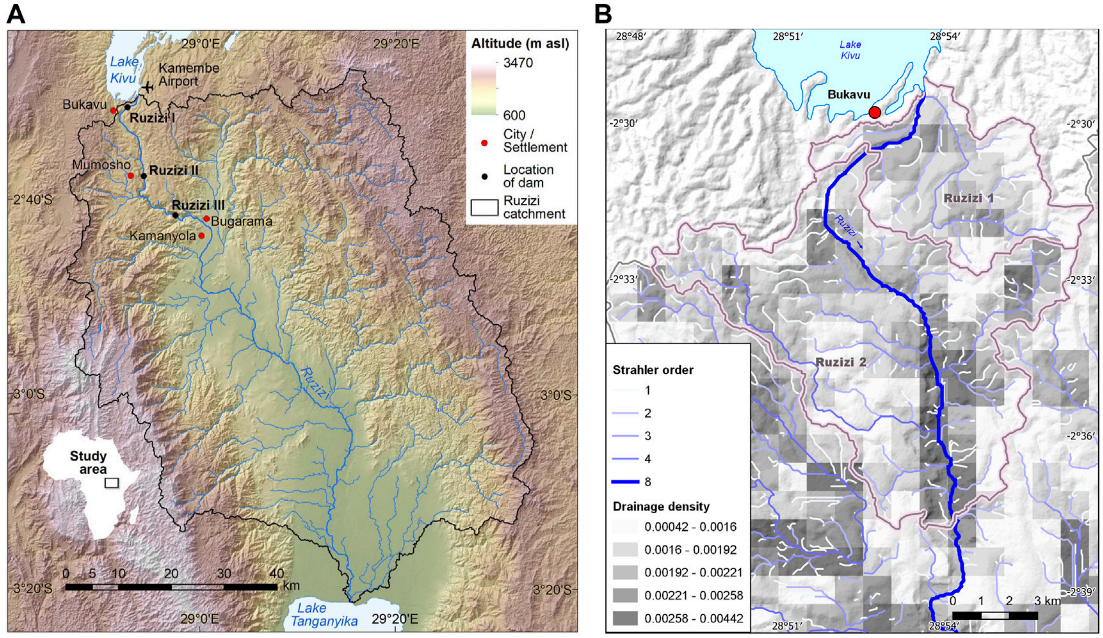

FIGURE 1 | (A) Map of the Ruzizi catchment showing hydropower dam locations modified after Eisenberg (2018) **(B)**. Drainage density and the Strahler order map for the Ruzizi River catchment according to Strahler (1952).

~1,100 mm/yr (Muvundja et al., 2014). The Lower Ruzizi catchment experiences lower precipitation (~800–900 mm/yr, Hyghes and Hyghes, 1992) and higher temperature differences (mean monthly minima: 14.5–17°C; mean monthly maxima: 30.5–32°C) relative to the Upper Ruzizi. The rainy season spans from September to May and the dry season from June to August.

The Ruzizi River basin also hosts a diverse range of endangered and legally protected aquatic fauna including mammals such as hippopotami and otters, reptiles (crocodiles, snakes, and turtles), fish (lungfish, endemic cichlids, cichlids, carps, and perch), birds (both resident and migratory), amphibians, and freshwater crustaceans and other invertebrates (Hyghes and Hyghes, 1992). The wetland vegetation around the basin is dominated by *Phragmites* sp., *Cyperus* sp., Love reed (*Typha* sp.), and *Panicum* sp. (Hyghes and Hyghes, 1992). The ecological uniqueness and biodiversity found in the Ruzizi River basin arise from its relief, varied land types, and climate (Lambinon and Reekmans, 1980).

Dam construction for electricity generation began on the Ruzizi River in the mid-20th century. **Table 1** lists information on existing and planned dam projects. This study addressed Ruzizi I and II dams and the downstream Ruzizi III project (**Figure 1**). Dams were designed assuming a theoretical usable discharge of 92–100 m^3/s (TRACTIONEL and RRI, 1980). Reservoir volumes of 1.46×10^6 and 1.75×10^6 m^3 (**Table 1**) give water residence times of ~4.7 and ~4.9 h, respectively. Land use in the Ruzizi I catchment consists mostly of urban activity. The Ruzizi II catchment is a mosaic of urban, peri-urban, and rural land use types. The reservoirs of both dams are polluted by urban wastewater.

The Ruzizi waters exhibit higher salinities than those of its tributaries. The salinity of the topmost Upper Ruzizi is the same as that of Lake Kivu surface waters (~1.1 g/L or 1,200 µS/cm of electrical conductivity (EC) at 20°C; Schmid et al., 2005). The Lower Ruzizi exhibits a salinity of ~0.5 g/L (~650 µS/cm) at Kiliba station (Kubaburhanzi, 2015). According to Kubaburhanzi (2015), most tributaries exhibit less than 2 m^3/s discharge during low water periods but become torrential during heavy rainfall with discharge up to 20 or 30 m^3/s. Values of pH range between 7.5 and 8.5 in the main channel. Tributary pH ranges between 7.2 and 7.5, and EC rarely exceeds 330 µS/cm (Kubaburhanzi, 2015; Cizungu, 2016; Hange, 2016; Kikuni, 2016; Muhindo, 2016).

MATERIALS AND METHODS

Hydrometeorology and Hydrologic Data Analysis

Monthly rainfall data obtained for a 2003–2010 study period from a local meteorological station located at Kamembe Airport (Rwanda, **Figure 1**) were compared with data covering the same period from three grid cells of the global satellite precipitation data (rainfall data by the U.S. National Oceanic and Atmospheric Administration). These span most of the Upper Ruzizi River catchment. The two data sets showed a strong linear correlation ($P_{Ground} = 1.14xP_{Sat}$; $R^2 = 0.96$; n = 12, where P_{Ground} and P_{Sat} are respective local and satellite precipitation estimates) at monthly resolution. The correlation coefficients indicate that satellite data underestimated precipitation by 14%. We obtained historical records (1941–2015) of river discharge

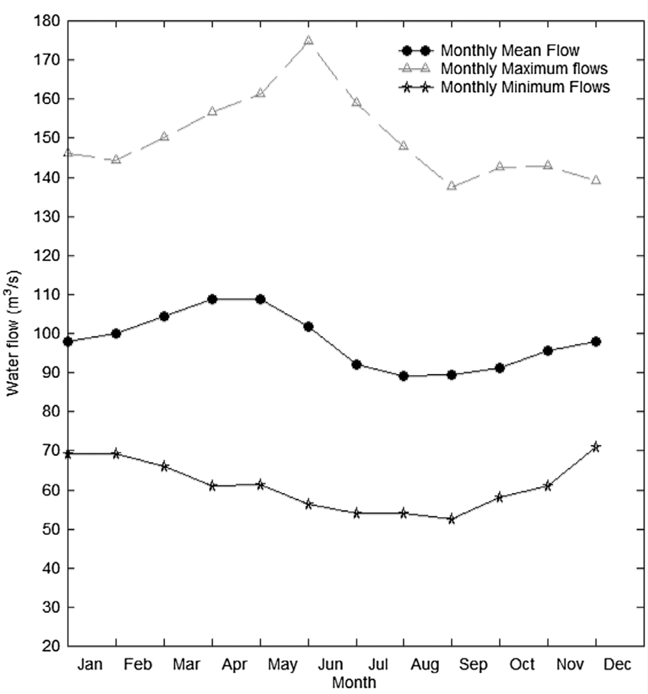

FIGURE 2 | Monthly (natural) water flow of the Ruzizi River at its Lake Kivu outflow (1941–2015).

from the Ruzizi I HP Operator (Société Nationale d'Electricité, SNEL for Ruzizi I; **Supplementary Table S1**). This data set was used for flood frequency analysis following a Gumbel (1941) distribution model.

Minimum Flow Analysis

This study used time series data from 1941 to 2015 and hydrological methods (look-up tables) to evaluate E-Flows at Ruzizi I (almost identical at Ruzizi II). Several hydrological indexes were calculated to evaluate minimum flow requirements. In addition to MAF, the indexes termed Q_{25}, Q_{50}, Q_{75}, and Q_{90} follow a format, where Q_i represents flows equal or exceeding the *i*th percentile of the recording period (Tennant 1976; Tessmann 1980; Pastor et al., 2014). Given its adequate performance for large rivers, this research used the Tessmann method to estimate minimum flow requirements (Karakoyun et al., 2018). The mean minimum flow was calculated as the difference between the long-term average river discharge and the long-term usable turbine discharge. The latter was derived from a regression model relating generated electric power to the turbine discharges for the period between 1989 and 2003 (**Figure 2**). The lack of ecological data precluded testing of other E-Flow methods.

Turbidity and Total Suspended Solids

Daily water turbidity measurements were performed upstream and downstream of the Ruzizi II dam from 10 September 2016 to 30 October 2017 using an HACH 2100Q turbidimeter. Several measurements were also made at Ruzizi I. Standard chemical solutions made for stabilized formazine turbidity values of 20, 100, and 800 NTU were used to calibrate the turbidimeter prior to field measurements (HACH 2009). Samples registering a turbidity value of more than 800 NTU were diluted with distilled water. Turbidity measurements are reported as mean values of triplicate analyses (coefficient of variation <6%). A calibration curve between turbidity of the standard solutions and total suspended solids concentration (TSS) was developed by preparing liquid samples from red clay Ferralsols (Muvundja et al., 2009), which typically occur in the catchment. In this procedure, soil samples were collected from the upper 10 cm of soil horizons and dried overnight in an oven at 110°C. Samples were thereafter stored in a desiccator until grinding and sieving at 1-µm mesh. Powdered samples were weighed and suspended (Minella et al., 2008; Perkins et al., 2017) in rainwater for calibration measurements.

Water Quality

Physicochemical parameters such as pH, dissolved oxygen (DO), water temperature, and EC were measured *in situ* at around 08:00 h on a monthly basis over the entire sampling period. *In situ* pH, water temperature, DO, and EC were measured using a Hydrolab probe (Data sonde ®4a USA). Water transparency (m) was measured with a 20-cm-diameter Secchi disk, with quadrants painted in black and white. Secchi depth was calculated as the average depth of disappearance and reappearance of the disk in water. Water samples were analysed for ammonium, soluble reactive phosphorus (SRP), nitrites, and silica. Biological oxygen demand (BOD) and chemical oxygen demand (COD) samples were collected using 4-L bottles downstream of Ruzizi I (twice per month) and Ruzizi II (once per month) over a 12-month period from January 2016 to February 2017. At each same location, equal sample volumes were collected from surface and middle depths before being mixed into a composite sample. Samples were preserved on ice during transport from the field to the laboratory and kept frozen until analysis. Standard methods were used for nutrient analysis. These included ammonia (NH_4^+) by the dichloroisocyanurate–salicylate method, SRP by the ascorbic acid method, nitrite (NO_2^-) by the colorimetric method, and soluble reactive silica by the molybdate complex method (APHA, 2005). Concentrations of these nutrients were estimated from UV/Vis spectrophotometry (Spectronic ®20 Genesys, USA). Total alkalinity concentration was estimated by titrimetric methods. Chloride concentrations in water samples were analysed based on titration with silver nitrate (Bartram and Ballance 1996; APHA, 2005; Rodier, 2009). BOD was determined using Oxitop bottles, while the analysis of COD was performed on $K_2Cr_2O_7$ oxidation-treated samples using spectrophotometric methods (Barttram and Ballance, 1996; Rodier, 2009; Lina, 2016). All analyses were performed at the Unité d'Enseignement et de Recherche en Hydrobiologie Appliquée (UERHA), Institut Supérieur Pédagogique de Bukavu. The electric charge-balance error of the water samples fell within <5% of measured values. The data were analysed with the help of the Statgraphics software. The water quality of the samples was assessed by calculating WQI values based on two WHO (2008) and TSI-266 (2005) standards.

Calculation of Water Quality Indexes

The Water Quality Index (WQI) is defined as a rating reflecting the composite influence of different water parameters (Sahu and Sikdar, 2008). In this study, the water quality of the Ruzizi River was evaluated for drinking and other purposes using the WQI method based on pH, HCO_3^-, Cl^-, COD, BOD, organic matter, NH_4^+, NO_2^-, PO_4^{3-}, SiO_2, turbidity, and total alkalinity. Interpretations used World Health Organization (WHO, 2008) limits for reference. To calculate WQI values at each sampling station, weightings (w_i) were assigned to each parameter according to their relative importance in the overall water quality expression for drinking purposes. The highest weight of five was assigned to nitrate (NO_3^-) and nitrite (NO_2^-) parameters due to their strong effects on drinking water (Şener et al., 2017). Consumption of water with high nitrate or nitrite concentrations can cause methemoglobinaemia disease (blue baby syndrome) in infants, gastric carcinomas, abnormal pain, central nervous system birth defects, and diabetes (Vasanthavigar et al., 2010; Varol and Davraz, 2015). The parameters pH, COD, BOD, SiO_2, and turbidity were assigned a weight of 4, while HCO_3^- and Cl^- received a value of 3. The minimum weight of 1 was assigned to total alkalinity. Relative weights (W_i) were then calculated for each parameter using **Eq. 1**. The WQI and overall WQI values for all samples were calculated using **Eqs 2–6** to establish water quality designations for each sampling point.

The relative weight (W_i) is calculated as follows:

$$W_i = \frac{w_i}{\sum_{i=1}^{n} w_i}, \tag{1}$$

where w_i is the weight of each parameter i (1 to n). A quality rating (q_i) for each parameter was calculated by dividing the concentration C_i (mg/L) of each sample by its WHO (2008) drinking water standard, S_i (mg/L), and then multiplied by 100 as follows:

$$q_i = \frac{C_i}{S_i} x100. \tag{2}$$

To calculate WQI, the SI_i subindex parameter values were first determined by the following equations (Ramakrishnaiah et al., 2009; Şener et al., 2017):

$$SI_i = W_i x q_i. \tag{3}$$

$$WQI = \sum_{i=1}^{n} SI_i. \tag{4}$$

Calculated WQI values were then parsed into five categories (Sahu and Sikdar, 2008; Yidana and Yidana, 2010; Şener et al., 2017) according to the WQI intervals:

WQI <50: excellent water quality.
50 < WQI <100: good water quality.
100 < WQI <200: poor water quality.
200 < WQI <300: very poor water quality.
WQI >300: unsuitable for drinking water.

In addition, the effective weight (EW_i) for each water quality parameter *i* was defined by dividing its subindex parameter value SI_i by the overall WQI value (**Eq. 4**) and multiplied by 100:

$$EW_i = \frac{SI_i}{WQI} x100, \tag{5}$$

The relative weights (W_i) reflect the significance of each parameter in relation to the other parameters used in WQI calculations. WQI is usually calculated in terms of a specific and intended use of water. In this study, the WQI was calculated relative to drinking water standards set as:

$$WQI = \frac{\sum_{i=1}^{n} w_i x q_i}{\sum_{i=1}^{n} w_i}. \tag{6}$$

RESULTS

Flood Frequency Analysis of Flow at Ruzizi I

Discharge records (1941–2015) suggest a long-term mean flow of 112 m^3/s for the Upper Ruzizi dam (**Figure 2**). Results of the flood magnitude–frequency analysis following Gumbel (1941) and Dalrymple (1960) indicate an exponential relationship between the 75 years of discharge data (1941–2012) and the recurrence time interval (**Figure 3A**; R^2 = 0.98). The high coefficient of determination indicates the suitability of Gumbel's distribution (also called extreme value distribution) in predicting river flow. The curve exhibits linear behaviour for smaller recurrence interval values. Discharges between 46 and 120 m^3/s can occur at any time within a 3-year time interval (**Figure 3A**). Longer recurrence interval values (t > 12.6 years) associate with discharge values of ~130 m^3/s (**Figure 3A**). The maximum flood event recorded occurred in 1998 with a peak flow of 143 m^3/s and recurs over a 76-year interval according to the flood frequency curve (**Supplementary Table S1**). Excessive flows like these likely occur or may be exceeded in a given year with a probability of ~1.3% (**Supplementary Table S1** and **Figure 3B**). The lowest flow of 46 m^3/s occurred in 1944 and represents a minimum recurrence value that will be exceeded with a probability of 100% (Supplementary material, **Table 1** and **Figure 3B**). The discharge–exceedance probability curve for natural Ruzizi flows gives values of 126, 96, 79, and 62 m^3/s for Q_{25}, Q_{50}, Q_{75}, and Q_{90}, respectively (**Figure 4**).

Minimum Hydrological Flow

The relationship between generated electric power and turbine discharges over a period of 14 years (1989–2003; **Figure 5**) yields 15.7 MW for a river discharge of 83.7 m^3/s. This is the long-term average power production at the corresponding long-term average flow of 112 m^3/s. The difference is 28 m^3/s (= 112–84), a value that should be implemented as the volume of permanently and freely flowing water in the bypass and fish ladders.

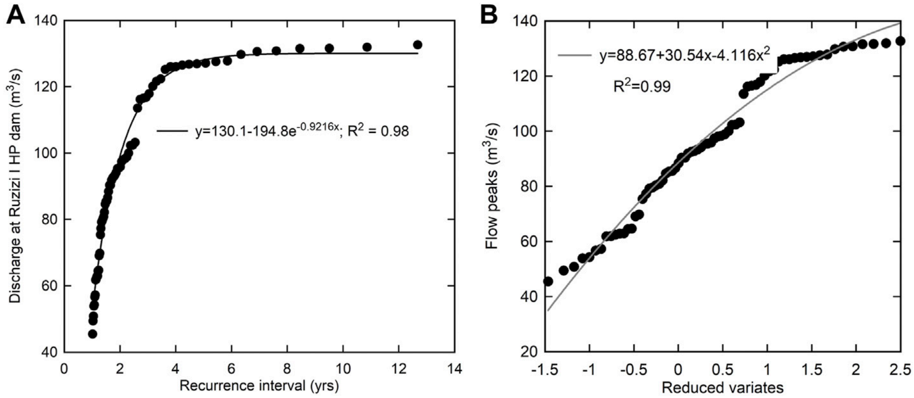

FIGURE 3 | (A) Magnitude–frequency curve for flows (Gumbel's curve) at the Ruzizi I HP dam (data from SNEL, 1941–2015). **(B)** Relationship between flood flows and reduced variates (same data set as in A).

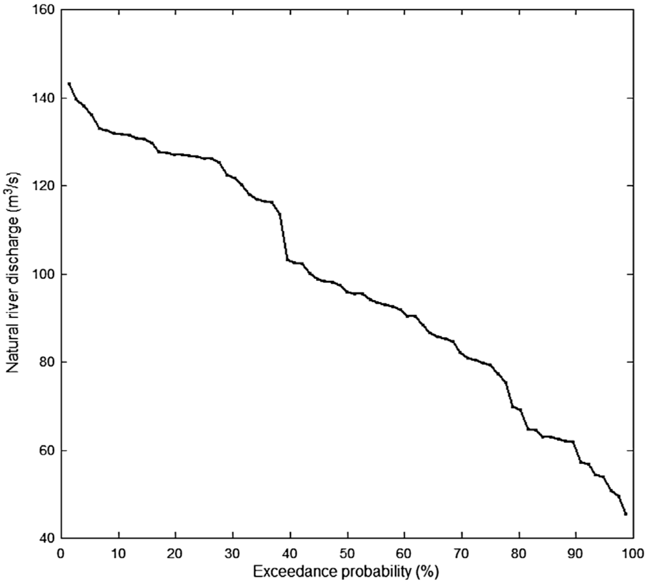

FIGURE 4 | River flows at Lake Kivu outflow vs. exceedance probability (same data set as in **Figure 3A**).

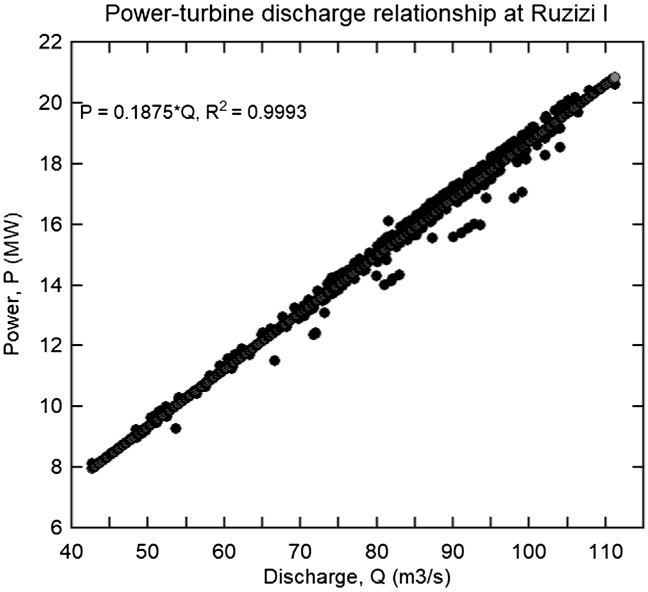

FIGURE 5 | Power–turbine discharge relationship at the Ruzizi I HP dam (SNEL, 1989–2009). Data sets represent monthly power production and monthly discharge averages of the turbines.

Surface Sediment Transport and Water Quality

We monitored turbidity and total suspended solids both upstream and downstream of Ruzizi II (**Figure 1**) over an annual cycle. Few data were available for Ruzizi I during the rainy season. The comparison between mean monthly turbidity at Ruzizi II and mean monthly rainfall (**Figure 6**) showed that turbidity and precipitation followed the same seasonal trend. The two parameters show moderate correlation (R^2 = 0.62 for upstream and 0.58 for downstream). The highest peaks of turbidity appeared during rainy seasons (September to June) with mean values exceeding 20 NTU. Maximum values were recorded in October (~30 NTU) and February (~36 NTU). The river gave lower turbidity values during the dry season. Values ranging from ~9 to ~13 NTU appeared from April to August with the lowest values in August (**Figure 5**). During the rainy season, the mean annual turbidity at the surface was 6.5 (upstream) and 5.1 NTU (downstream) of Ruzizi I (Supplementary material, **Table 2**). The annual mean was 24.5 upstream and 22.9 NTU downstream of Ruzizi II during rainy seasons, whereas dry season turbidity fell to 10.8 and 9.4 NTU upstream and downstream, respectively. In the Ruzizi I reservoir, turbidity measurements indicated 8.5, 7.6, 7.7, 8.3, 8.1, 5.8, and 6.9 NTU for 1, 2, 3, 4, 5, 6, and 7 m depth, respectively. For the Ruzizi II reservoir, the measurements yielded 11.3 NTU at 1 m, 13.1 NTU at 2 m, and 22.0 NTU at 3 m depths.

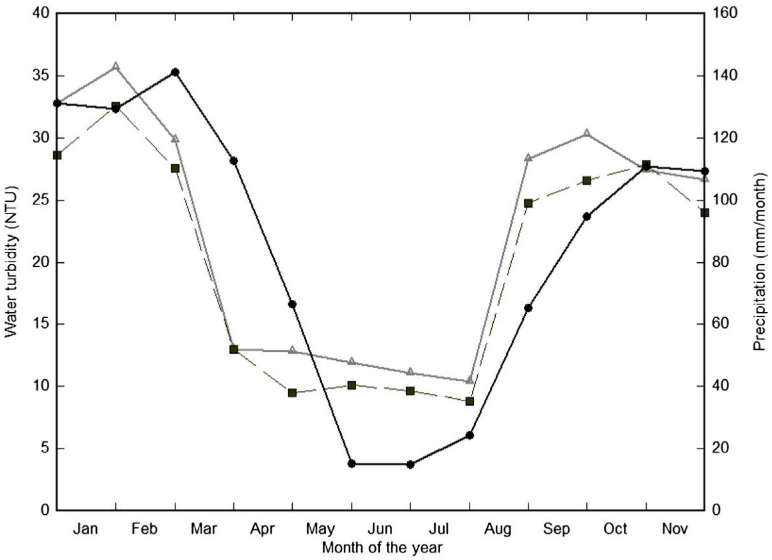

FIGURE 6 | Seasonal variation of precipitation and water turbidity upstream and downstream of the Ruzizi II dam. Data sets represent monthly averages of turbidity and catchment rainfall from September 2016 to October 2017.

TABLE 2 | WQI values and water types at sampling sites.

	WHO standards	Ruzizi I Upstream	Ruzizi I downstream	Ruzizi II Upstream	Ruzizi II downstream	Mean
WQI	<100	605	792	681	676	688
Water type	Suitable drinking water	Unsuitable drinking water	Unsuitable drinking water	Unsuitable drinking water	Unsuitable drinking water	Unsuitable drinking water

Waters generally appeared more turbid upstream than downstream of the dams. The difference was not significant for Ruzizi I (p-value = 0.6818 at $p < 0.05$, N = 22, two-tailed Mann–Whitney U test). However, the Ruzizi II site gave higher turbidity values by a factor of two than the Ruzizi I site. Upstream and downstream values showed statistically significant differences (p-value = 0.008 at $p < 0.01$, N = 308, two-tailed Mann–Whitney U test). The Ruzizi II reservoir exhibited higher turbidity (by ~2x) than the Ruzizi I reservoir. Daily turbidity data indicated differences of about 10% between the reservoirs, 10% above and below the Ruzizi II dam, and 22% above and below the Ruzizi I dam. This indicates a higher sedimentation rate at Ruzizi I relative to Ruzizi II.

Figure 7 shows a model used to convert turbidity measurements to total suspended solids (TSS). The TSS concentrations at Ruzizi I averaged 10 mg/L (both at upstream and at downstream; applicable to the rainy season only). For Ruzizi II, the average turbidity values found during the rainy season reached 60 and 50 mg/L upstream and downstream, respectively, compared to 20 mg/L upstream and downstream during the dry season. Annual mean TSS at Ruzizi II was 50 mg/L.

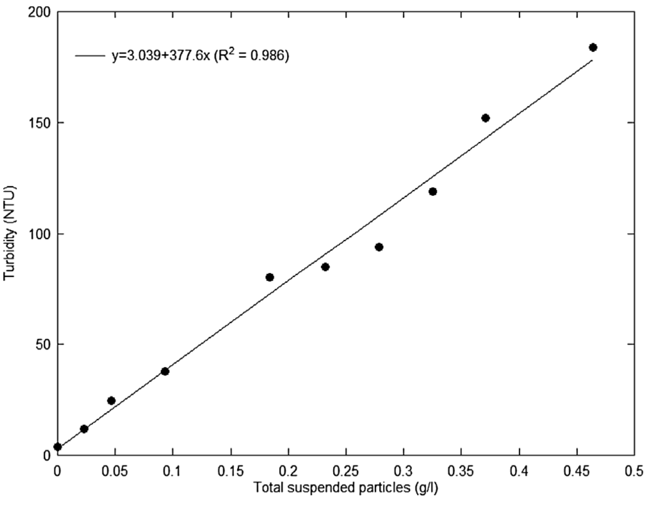

FIGURE 7 | Calibration curve between turbidity and catchment soil particle concentrations (No. of samples = 12). Soil samples (Ferralsols) were collected at ISP Bukavu in the town of Bukavu (**Figure 1A**).

A mean discharge of 98 m^3/s corresponds to an annual particle transport of 155 kt/yr and 1 $kt/km^2/yr$ over the entire Ruzizi II subcatchment.

During the sampling period, the pH values varied from 6.1 to 10.0 with a mean value of ~9. Water temperature ranged from 23 to 26°C, and EC varied over 1,021 to 1,225 μS/cm (**Supplementary Table S3**). *In situ* measured DO values of water samples ranged from ~0 to 9 mg/L, with an average value of ~5–6 mg/L depending on the site (**Supplementary Table S3**). Low DO values were measured in all reservoirs but not consistently. The DO saturation varied between 3 and 122% with averages between 60 and 86% depending on the site (**Supplementary Table S3**).

The mean COD ranged from 527 to 783 mg/L, whereas BOD varied from 8 to 26 mg/L, with an average of 19 mg/L (**Supplementary Table S3**). The concentration of total alkalinity ranged from ~0 to 2 mg/L (**Supplementary Table S4**). Both minimum and maximum values were measured upstream and downstream of Ruzizi I, respectively.

Ammonium (NH_4^+) contents of the Ruzizi River ranged from 0.01 to 1.60 mg NH_4^+/L. The higher value was measured upstream of Ruzizi I. Nitrite (NO_2^-) contents ranged from ~0 to 9.80 mgNO_2^-/L during the sampling period. Elevated values may reflect leaching of waste disposal, sanitary landfills, overdosage of inorganic nitrate fertilizer, or improper manure practices (Chapman, 1996). Phosphate concentrations ranged from 0.01 to 0.73 mgPO_4^{3-}/L. The highest phosphate values occurred upstream of the dam. The silica contents of the water samples ranged from ~0 to 31 mgSiO_2/L.

The WQI-relevant parameters included COD (with mean effective weights between 55 and 73% depending on the sites), BOD (3–11% mean effective weight), and finally nutrients and pH (**Supplementary Table S4**). The results obtained from this study (**Table 2**) gave WQI values for the Ruzizi River ranging from 605 to 792 over all sampling stations. These significantly exceeded drinking water limits (WQI <100).

DISCUSSION

Minimum Hydrological Flow Under Current Ruzizi HP Dam Operations

The Ruzizi I and II HP plants never reach their installed capacity (28 and 44 MW, respectively; SHER and ARTELIA, 2017) due to recurrent technical problems caused by ageing or outdated technology, and poor management and maintenance. Ruzizi I operates at an average power of ~16 MW (**Figure 5**), whereas Ruzizi II averages at 25 MW (EGL 2015). This means that if efficiently managed, they would require only ~85 m^3/s of the long-term discharge of ~112 m^3/s.

The recorded base flow is ~65 m^3/s, while the maximum flow is ~140 m^3/s (**Figure 2**). The base flow is almost the same as the Q_{90} value (62 m^3/s; **Figure 4**) according to the percentile of discharge modelling by Smakhtin et al. (2004). This E-Flow requirement represents fair hydrological conditions below which river ecological integrity will start to decline (Smakhtin et al., 2004). Excess flow beyond the optimal power generation discharge (85 m^3/s) is 28 m^3/s, which should flow freely and permanently outside the turbines. This means that turbines can operate efficiently on average without closing the bypasses and fish ladders. This water amount can likely sustain short-term aquatic life when hydropeaking is required for dam operations and turbine maintenance. This water flow is equivalent to ~25% of the average river discharge and can be considered as an ideal potential E-Flow. Minimum flow represents more than the 10% required to sustain short-term preservation of habitat for most aquatic life forms, and approaches the 30% flow value recommended for sustaining the biological integrity of riverine ecosystems (Tenant, 1976). The ~25% value also approaches the 30% of MAF E-Flow suggested by Pastor et al. (2014) for high-flow seasons. The calculated minimum hydrological flow represents 61% of the lowest natural flow (i.e., 46 m^3/s) ever observed from the Ruzizi River (**Supplementary Table S1**; **Supplementary Table S4**). This minimum flow fits with the 60% MMF value recommended for E-Flow during low-flow seasons (Pastor et al., 2014). For Ruzizi dams, respective Q_{50}, Q_{75}, and Q_{90} that equal 96, 79, and 62 m^3/s represent good, moderate, and fair ecological conditions (**Figure 4**).

Environmental Impacts

According to Lidec and Quintero (2003), large dams can vary considerably in terms of their adverse environmental impacts and can thus be classified as "good" or "bad" dams. Given their site selection features, Ruzizi dams were categorized as "good." These features include the following: 1) minor evaporation loss from a small reservoir surface area, 2) minor losses of natural habitat and wildlife, 3) a relatively small river with limited aquatic biodiversity at risk, 4) a deep reservoir with low sedimentation rate, 5) many downstream tributaries, 6) little or no flooding of forests, 7) no tropical diseases, and 8) no floating weed problems. The typical "bad" dam features around Ruzizi dams include extensive soil erosion (Eisenberg, 2018; Eisenberg and Muvundja, 2020) and solid waste pollution from urbanized areas of the catchment. The solid waste in urban and peri-urban areas accumulates in the reservoirs, particularly from Bukavu, and disturbs electricity production. This waste consists primarily of plastics and plant debris. Soil erosion of the surrounding hillsides and deeply incised tributaries, where poor soil protection prevails, also contributes sediment to the reservoirs (Eisenberg and Muvundja, 2020). Water run-off from impermeable urban surface triggers significant landslides and gullies, which contribute to soil erosion and reservoir siltation.

Hydropeaking Effects

Despite environmental obligations of the dam operating companies, the fish ladders operate only occasionally. When facing technical problems, such as solid waste blockage, typical management practices consist of changing river flows. According to the HP management at Ruzizi II, downstream flow is emptied every Wednesday and Sunday for almost two hours for purposes of turbine maintenance and solid waste evacuation leading to hydropeaking in the riverine ecosystem (Liévin Cizungu, Pers. Comm.). **Figure 6** shows operating discharges at Ruzizi I ranging between 60 and 100 m^3/s for ~86% of the time (**Figure 6**). Extreme values occur only ~14% of the time. From 1989 to

2003, Ruzizi I operated within its expected range of electricity production (**Figure 5**) with enough water in the bypass (35 m^3/s, i.e., ~30% of the average river flow; **Figure 6**). According to **Figures 5**, **6**, all incoming water was used from 2004 to 2009 without any perceptible increase in power production (**Figures 5**, **6**). This reveals that technical problems including maintenance and ageing equipment have increased the environmental impact of these dams.

Flood frequency analysis of the Lake Kivu outflow shows that the natural Ruzizi discharge remained stable and did not experience any important interannual or seasonal perturbations. The highest flood flow over the last 5 decades occurred in 1998 at a peak of 143 m^3/s with a recurrence interval of 76 years (**Supplementary Table S1**). A similar peak flow (140 m^3/s) occurred once in 1963 simultaneous with the so-called "Centennial" rising of the Rivers Congo and Nile (Bergonzini et al., 2002; Muvundja et al., 2014) but associated with a recurrence interval of 38 years (**Supplementary Table S1**). In terms of power production, neither Ruzizi I (**Figure 5**) nor Ruzizi II ever reached their installed capacity (SHER and ARTELIA, 2017). At Ruzizi I, turbine discharges ranged between 43 and 112 m^3/s from 1989 to 2009 (**Figure 5**), a period during which lake water levels allowed full utilization capacity, except during the 2004-to-2006 dry spell (Muvundja et al., 2014). By contrast, electricity production trends during this period (**Figure 5**) indicate that power production fluctuations depend on lake outflow and dam operation. HP plants work only at ~60% of their capacity regardless of available flow. The 16 and 25 MW (**Table 1**) production thresholds for Ruzizi I and Ruzizi II can each be reached without emptying the bypasses and/or closing the fish ladders. This allows reserving enough E-Flow to bypass the dams. Our calculations yielded a flow of 42 m^3/s as representing 40% of MAF, a value widely accepted by E-Flow experts (Tennant, 1976; Acreman and Dunbar, 2004; Linnansaari et al., 2013; Pastor et al., 2014). This volume of water supports ecosystems by maintaining aquatic habitats for native biota (plankton, macroinvertebrates, macrophytes, fish, and mammals; Annear et al., 2004).

The ecosystem disturbance caused by dams depends on both the quantity of water and its flushing rates. Hydropeaking floods and scours the downstream riverbed for up to two hours twice per week, flushing out plankton, macroinvertebrates, fish, food sources, eggs, and larvae (Bruder et al., 2016; Tonolla et al., 2017). During these events, mobile or nektonic organisms can take refuge in isolated waters. At these extreme flow rates, fragile organisms washed downstream suffer low survival rates. Even accidental drowning events (especially affecting children) have occurred several times downstream of Ruzizi II. Hydropeaking often results in poor macroinvertebrate diversity in dammed rivers (Bruder et al., 2016). Habitat changes caused by upstream floods resulted in declines for 1) fish species in the Ruzizi River (e.g., haplochromines and cyprinids; Munini et al., 2011), 2) zooplankton such as *Alona* sp. and *Ascormopha ecaudis* (Kisekelwa and Isumbisho, 2009), and 3) macroinvertebrate species (Hyangya et al., 2011).

Management practices impact the distribution of biota downstream of dams (Mantel et al., 2010). Declines in lake fish stocks reported by fishermen result from disruption due to hydropeaking during critical developmental stages. Overfishing may have also exacerbated the situation. Fishing communities argue that some species have become extinct and no longer appear either upstream or downstream (Bahimba Janda Morgan, Mumosho, pers. comm.). Despite their adaption to unstable hydrology, even cyprinid fish (*Barbus* spp.) appear impacted. These fish can swim through rapids and temporarily migrate into small tributaries for spawning and refugia (Masilya et al., 2020). Following E-flow protocols and other practices can mitigate these impacts (Tennant, 1976; Pastor et al., 2014). Specifically, Leclerc and Quintero (2003) suggest the following for hydroelectric projects:

(1) Always maintain between 28 m^3/s (40% MAF) and 62 m^3/s (Q_{90}) of water flow in the bypasses and fish ladders to sustain permanent outlets and allow upward and downward fish migration at any time. Dam operators should always keep fish ladders operational to help migratory fishes move up and down the river bypass. The E-Flow will also help rehabilitate the cultural and religious site of Butagarura Falls near the Ruzizi II dam.
(2) Improve the management of water releases to reduce the impacts of hydropeaking on biota. Water can be released incrementally allowing motile organisms in and below the reservoir to survive such events (Ledec and Quintero, 2003).
(3) Fish hatcheries should be implemented to support populations of native fish species such as tilapia and African catfish in the reservoirs. Fishing regulation should be enforced to maintain viable populations of commercial fish species.
(4) The area needs a long-term and integrated waste management plan for towns and facilities within the catchment and adjacent to the reservoir.

Forthcoming dam construction projects should install adequate reservoir volumes for flow requirements. Project planning should specifically include a minimum flow of ~28 m^3/s (outside the turbines) to avoid unrealistic estimates of power production. A 10% of MAF E-Flow estimate suggested for Ruzizi III (ONEC-BAD 2015) will not suffice and should be revised prior to project implementation.

Surface Sediment Transport

Sedimentation reduces live storage and power generation over time and thus compromises the HP and environmental services provided by the dam (Ledec and Quintero, 2003). Greater turbidity and longer stagnation in the reservoir lead to higher sedimentation rates.

Ruzizi River turbidity varies from levels appropriate for natural waters (e.g., T < 25 NTU; domestic use, fisheries, and recreation) to high suspended particle concentrations of T > 25 NTU (MPCA, 2008). According to MPCA (2008), turbidity levels of 25 NTU in rivers and streams equal a suspended particle concentration of ~58 mg/L. This value can vary significantly for individual streams and rivers even within the same large-scale catchment (MPCA, 2008). For the Ruzizi River basin, 25 NTU was surprisingly consistent with the empirical value of 58 mg/L.

Population density (>400 inhabitants/km^2), topographic relief in the Kivu region, intense pluviosity, and soil degradation cause considerable erosion and frequent landslides in the Ruzizi catchment (Muvundja et al., 2009; Eisenberg and Muvundja, 2020). This contributes to high levels of turbidity in the Ruzizi River. A high correlation (r = 0.79; N = 12) between precipitation and turbidity (**Figure 6**) confirms this interpretation. Turbidity recorded during the dry season (~10 NTU) represents a baseline more or less corresponding to Lake Kivu seston, which matches surface water turbidity (10–20 NTU; Pasche et al., 2013).

Soil erosion represents a major environmental issue in this region given the impacts of reservoir siltation. A related study reported an erosion rate of ~577 kt/yr for the entire catchment (Eisenberg, 2018). A corresponding area-normalized value of ~5 $kt/km^2/yr$ falls within the range of an 8.4 $kt/km^2/yr$ estimate reported in Nambajimana et al. (2020) for soil losses in the same river catchment of the Rwandan District of Rusizi. The present study estimated surface sediment transport of ~1 $kt/km^2/yr$ (see above). This represents only a quarter of the loss estimated by Eisenberg (2018). The discrepancy may reflect the large quantity of soil particles deposited as sediment within the catchment along tributaries and within the reservoirs. Comparing the Ruzizi catchment with neighbouring subcatchment of Lake Kivu having similar soil types, land use, and geological and climatic conditions can help constrain the understanding of soil loss risk as it applies to the Ruzizi catchment. The area-specific TSS load transported by the Upper Ruzizi reaches values three to four times higher than those reported for Kawa, Mugaba, and Murhundu Rivers and 14 times higher than those reported for the whole Lake Kivu catchment average (Muvundja et al., 2009).

Reservoir siltation implies nutrient removal from waters downstream of dams. Data given in Muvundja et al. (2009) exhibited a high correlation ($TP = 0.5989*TSS - 38.893$, R^2 = 0.94; n = 6) between area-specific TSS load and the total phosphorus (TP) load for Lake Kivu tributaries close to Bukavu (excluding the polluted urban stream of Kahuha). This confirms that turbidity matches soil-based nutrient losses and nutrient enrichment in rivers. This confirms the interpretation that the dams act as barriers for both sediment and nutrients, and they facilitate longitudinal transport of dissolved nutrients compared to nutrients deposited by settling the particulate matter.

The Ruzizi II reservoir suffers from greater soil erosion, siltation, and flooding. The bottom waters of the reservoir were 62% more turbid than surface waters. This indicates that incoming particulate material and nutrients predominantly sink to bottom water areas but remain susceptible to transport. Based on their work on Lake Brienz (Switzerland), Finger et al. (2006) reported that HP dams drastically diminish particle fluxes and filter solid particulates according to size and density. Coarser particles settle in upstream areas of the reservoir, while fine-grained particles remain suspended and pass downstream. These researchers found that only ~3% of the fine-grained materials were deposited as sediment. We therefore assert that the suggested minimum flow would help maintain sediment transport downstream of dams even though some siltation would still occur.

Measures to mitigate reservoir sedimentation require effective catchment management. Management can include the implementation of conservation practices on agricultural land such as terracing and planning of road construction, mining projects, and other land use changes using sustainable best practices (Ledec and Quintero, 2003). A protected area established in the Ngomo mountainous region, which abuts the Ruzizi IV (Sisi V; **Figure 1**), would reduce sediment flows into reservoirs of planned future dams (SHER and ARTELIA, 2017).

Water Quality

Low DO concentrations likely arise from waste discharges along the course of the river, which stimulate increased microbial oxidation of organic matter (Patnaik, 2005). While the average measured DO falls within ‘good’ water quality standards (Riziki, 2016), water samples gave a mean COD of 623 mg/L (**Supplementary Table S4**). Values indicate voluminous quantities of oxidizable organic and inorganic pollutants (Otukune and Biukwu, 2005). The total alkalinity contents of water samples fell within TSI-266 (2005) and WHO (2008) limits.

Nutrients, such as bioavailable forms of phosphorus and nitrogen (ammonia, nitrate, and nitrite), can strongly impact lake water quality (Şener et al., 2013) due to their role in eutrophication (Soulsby et al., 2001). Orthophosphates can rapidly be absorbed by plants and generally exert a greater influence on eutrophication than nitrogen (Sharpley et al., 2001). The pH- and temperature-dependent, unionised forms of ammonia, however, pose high toxicity risk to fish even at low concentrations (Debels et al., 2005). Most of NH_4^+ concentration measurements fell within acceptable standard limits (0.5 mg/L, WHO, 2008; Rodier, 2009). The NO_2 contents of water samples collected upstream of Ruzizi I exceeded permissible TSI-266 (2005) and WHO (2008) limits. Most sample sites yielded phosphate values higher than eutrophication-limiting values (0.1 mg PO_4^{3-}/L; Bartram and Balance, 1996; Rodier, 2009; Şener et al., 2017). All silica values fell within permissible WHO (2008) limits. The highest silica concentration value occurred upstream of Ruzizi I likely reflecting the Lake Kivu origin of Ruzizi waters.

Calculated WQI values ranged between 600 and 800 (**Table 2**). The computed WQI average for all monitored stations was 271. This value exceeds WHO standard values (**Table 2**) and falls within the category of poor quality for drinking water (Sahu and Sikdar, 2008; Yidana and Yidana, 2010). The high WQI likely arises from municipal waste and organic loads generated by agricultural activity. While solid waste was not investigated by this study, solid waste accumulation is a significant problem. The Ruzizi I and II reservoirs respectively receive 1,200 and 1700 m^3/yr consisting of 60% domestic organic waste and 40% industrial solid waste (EGL, 2015). This material limits power generation at the two dams (SNEL and SINELAC, Pers. Comm.).

Sustainable Management of Ruzizi and Other African HP Dams

Sustainable management of HP resources requires a minimum flow to maintain (as best as possible) the “natural river course.” This limits sediment accumulation in reservoirs. Local government and dam managers need to implement agricultural practices that reduce land degradation and soil/nutrient loss. This will improve the river water quality and expand the ecosystem services provided by both the land and rivers. Soil conservation and habitat expansion can support riparian communities. Dam maintenance requiring flow cessation (repairs and cleaning) should use incremental changes in flow to avoid scouring of the channel or catastrophic downstream transport of aquatic organisms (juveniles and even adult fishes, macroinvertebrates, and zooplankton). According to Welcome et al. (2006), poor flow control can devastate species that produce semi-pelagic eggs. Consistent, natural flows disperse eggs and larvae to new habitats representing appropriate juvenile nursery grounds on floodplains or in backwaters.

The plastics and other solid pollutants in Lake Kivu and the Ruzizi River require a comprehensive and integrated waste management plan. Such an initiative could also support Lake Tanganyika, a body fed by the Ruzizi River and a hot spot of freshwater biodiversity. Dam management could fund waste management by allocating resources equivalent to what they lose in electricity production caused by waste evacuation towards recycling or waste capture/reduction programmes. Current initiatives of converting plastic waste to cobblestones and building blocks offer examples of creative and economically lucrative waste diversion. The river and reservoir projects face the dual problem of poverty and human encroachment along the riparian corridor and reservoir areas. Initiatives wherein HP companies work with local communities to reduce poverty, conduct restoration and protect buffer zones between the river and human settlements or cultivated land along the HP dam cascade could reduce reservoir siltation and pollution. Specialized agricultural methods such as terracing, afforestation, and pasture improvement for steeply sloping areas can reduce landslides, gully formation, and erosion. Each of these compromises the environmental integrity of tributaries and the Ruzizi dam operations. Finally, government agencies should enforce EIA policies and regulations regarding forthcoming HP dams.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**; further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

FM, AK, CA, JE, and AW actively contributed to the research elaboration and methodological approach design. Except AK, CA, and AW, all other authors contributed to fieldwork and laboratory work. All authors substantially contributed to analysis and interpretation, which led to consensus and agreement on publication.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2022.892591/full#supplementary-material

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Lake Victoria's bounty: A case for riparian countries' blue economic investment

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People residing in Lake Victoria's basin and riparian countries benefit from ecosystem services provided by the lake. However, the lake's resources, particularly fish, are under threat from pressures caused by humans such as overfishing, alien species invasion, rising eutrophication, and climate change. In this assessment, we look at how to maximize the lake's benefits through product diversification, value addition, and sustainable use of its fisheries. We show that minimizing post-harvest losses of the silver cyprinid (*Rastrineobola argentea*), locally known as *Dagaa*, will provide more high-quality fish for human consumption, while significantly increasing fishing earnings. Furthermore, by utilizing biowaste (frames, skin, and fish mouth), revenues from Nile perch could be increased up to thrice. Furthermore, if fishing could target maximum sustainable yield (MSY), landings of Nile perch and *Dagaa* could increase significantly in the long run. These, combined with investments in other areas such as lacustrine tourism and recreation, as well as fish cage culture, can help the Lake Victoria region's blue economy expand.

KEYWORDS

ecosystem services, blue growth, sustainable, value addition, fish maws

Introduction

The blue economy (BE) concept is widely being promoted as a means of gaining both concrete (economic development, improved livelihoods, and employment) and intangible (carbon storage, coastal protection, cultural values, and biodiversity) benefits through the sustainable use of ocean resources (Okafor-Yarwood et al., 2020; The World Bank, 2017). The African Union (AU) expands this to encompass "commercial exploitation of oceans, lakes, rivers, and other bodies of water and aquatic ecosystem conservation" (AU-IBAR, 2019). The key components of the AU BE strategy for promoting blue economy development on the continent are fisheries, aquaculture, and ecosystem conservation; shipping, maritime safety, and trade; climate change, environmental sustainability, and ecotourism; sustainable energy and extractive mineral resources; and governance, institutions, gender, and job creation (AU-IBAR, 2019).

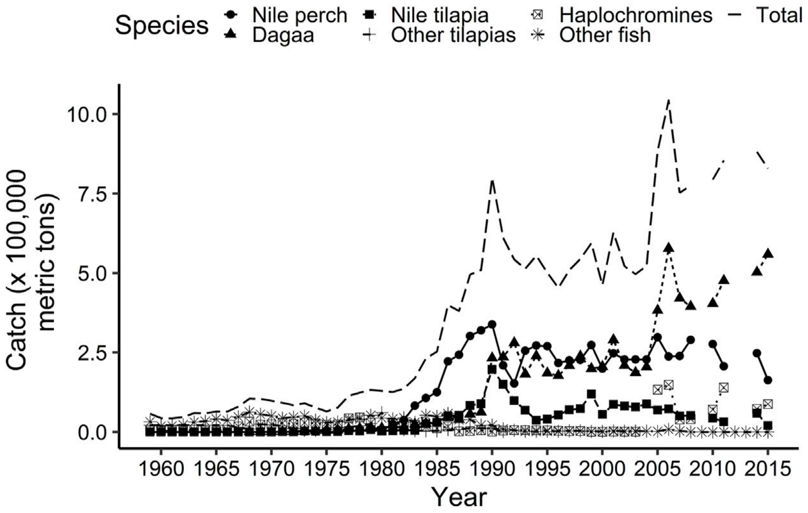

FIGURE 1
Annual fish landing trend in Lake Victoria. Source LVFO (2016).

Under the East African Development Strategy, the Lake Victoria basin has been identified as an area of common economic interest and an economic development zone to be developed collectively by the partner states. Lake Victoria is the largest freshwater lake in the tropics and second in the world. The lake provides ecosystem services such as water for domestic and industrial use, transport, hydropower generation, and food to over 40 million people (Swallow et al., 2009; Downing et al., 2014; Nyamweya et al., 2016; Awange, 2021). Because of its large size (surface area of approximately 680,000 km^2), the lake also plays an essential role in modulating regional climate (Stager and Johnson, 2008; Nyamweya et al., 2016). By far, the most important economic activity for lakeside inhabitants is fishing (UNEP, 2006). In 2021, around 1.48 million tonnes of fish were landed from the lake, with a beach value of USD 1.14 million (LVFO, 2022). The fishery employs over three million people in fisheries-related activities (Awange, 2021). Despite an abundance of resources, residents in the Lake Victoria region are among the poorest in the world, with the majority surviving on less than USD 1.25 a day, as a result of inefficient and unsustainable resource use. Such unsustainable practices deteriorate ecosystem functioning and precipitate the collapse of resource elements (Mackinson, 2010).

Over the last century, Lake Victoria has undergone many changes, stemming from human activities such as overfishing (Graham, 1929; Nyamweya et al., 2020), the introduction of Nile perch (Ogutu-Ohwayo, 1990; Pringle, 2005; Marshall, 2018), population growth, pollution (Juma et al., 2014), and climate change (Awange et al., 2013). For the last two decades, the three riparian countries have harvested 1 million tonnes of fish annually despite the increasing human population and fishing pressure. The national population growth rates in Kenya, Uganda, and Tanzania are 2.2, 3.6, and 3.0%, respectively (Population Reference Bureau, 2021), while the Lake Victoria region is 3.8% (Bremner, 2013). Projections show that the population in the three riparian countries will at least double by 2050. Herein, we examine exploitation trends (emphasizing fisheries) and point out possibilities of improvement and diversification of benefits derived from the lake to drive blue economic development.

Fishery evolution in Lake Victoria

Lake Victoria fishery has transformed over time from the one dominated by indigenous fish species to the one dominated by the introduced Nile perch (*Lates niloticus*) and Nile tilapia (*Oreochromis niloticus*), and the native silver cyprinid (*Rastrineobola argentea*) locally known as *Dagaa* (Figure 1). According to Opondo (2016), during the precolonial era, the main fish species caught by the local communities were *Oreochromis esculentus*, *Labeobarbus victorianus*, *Synodontis* spp., *Mormyrus kanume*, and *Clarias gariepinus*. Fishing at that time was managed by clan-based rules, for fishing had to be observed by all fishers. Compliance to the controls included target species, sizes, fishing gear, fishing areas, and season-guaranteed future supply of fish (Opondo, 2016).

The advent of new fishing gear and an influx of immigrants resulted in fish harvest rates that threatened the sustainability of the fishery, with evident signs of overfishing by the 1920s (Graham, 1929). The decline in the catch of native tilapias (*O. esculentus* and *O. variabilis*) necessitated dialogue and the eventual introduction of new species (*L. niloticus* and *O. niloticus*) in the 1950s and 1960s to boost the fishery that was yielding ca 100,000 tonnes annually. The consequences of the

TABLE 1 Proportion and average regional processors' purchase prices for Nile perch products from Lake Victoria.

Item	Proportion by weight (%)	Cost per kg (USD)	Total cost (USD)
Frame	42	0.61	11.7
Skin	8	0.26	3.2
Gut	5.7	0.6	1.71
Swim bladder	2.3	187	172.5
Fillet	42	4	84

species introduction are well-documented (Marshall, 2018), but notably, total fish landings increased significantly, steadying at over 1 million tonnes (LVFO, 2018) amid increasing fishing capacity. The initial boom of catches of the introduced species attracted investment mainly in industries that support the fishery value chain. The catches for the lucrative Nile perch peaked in the 1990s and started declining from 2005 (Nyamweya et al., 2020), as attested by the closure of some fish processing factories throughout the region (Njiru et al., 2008; Kayanda et al., 2009; Nunan, 2010).

Diversification of fish and fishery products

An analysis of landings and fishing effort trends reveals that Nile perch in Lake Victoria are currently overfished (Nyamweya et al., 2020), with no chance of increasing landings unless harvesting pressure is reduced in the short-term to allow stock recovery and achieve maximum sustainable yield (MSY) in the long term (Nyamweya et al., 2017). That notwithstanding, more value can be derived from Nile perch by diversifying the products. Fillet accounts for 37–40% of the total Nile perch and the remaining 60–63% are byproducts. Frames or skeletons with adhering flesh account for 40–43% of the byproducts' weight, followed by skin (8%), gut (5.7%), and swim bladder or fish maw (2.3%) (Kabahenda and Hüsken, 2009). Table 1 shows a theoretical breakdown of monetary value that a 50 kg Nile perch can attract. The potential earnings from Nile perch byproducts could be two times more than those from the fillet (Bagumire et al., 2018). With appropriate technology and investment, these "wastes" can be transformed into premium products that attract high-end consumers. For instance, when handled properly, fish skin can produce high-quality leather (Muyonga et al., 2004; Muralidharan et al., 2013). Various studies have shown that viable quantities of oil can be extracted and refined from Nile perch viscera (Okoth et al., 2015), belly flaps (Ogwok et al., 2009), and heads (Turon et al., 2005). The resulting oils are rich in Omega 3 fatty acids, which are in great demand globally. (Okoth et al., 2015).

Nile perch swim bladder (maw) is among the precious aquatic products (Shelley, 2004). There are about 500 million consumers of fish bladder in China and Hong Kong (LVFO, 2015). Fishers can be paid ten times more for the bladder than the price they can achieve for fish flesh (Brierley, 2018). Currently fish maws on an average attract a retail value of USD 127 to 287 per kilogram. Although this trade has been going on in Lake Victoria since the 1990s, it is restricted to a few traders. If this value chain is formalized and optimized, it could give the lake edge communities an additional income.

Optimization of fish harvesting and post-harvest management

The catch per unit effort (CPUE) for Nile perch in Lake Victoria has progressively dropped since effort has increased. Indeed, the species' lowest annual landings in the previous two decades were 165,083.4 tonnes in 2015, significantly below the predicted maximum sustainable yield (MSY) of roughly 212,000–323,000 tonnes (Kayanda et al., 2010). A gradual increase in fishing effort in a fishery will increase the fish output until a maximum is achieved (MSY level). If fishing effort is increased further, the overall fish output will more or less plateau around the MSY level for a time before beginning to fall, as shown with Nile perch in Lake Victoria. According to Kayanda et al. (2017), the Nile perch is overfished. Fortunately, in 2017, authorities in Uganda and Tanzania, where the bulk of Lake Victoria is situated, initiated a strict enforcement to eradicate illicit fishing gear (LVFO, 2021), offering a chance to examine the effect of such efforts on fish populations. In 2021, the species' catches were much higher (221,640 tonnes) and within the MSY level. The increase in landings might have been a response to the decrease in effort as a result of the management initiatives; thus, implying that production and income can be improved if the fishery is sustainably managed.

On the other hand, the catch of *Dagaa,* which accounts for most of the landings in the lake, is lower than the MSY. Natugonza et al. (2016) indicate that *Dagaa* and haplochromines are underexploited, having exploitation rates of less than 15%. Nonetheless, haplochromines are considered as low-value fish. Their true worth is found in their ecological function. Indeed, simulations using the Atlantis ecosystem model have revealed that minimizing haplochromine harvesting results in optimum ecosystem function, improved yield of economically significant species, and probably the least "socioeconomic" cost implications. Given these findings, there is an opportunity to increase landings from Lake Victoria that currently produces about 8% of the total inland capture landings globally (Nyamweya et al., 2017).

Dagaa catches have been on an upward trajectory since the early 1980's. This species has a high turnover rate and can be resilient to heavy predation and exploitation (Irvine et al., 2019; Kolding et al., 2019). Currently, *Dagaa* accounts for ca 60% of the total catch, but substantial quantity and quality is lost due to poor post-harvest handling (SmartFish, 2011; Odoli et al., 2019). Lake Victorian *Dagaa* is traditionally dried in the Sun on the ground, grass, matting, or old fishing nets. The procedure provides no protection for the product against rain, animals, insects, and soil contamination. As a consequence, *Dagaa* fishermen face significant post-harvest losses (Ibengwe and Kristofersson, 2012). Post-harvest loss types for the species are mainly rotting or spoilage (79.3%), followed by *Dagaa* being swept back to the lake by rain (10.3%) and loss of color (3.4%) (Odongkara et al., 2016). The reduction of post-harvest losses for the species will provide more quality fish for human consumption and contribute to food and nutrition security for the riparian communities. If the deterioration of *Dagaa* is halted right from capture, earnings from the fishery could increase appreciably.

Other areas of potential investment

In the last 10 years, landings of the main commercial species in Lake Victoria have either declined or stagnated. The stagnation of production from the captured fisheries presents an opportunity for entrepreneurs to bridge the large gap between the amount of fish consumed (≤6 kg person^{-1} year $^{-1}$) and that recommended by the World Health Organization (17 kg person^{-1} year^{-1}) (Kirema-Mukasa, 2011). The growing number of fish in cages in Lake Victoria could be a response to fill the deficit in fish production (Hamilton et al., 2019). Sustainable cage-fish farming offers an opportunity for investment in the venture's value chain. Presently, cage-building materials are sourced from Far East and Europe, an undertaking that is quite costly. Potential entrepreneurs can take on producing these materials locally and cheaply. Other potential investment areas in the cage culture value chain include fish seed and feed production, cold storage, value addition, consultancy, and marketing (Njiru et al., 2019).

The diversity of haplochromine cichlids in Lake Victoria is among the greatest found elsewhere in the world (Goldschmidt and Witte, 1992). Because of the vivid colors they display, haplochromine cichlids are considered to have a high level of aesthetic value (Theis et al., 2012; Maan and Sefc, 2013; Sefc et al., 2014). Despite this, people in East Africa often see them as being of little economic worth or as trash fish. There is a window of opportunity to convert the haplochromines, which are currently on the path to recovery (Figure 1), into a cash crop by capitalizing on the more profitable ornamental fishery (Andrews, 1990; Chapman et al., 1997; Wabnitz et al., 2003).

In many parts of the world, lakes play a vital part of recreation and tourism as both are locations for leisure activities, as well as an attraction in their own right (Hall and Härkönen, 2006). Certainly, the lacustrine tourism has potential in Lake Victoria, which has a shoreline of more than 7,000 km, offering a huge waterfront (Hamilton, 2016), and a desirable attribute in the tourism and hospitality industry (McCarthy, 2004). The lake offers unique recreation opportunities like sport fishing for the Nile perch, windsurfing in the expansive open waters, boat racing, and getaway holidays in many of its exotic islands (Awange and Ong'ang'a, 2006).

Conclusion

For a long time, Lake Victoria has been known for its vibrant fishery. However, considering the population growth rate, it is unlikely that fishing activities will satisfy the nutritional, income, and employment needs for the region. Fortunately, amid the looming crisis occasioned by declining wild stocks of some commercial species lie tangible solutions to propel the region to economic prosperity. Possible solutions include but are not limited to optimizing fish harvesting, eliminating or reducing post-harvest losses along the fishery value chain, and biowaste utilization. It will also entail diversification of investments to include the development of fish maw trade, ornamental fishery, tourism and recreation, and fish cage culture.

Data availability statement

The original contributions presented in the study are in the article. Further inquiries can be directed to the corresponding author.

Author contributions

CN conceptualized, analyzed the data, and wrote the manuscript; HN analyzed the data and wrote sections of the manuscript; CA interpreted the data and participated in writing; KM conceptualized and participated in writing; EM provided the data and participated in the analysis; CO interpreted the data and participated in writing; and JN interpreted data and participated in writing.

Conflict of interest

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Niche expansion by indigenous fish species following the introduction of rainbow trout (*Oncorhynchus mykiss*) in a subtropical river system, upper Blyde River, South Africa

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The introduction of alien predatory fish such as rainbow trout (*Oncorhynchus mykiss*) can have a significant effect on indigenous fish communities such as altering the structure and dynamics of food webs. Quantifying the trophic niche utilised by the alien fish species is therefore important to aid our understanding of how their feeding strategies might aid establishment, define their functional role and inform on potential impacts. This study assessed food web interactions between fish communities in river reaches that are invaded by *O. mykiss* and sections that are free of invasions in the upper Blyde River catchment, South Africa. It specifically evaluated the hypothesis that *O. mykiss* invasion is likely to lead to a decrease in the trophic functional diversity through predation and that indigenous fish will shift their trophic niche to either minimise competition with, or avoid predation, by *O. mykiss.* Gut content and stable isotope analyses were used to determine trophic interactions. Fish communities in uninvaded areas utilised fewer and similar food sources and occupied lower trophic levels, than fish communities in invaded areas. *Oncorhynchus mykiss* fed mainly on invertebrates and at trophic levels similar to and or lower than indigenous fishes. This suggests that their current impact on indigenous fish communities is mainly through competition for resources. We posit that *O. mykiss* invasions reduced the abundance of indigenous fishes and thereby reduced predation pressure on aquatic invertebrates, with a knock-on effect on the trophic interrelationships among fish assemblages. Our findings are consistent with other studies in South Africa that have shown that the impacts of *O. mykiss* invasion can lead to a decline and fragmentation of indigenous species populations and niche shifts.

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Introduction

Predatory freshwater fish species have been introduced worldwide to enhance angling and inland fisheries (Cambray, 2003; Arlinghaus and Cooke, 2009). Rainbow trout (*Oncorhynchus mykiss*) is one of the most widely distributed fish species for angling (Crawford and Muir, 2008). It is native to the western seaboard of the United States, Canada, and northern Mexico (Page and Burr, 2011). In its introduced range, *O. mykiss* has been implicated in causing major impacts on indigenous fauna and ecosystems mainly through competition and predation (Drenner and Hambright, 2002; Simon and Townsend, 2003; McDowall, 2006; Fausch, 2007), modifying aquatic-terrestrial linkages (e.g., Epanchin et al., 2010; Jackson et al., 2016), altering food-web structure (Flecker and Townsend, 1994), and disrupting nutrient cycles (Schindler et al., 2001; Eby et al., 2006).

Oncorhynchus mykiss was introduced into South Africa in 1897, mainly for angling and aquaculture (Cambray, 2003; Ellender et al., 2014). Currently, *O. mykiss* has established populations in the mountain streams and upland reservoirs in over 80% of the major drainage basins in the country (Weyl et al., 2020). There is, however, limited information on the impacts of *O. mykiss* invasions in South Africa (Zengeya et al., 2020; Van Wilgen et al., 2022). The few available studies have, however, shown that *O. mykiss* can cause a decline, and in some cases local extirpation of indigenous invertebrates, frogs, and fishes (Karssing et al., 2012; Rivers-Moore et al., 2013; Shelton et al., 2015a; Jackson et al., 2016; Weyl et al., 2020). For example, *O. mykiss* invasions in the uKhahlamba Drakensberg Park in Kwa-Zulu Natal have been implicated in the reduction of macroinvertebrate communities and the abundance of amphibians probably likely through predation (Karssing et al., 2012; Rivers-Moore et al., 2013). Similarly, *O. mykiss* invasions have also been implicated in the decline in the abundance of populations of amphibians in the upper Krom River in the Western Cape (Avidon et al., 2018). *Oncorhynchus mykiss* invasions have also led to a decline in the abundance of river minnows such as the Breede River redfin (*Pseudobarbus burchelli*), the Cape kurper (*Sandelia capensis*), and the Cape galaxies (*Galaxias zebratus*) in the Breede River system, Western Cape (Shelton et al., 2015a). Also, in the Breede River, *O. mykiss* invasions were shown to induce a trophic cascade by releasing some herbivorous invertebrates from predation, leading to an increase in grazing pressure and lower algal biomass at invaded sites (Shelton et al., 2015b). *Oncorhynchus mykiss* invasions in several river systems such as the Keiskamma River (Eastern Cape), the Thukela and the Umgeni River systems (KwaZulu Natal) have been shown to modify aquatic-terrestrial food-web linkages by competing for and reducing the trophic subsidies available for riparian consumers such as spiders (Jackson et al., 2016).

In this study, we examined the trophic structure of fish communities in the upper Blyde River catchment in Mpumalanga Province where *O. mykiss* was introduced into several headwater streams of the catchment in the early 1900s for sport fishing (Engelbrecht and Roux, 1998). The subsequent establishment of naturalised populations of *O. mykiss* poses a risk to endangered indigenous fish species through predation and competition for habitat and food resources as a result of resource overlaps with *O. mykiss*. For example, the Treur River barb (*Enteromius treurensis*) is endemic to the upper reaches of the Blyde River (Kleynhans, 1987). It was extirpated from several sections of the Blyde River system invaded by *O. mykiss*, brown trout (*Salmo trutta*), and smallmouth bass (*Micropterus dolomieu*) (Gaigher, 1969; Kleynhans, 1987; Engelbrecht and Roux, 1998). The remnant populations of the Treur River barb are fragmented but largely intact, restricted to the upper river sections of the catchment because a series of waterfalls have prevented upstream migrations of invasive predatory fishes from lower river sections where introductions occurred. A recent study in the upper Blyde River catchment demonstrated that *O. mykiss* invasions have caused a decline in the abundance of populations of indigenous fish species such as *E. treurensis* (Maimela et al., 2021). However, the mechanism(s) through which these impacts have manifested remains unknown. Trophic niche analysis has been used to assess the ecological opportunities available to alien species in terms of food resources and how factors that affect access to, and utilisation of, food resources facilitate the successful establishment and adverse impacts in a recipient ecosystem (e.g., Tonella et al., 2018). This study, therefore, investigated the trophic interrelationship between *O. mykiss* and the indigenous fish species in the upper Blyde River catchment, by assessing whether *O. mykiss* invasion altered community structure and function, such as trophic functional diversity and trophic niche size of indigenous fish species. It is likely, as demonstrated elsewhere, that *O. mykiss* invasions could lead to niche shifts and a decline in indigenous fish populations through either predation or competitive exclusion from food resources (e.g., McDowall, 2006; Kadye et al., 2013; Shelton et al., 2015a; Weyl et al., 2020). This study, therefore, tested the hypotheses that the *O. mykiss* invasion in the upper Blyde River catchment has decreased the trophic functional diversity of indigenous fish species through predation and that indigenous species have shifted their niche width (i.e., the variety of food items utilised by a species) and position (i.e., type of food resources utilised) to minimise competition with, or avoid predation, by *O. mykiss*.

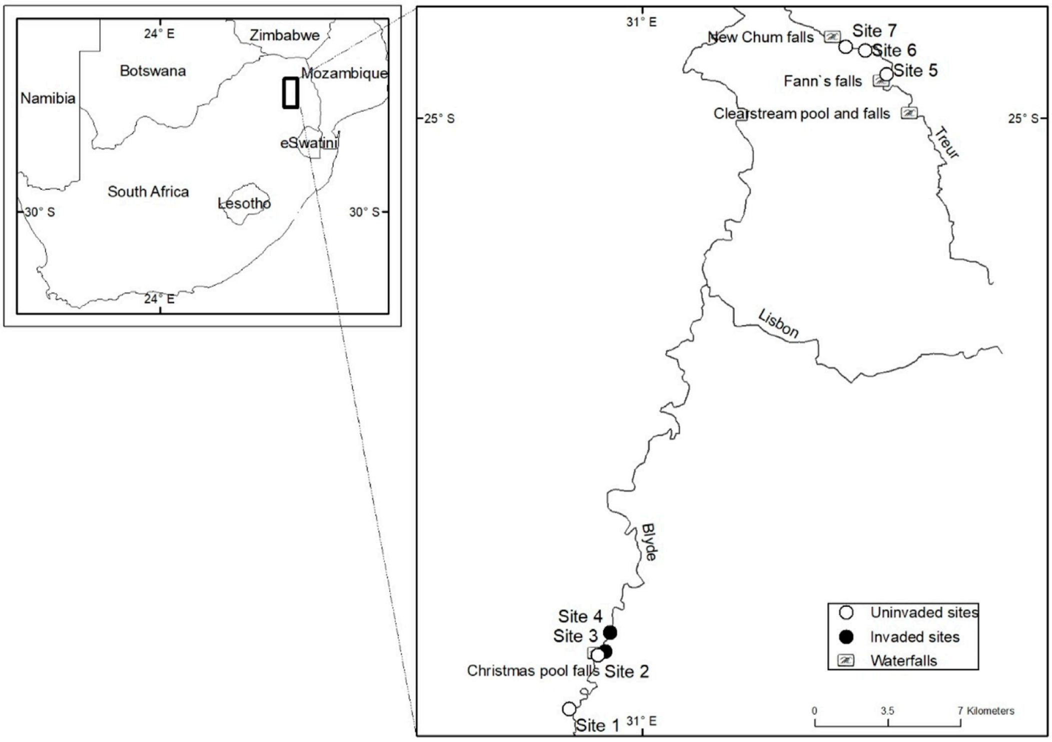

FIGURE 1
A map showing the location of sites sampled. Sites invaded by rainbow trout (*Oncorhynchus mykiss*) (•) and sites free of invasion (o) in the Blyde and Treur rivers in Mpumalanga Province, South Africa.

TABLE 1 Sampling sites on the Blyde and Treur rivers in Mpumalanga Province, South Africa and their associated rainbow trout (*Oncorhynchus mykiss*) invasion status, geographic coordinates, and elevation.

River	Site	Location	Invasion status	Geographic coordinates	Elevation (m)
Blyde River	1	5 km above the first Christmas Pools waterfall	Uninvaded	25°00'14.2"S 30°43'06.3"E	1,432
	2	Christmas Pools below the first waterfall	Uninvaded	24°58'51.0"S 30°43'50.4"E	1,372
	3	Christmas Pools below the second waterfall	Invaded	24°58'45.6"S 30°44'01.9"E	1,362
	4	3 km downstream of the second waterfall	Invaded	24°58'15.9"S 30°44'09.5"E	1,341
Treur River	5	Pools below Treur River waterfall, 2.5 km upstream of Potluck Boskombuis	Uninvaded	24°43'52.2"S 30°51'18.5"E	1,293
	6	Pools next to Potluck Boskombuis restaurant	Uninvaded	24°43'15.0"S 30°50'45.0"E	1,272
	7	Pools downstream of the bridge to Potluck Boskombuis restaurant	Uninvaded	24°43'09.4"S 30°50'14.5"E	1,246

Materials and methods

Study area and sampling

The study area included the headwaters of the Blyde and Treur rivers in the northern part of the Great Drakensburg escarpment, Mpumalanga Province, South Africa (Figure 1). The study sites were located on the upper slopes of the escarpment (1,200–1,500 m above sea level (a.s.l.)) that are characterised by grassland vegetation that is interspaced by Afromontane forests. The climate is cool to moderate (10–18°C), moderate to high annual rainfall (800–1,200 mm), and mean annual precipitation of more than 1,000 mm (WRC, 2001).

Fish were sampled between September 2017 and October 2018 from 7 sampling sites (Figure 1; Table 1) and each site was sampled once every three months on five different occasions. To assess the impact of *O. mykiss* invasion on trophic niche utilised by indigenous fishes, sites were grouped based on their invasion status. A site was categorised as invaded if *O. mykiss* was present at the site or at upstream sites where downstream dispersal is possible. Four sampling sites were selected from the upper Blyde River, two in uninvaded sites (sites 1 and 2) and two in invaded river sections (sites 2–4) (Table 1). Three additional sites (sites 5–7) were located in the upper sections of the Treur River that are free of alien fish. All the sites were sampled using a SAMUS725MP electrofisher (Samus Special Electronics, Warsaw, Poland), while fyke nets were used at all sites on the Blyde River but not in the Treur River because of low water levels. A river stretch of ~50 m was blocked off to prevent fish escapes, and electrofishing was conducted for ~30 min, with the electrofisher being operated in an upstream direction with a 1 m deep x 2 m wide, 5 mm mesh mobile block net behind to catch stunned fish missed by the operator (Kimberg et al., 2014). Fyke nets were set in the evening and retrieved the following morning. Fish were sorted according to species, weighed (g), and total length (TL, mm) recorded, and then preserved in ethanol for further analysis.

Determination of diet and trophic interactions

Gut content and stable isotope analyses were used to determine diet and trophic interactions between *O. mykiss* and indigenous fish species. The simultaneous use of gut content analysis and stable isotope analysis (SIA) allows for a better estimate of the overall fish diet and better insights into the trophic levels of fish communities that reflect different spatial and temporal scales (see Hyslop, 1980; Fry, 2006; Finlay and Kendall, 2007; Marshall et al., 2007; Zengeya et al., 2011; Kadye and Booth, 2012). Gut content analysis was used to identify possible food sources, and this was achieved by sampling fish from both invaded and uninvaded sites to obtain an overall diet spectrum for each sampled species, SIA was then used to differentiate food niches among fish species concerning trophic space, width, and the degree of overlap.

Fish gut contents were examined using a microscope and identified to the lowest possible taxonomic level. The diet of each fish species was determined using the frequency of occurrence (% F) (Hyslop, 1980). The approach provides estimates of the number of stomachs containing a specific prey item as a percentage of all analysed stomachs. It has been shown to provide a more robust and interpretable measure of diet composition than other quantitative approaches (Baker et al., 2014; Buckland et al., 2017; Amundsen and Sánchez-Hernández, 2019). The observed food items included algae, plant material and insects (Supplementary Table S1). The food items identified in the gut content analysis were then collected from the environment to delineate the food web structure in the river system. Algae and plant material were collected by hand, while aquatic invertebrates were collected using kick-net sampling (mesh size = 1 mm) on all available substrates at each site following the SASS protocol (Dickens and Graham, 2002). The aquatic invertebrates were identified up to family level using a photographic identification manual developed for South African river systems (Gerber and Gabriel, 2002). The differences in community structure of fish and invertebrates among the sites were tested using multivariate PERMANOVA based on a Bray–Curtis similarity matrix of fourth root transformed abundance data with 9,999 permutations. The PERMANOVA was based on algorithms in PAST v3 (Hammer et al., 2001).

Stable isotope analysis

Muscle tissue was excised from all fish samples while insects were analysed whole following similar studies done in the region (e.g., Lübcker et al., 2016; Taylor et al., 2017; Lombard et al., 2018; Bokhutlo et al., 2021). Insects were combined so that they included representatives from the following seven orders: Coleoptera, Diptera, Ephemeroptera, Hemiptera, Lepidoptera, Odonata and Trichoptera. Algae, plant material, insect samples and muscle tissue were oven-dried at 70°C for 24 h. SIA was undertaken on a Flash EA 1112 Series coupled to a Delta V Plus stable light isotope ratio mass spectrometer via a ConFlo IV system (all equipment supplied by Thermo Fischer, Bremen, Germany), housed at the Stable Isotope Laboratory, Mammal Research Institute (MRI), University of Pretoria, Pretoria, South Africa. A laboratory running standard (Merck Gel: $\delta^{13}C$ = - 20.57%; $\delta^{15}N$ = 6.8%; C% = 43.83; N% = 14.64) and blank sample were run after every 12 samples. The standards were Vienna Pee Dee Belemnite limestone for $\delta^{13}C$ (Craig, 1957) and atmospheric nitrogen for $\delta^{15}N$ (Ehleringer and Rundell, 1989). Results were expressed in delta notation using a parts per thousand scale following the standard equation by Craig (1961) (Eq. 1):

$$\delta X(‰) = \left[\left(R_{sample} - R_{standard}\right) \middle/ R_{standard} \right] \times 1000 \qquad (1)$$

Where X = ^{15}N or ^{13}C and R = $^{15}N/^{14}N$ or $^{13}C/^{12}C$, respectively. Analytical precision was <0.05‰ for $\delta^{13}C$ and <0.09‰ for $\delta^{15}N$.

Isotope baseline corrections

Stable isotope values for basal resources can vary considerably along spatial and temporal gradients, and to correct for these differences, nitrogen values ($\delta^{15}N$) were converted to the trophic position of each consumer (TP), and carbon values ($\delta^{13}C$) were corrected to the carbon of each consumer (Ccorr) following Olsson et al. (2009); (Eqs 2, 3)

$$\mathrm{TP} = \left(\frac{\delta^{15}\mathrm{N}_{consumer} - \delta^{15}\mathrm{N}_{\mathrm{baseline}}}{3.4} \right) + 2 \qquad (2)$$

where δ^{15}Nconsumer = the isotopic ratio of each consumer, and δ^{15}Nbaseline = the isotopic ratio and trophic position of a primary consumer. The trophic enrichment factor was set 3.4 following the meta-analysis of Post (2002).

$$\delta^{13}\mathrm{C}_{corr} = \left(\frac{\delta^{13}C_{consumer} - \delta^{13}\mathrm{C}_{\mathrm{meaninv}}}{CR_{inv}} \right) \qquad (3)$$

where δ^{13}Cconsumer = the isotopic ratio of each consumer, δ^{13}Cmeaninv = the mean invertebrate isotope ratio, and CRinv = the invertebrate carbon range (δ^{13}Cmax - δ^{13}Cmin).

Ideally, the δ^{15}Nbaseline is estimated using the isotopic signature of a long-lived filter-feeding primary consumer (e.g., bivalves; Vander Zanden and Rasmussen, 1999; Post, 2002), but the absence of long-lived filter-feeding primary consumers at the study sites necessitated the use of mean nitrogen values of aquatic insects as recommended by Jackson and Britton (2014). These include several families of dragonflies (Odonata), damselflies (Odonata), mayflies (Ephemeroptera), crane flies (Diptera), giant water bug (Hemiptera), and whirligig beetles (Coleoptera). The insect families that occurred at all sampling sites were dragonflies, mayflies, crane flies, and whirligig beetles. The isotopic signatures of mayfly insects showed no significant differences for δ^{15}N (Welch: F = 1.12, df = 12.92, $p > 0.05$) and δ^{13}C values (Welch: F = 0.02, df = 13.62, $p > 0.05$) between sampling sites, and were used to determine nitrogen baseline (δ^{15}Nbaseline), mean carbon invertebrate ratio (δ^{13}Cmeaninv), and the invertebrate carbon range (CRinv). The isotopic baseline correction for nitrogen (δ^{15}N) and carbon values (δ^{13}C) to trophic position (TP) and carbon corrected values (Ccorr) allowed for comparisons of trophic interactions among fish communities across a stream where sampling sites were grouped into qualitative categories based on their invasion status (i.e., free of, or invaded by *O. mykiss*) and location (Figure 1).

Stable isotope mixing models to quantify consumer diets

A Bayesian mixing model (simmr, Parnell et al., 2010; R Core Team 2021) was used to estimate the relative proportion of basal food resources that were assimilated by *O. mykiss* and indigenous fish species. The mixing model was calibrated using food sources that were identified from the gut content analysis. Food items were grouped into broad taxonomic categories: algae, plant material, and insects were grouped into orders (Coleoptera, Diptera, Ephemeroptera, Hemiptera, and Odonata). Models were constructed using uncorrected stable isotope data. The trophic enrichment factors were set at 3.37 ± 1.30% for nitrogen and 0.54 ± 0.53% for carbon following Taylor et al. (2017) and Bokhutlo et al. (2021). Model performance and fit were checked using the convergence diagnostic tool and by visually checking the posterior probability distributions.

Trophic niche position and dispersion

Shifts in trophic niche position for each fish species were determined using measures of central tendency (i.e., the difference in centroid position between species) following the approach by Turner et al. (2010). Several metrics, trophic position range (TP range), corrected carbon (Ccorr range), mean distance to the centroid (CD) and mean nearest neighbour distance (NND) were used to calculate niche dispersion (see Layman et al., 2007). TP range provides an estimate of the trophic length of the community, the Ccorr range provides an estimate of the diversity of food resources, the CD represents the mean of Euclidean distance of each species to the sample bi-plot centroid, where the mean of TP values and Ccorr of all species in the food web represent the sample centroid. NND represents the mean of Euclidean distances to each species' nearest neighbour in a TP and Ccorr - bi-plot. A lower dispersion in the two dispersion metrics of trophic diversity of a system indicates trophic redundancy. The significance of the test statistics for the niche position and dispersion metrics were then evaluated using null distributions generated from nested linear models and a residual permutation procedure (Turner et al., 2010). Post-hoc analysis of significant differences was done using one-way analysis of variance (ANOVA) posthoc test.

A residual permutation procedure was used to assess if changes in the trophic niche position of a species were due to the invasion status of the sampling site (see Turner et al., 2010). This was done by comparing the magnitude (path length) and direction of change (path direction) in centroid positions for each species sampled in the invaded and uninvaded sites. Path length was taken as the Euclidean distance between a pair of centroid positions sampled from different sites, and path direction was defined as the angle (θ) between the first principal component (PC1) vectors (see Collyer and Adams, 2007; Adams and Collyer, 2009).

Niche size and overlap

The niche space utilised by the fish species was delineated using sample size-corrected standard ellipse area ($\mathrm{SEA_c}$) that were derived using the R package *Stable Isotope Ellipses* (SIBER) (Jackson et al., 2011). The SEAc were constructed using variance and covariance of the isotope biplot that contained only 40% of the data, which represents the core isotopic and typical resources utilised by a species. A Markov Chain Monte Carlo (MCMC) simulation with 10^4 iterations that provides 95% confidence limits of the isotopic niche size for each species sampled was used for a Bayesian estimate of $\mathrm{SEA_c}$ and its area (Jackson et al.,

TABLE 2 The composition and abundance (by numbers) of invertebrate and fish taxa sampled from sites invaded by rainbow trout (*Oncorhynchus mykiss*) on the Blyde River and sites free of invasion both the Blyde River and Treur rivers. The numbers of fish outside parentheses indicate the total number of individuals collected using electrofishing and those in parentheses using fyke nets.

	Taxa	Blyde river (uninvaded)		Blyde (invaded)		Treur (uninvaded)		
		Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7
Aquatic invertebrates	Coleoptera					1		
	Diptera		3		13		6	
	Ephemeroptera	3		3		5	1	
	Hemiptera	2			1	2	2	1
	Odonata	16	19	13	23	7	27	15
	Opisthopora						5	
	Trichoptera						4	
Fishes	*Amphilius uranoscopus*		1 (1)		3 (1)	15	9	
	Enteromius anoplus					15	4	
	Enteromius lineomaculatus					13	4	3
	Enteromius treurensis	50 (22)	40 (47)	1 (1)	4	3	6	14
	Oncorhynchus mykiss			10 (5)	2 (13)			
	Tilapia sparrmanii					4	17	14

2011). A maximum likelihood-fitted SEA_c was used to quantify the magnitude of niche size overlap between species and was expressed as the proportional area that overlapped between a pair of SEA_c divided by the total area of both ellipses (Jackson et al., 2011). Extent of overlap was classified as low (<40%), intermediate (40–60%) and high (>60%) (Langton, 1982).

Results

Species composition and abundance

There were no significant differences in composition (PERMANOVA: $p > 0.05$) and abundance (PERMANOVA: $p > 0.05$) among the invertebrate assemblages based on the invasion status of the site (Table 2). The most abundant taxa across all sampling sites were Odonata (64% of the total) and Diptera (11%) which collectively made up 76% of the total number of invertebrates collected (Table 2). There were however, significant differences in species composition, (PERMANOVA: $p < 0.05$) and abundance (PERMANOVA: $p < 0.05$) among fish assemblages based on invasion status and site location (Blyde or Treur River) (Table 2). In the Blyde River, sites (1 and 2) were uninvaded by *O. mykiss* and only two species were collected from these sites, *E. treurensis* and the stargazer mountain catfish (*Amphilius uranoscopus*), of which *E. treurensis* was highly abundant. In contrast, *O. mykiss* was abundant at sites 3 and 4 but *A. uranoscopus* and *E. treurensis* were sparse. Sites 5-7 were located on the Treur River and were uninvaded by *O. mykiss*. The sites had a higher species diversity because of three additional species chubbyhead barb (*E. anoplus*), *E. lineomaculatus* (line spotted barb) and banded tilapia (*Tilapia sparrmanii*) that were caught from these sites but not on the Blyde River (Table 2).

Niche position and size

Fish communities in the uninvaded river sections of the Blyde River comprised only two species, *E. treurensis* and *A. uranoscopus*, that both primarily fed on Diptera and Ephemeroptera (Table 3). The two species, however occupied significantly different centroid positions ($p < 0.05$), because of differences in Ccorr values (ANOVA: $F_{2,\ 56} = 29.8$; $p < 0.05$) but the TP values were similar (ANOVA: $F_{2,\ 56} = 3.3$; $p > 0.05$) (Figure 2A).

Fish communities in the invaded river reach of the Blyde River comprised three species that fed mainly on insects but also consumed algae, and macrophytes (Supplementary Table S4). The three species occupied significantly different centroid positions ($p < 0.05$) because of differences in TP values (ANOVA: $F_{2,\ 37} = 9.29$; $p < 0.05$) but Ccorr values were similar (ANOVA: $F_{5,\ 120} = 3.9$; $p > 0.05$) (Figure 2B). *Enteromius treurensis* ($\text{SEA}_c = 0.05$) and *O. mykiss* ($\text{SEA}_c = 0.03$) utilised similar-sized isotopic niche spaces ($p > 0.05$) that were significantly larger than the isotopic niche space utilised by *A. uranoscopus* ($\text{SEA}_c = 0.01$) (Figure 2E). The extent of overlap between *A. uranoscopus* - *E. treurensis* (0.11), and *A. uranoscopus*

TABLE 3 Proportion (% of isotopic value) of food sources utilised by rainbow trout (*Oncorhynchus mykiss*) and indigenous fish species in the catchments of the Blyde and Treur rivers, Mpumalanga Province, South Africa. ± denotes standard deviation.

Site and invasion status	Species	Algae	Macrophytes	Coleoptera	Diptera	Ephemeroptera	Hemiptera	Odonata
Blyde River, sites 1–2 (uninvaded)	*A. uranoscopus*	0.05 ± 0.05	0.04 ± 0.03		0.23 ± 0.24	0.61 ± 0.27		0.07 ± 0.06
	E. treurensis	0.07 ± 0.03	0.03 ± 0.02		0.11 ± 0.08	0.75 ± 0.06		0.05 ± 0.03
Blyde River, sites 3–4 (invaded)	*A.uranoscopus*	0.21 ± 0.13	0.09 ± 0.06		0.16 ± 0.13	0.15 ± 0.11	0.21 ± 0.16	0.17 ± 0.14
	E. treurensis	0.25 ± 0.11	0.06 ± 0.04		0.15 ± 0.11	0.15 ± 0.09	0.23 ± 0.16	0.16 ± 0.12
	O. mykiss	0.25 ± 0.08	0.03 ± 0.02		0.11 ± 0.07	0.23 ± 0.09	0.28 ± 0.14	0.11 ± 0.07
Treur River, sites 5–7 (uninvaded)	*A. uranoscopus*	0.04 ± 0.03	0.04 ± 0.03	0.18 ± 0.13		0.10 ± 0.10	0.24 ± 0.13	0.40 ± 0.23
	E. anoplus	0.04 ± 0.03	0.05 ± 0.03	0.13 ± 0.08		0.10 ± 0.09	0.49 ± 0.11	0.18 ± 0.14
	E. lineomaculatus	0.06 ± 0.03	0.06 ± 0.04	0.15 ± 0.08		0.17 ± 0.11	0.43 ± 0.09	0.14 ± 0.08
	E. treurensis	0.04 ± 0.02	0.04 ± 0.03	0.12 ± 0.07		0.10 ± 0.08	0.57 ± 0.09	0.14 ± 0.10
	T. sparrmanii	0.14 ± 0.06	0.08 ± 0.05	0.05 ± 0.03		0.12 ± 0.10	0.53 ± 0.08	0.08 ± 0.06

- *O. mykiss* (0.11) was low but the was an intermediate overlap between *E. treurensis* and *O. mykiss* (0.55) (Figure 2E).

The fish species from the uninvaded Treur River could be separated into two groups. Four insectivores, *A. uranoscopus, E. anoplus*, *E. treurensis*, and *E. lineomaculatus,* fed mainly on insects while *T. sparrmanii* was an omnivore and fed on algae and insects (Table 3). *Amphilius uranoscopus* had the highest TP values (TP = 2.72 ± 0.14, Ccorr = 0.53 ± 0.09) that was significantly different from *E. lineomaculatus* (TP = 2.35 ± 0.12, Ccorr = 0.57 ± 0.11) and *E. treurensis* (TP = 2.51 ± 0.11, Ccorr = 0.63 ± 0.10), while *T. sparrmanii* (TP = 2.15 ± 0.27, Ccorr = 0.58 ± 0.12) had the lowest TP values that were significantly different from all the other species (ANOVA: $F_{5,120} = 18.24$; $p < 0.05$) (Figure 2C, Supplementary Table S2). There were no significant differences in Ccorr values (range = 0.41–0.63) among the five species (ANOVA: $F_{5,\ 120} = 3.9$; $p > 0.05$). *Tilapia sparrmanii* utilised the largest isotopic niche (SEAc = 0.11 ± 0.1) while the rest of the species utilised smaller but similar-sized isotopic niches (*A. uranoscopus* = 0.03 ± 0.03, *E. anoplus* = 0.03 ± 0.02, *E. lineomaculatus* = 0.04 ± 0.04, and *E. treurensis* = 0.04 ± 0.03) (SIBER $p < 0.05$) (Figure 2F, Supplementary Table S2). The extent of overlap was low for most species except for intermediate overlap between *E. lineomaculatus* and *E. treurensis* (45%) and high overlap between *E. anoplus* and *E. treurensis* (62%) (Supplementary Table S4).

Spatial differences in food web structure

The isotopic food web varied in structure across the invaded and uninvaded sites from the Blyde and Treur rivers. The fish communities from the invaded section of the Blyde River had smaller niche metrics [total area of the convex hull (TA), trophic range (TP range), and Ccorr range] than fish communities in the uninvaded sections in the Blyde and Treur rivers (Supplementary Table S5). In addition, there were significant differences ($p < 0.05$) in the mean community centroid positions across sites in the uninvaded (Ccorr = 0.19, TP = 2.55) and invaded (Ccorr = 0.38, TP = 2.88) sections of the Blyde River and invasion free Treur River (Ccorr = 0.58, TP = 2.37). The mean distance to the centroid of fish communities in uninvaded sections of the Blyde (CD = 0.22) and Treur River (CD = 0.24) was higher and significantly different ($p < 0.05$) from fish communities in the invaded section of the Blyde River (0.15). In contrast, the nearest-neighbour distance (NND) was not significantly different ($p > 0.05$) (Supplementary Table S5).

Only two species (*A. uranoscopus and E. treurensis*) were sampled across invaded and uninvaded sites in the Blyde and Treur rivers. The two species occupied significantly different centroid positions from each other across the uninvaded and invaded sites (Figure 3). The centroid position of *A. uranoscopus* from uninvaded sections of the Blyde River was characterised by depleted TP (2.37 ± 0.06) and Ccorr values (0.16 ± 0.01) relative

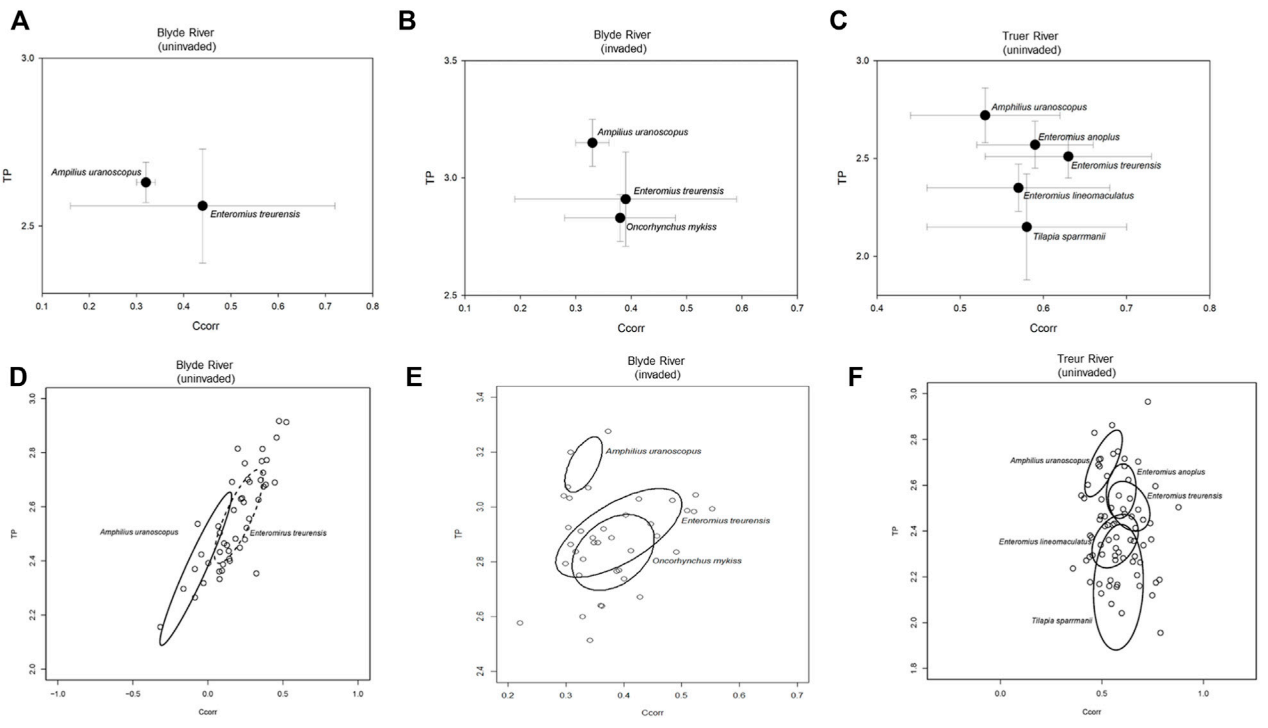

FIGURE 2
Centroid positions, and estimates of niche size and overlap using sample size-corrected standard ellipse areas (SEAc) of fish species sampled from sites invaded by rainbow trout (*Oncorhynchus mykiss*) on the Blyde River **(B,E)** and sites free of invasion both the Blyde **(A,D)** and Treur rivers **(C,F)**.

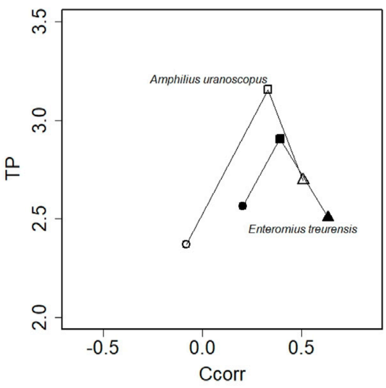

FIGURE 3
The magnitude and direction of change in centroid positions of *Enteromius treurensis* and *Amphilius uranoscopus* across invaded and uninvaded sites on the Blyde and Treur rivers in Mpumalanga Province, South Africa.

to invaded sections (TP = 3.15 ± 0.09 and Ccorr values = 0.33 ± 0.03) and invasion free Treur River (TP = 2.72 ± 0.14 and Ccorr = 0.53 ± 0.09). A similar trend was also observed for *E. treurensis* were the centroid position in invaded section of the Blyde river had enriched TP and Ccorr values (TP = 2.91 ± 0.11 and Ccorr = 0.39 ± 0.15) relative to invasion free sections of both the Blyde (TP = 2.56 ± 0.17, Ccorr = 0.22 ± 0.11) and Treur rivers (TP = 2.51 ± 0.11 and Ccorr values = 0.63 ± 0.1). There were no significant differences ($p > 0.05$) in path length, direction and shape between the centroid positions of the two species among invaded and uninvaded sites across the two rivers (Figure 3).

Discussion

This study assessed food web interactions between fish communities in river sections invaded by *O. mykiss* and sections that are still free of invasion in the upper reaches of the Blyde River catchment. *Oncorhynchus mykiss* was an insectivore and fed at a similar trophic level to that of indigenous fish species. Similar results have been reported elsewhere in the uKhahlamba Drakensberg Park and Cape Floristic Region of South Africa where introduced trout (*S. trutta* and *O. mykiss*) have been observed to feed at similar and or lower trophic levels to indigenous fishes (e.g., Rivers-Moore, et al., 2013; Jackson et al., 2016; Weyl et al., 2020). *Oncorhynchus mykiss* is known to impact indigenous fish fauna through various mechanisms such as competition for resources (food and space), predation, alteration of food web structures and changes in habitat coupling, as well as overall ecosystem-level effects (e.g., Eby et al., 2006; McDowall, 2006; Karssing et al., 2012; Ellender, 2013; Shelton et al., 2015a). Isolating the relative individual contribution of each of these mechanisms to the overall impact on the food web is difficult (e.g., see Crowl et al., 1992; Townsend, 2003). The fact that *O. mykiss* and indigenous fish communities were feeding at the same trophic level suggests that their present impact on indigenous fish communities in the upper Blyde River Catchment is more likely through competition for resources.

Competition for food and space between *Oncorhynchus mykiss* and indigenous species can lead to adverse impacts on community structure and function (e.g. Eby et al., 2006). Such impacts include a decline and fragmentation of indigenous species populations and niche shifts (e.g., Kadye et al., 2013; Shelton et al., 2015a). *Oncorhynchus mykiss* and *E. treurensis* utilised a similar-sized isotopic niche that had intermediate overlap (55%). *Oncorhynchus mykiss* is a general predator but it has been shown to feed primarily on terrestrial invertebrates, while *E. treurensis* utilises a wider variety of invertebrate prey (Kleynhans, 1987). This is consistent with observations from gut contents in this study where *O. mykiss* fed mainly on aquatic invertebrates but the *E. treurensis* had a broader diet spectrum that included algae and aquatic invertebrates. The high proportion of aquatic invertebrates in the diets of *O. mykiss* and *E. treurensis* likely lead to the observed high niche overlap between the two species. This indicates that *O. mykiss* is not using a vacant trophic niche in this system, but is instead using similar food resources to *E. treurensis*, potentially competitively displacing it. This notion is supported by the fact that river sections with high *O. mykiss* densities were characterised by a low abundance of *E. treurensis* (96% lower) relative to uninvaded areas.

In addition, competition often leads to niche shifts by the weaker competitor to minimise competition (Kramer and Drake, 2014). However, the effects of interspecific competition on the niche widths of consumers are often context-specific (Araújo et al., 2011). For example, competing species can utilise smaller niche spaces to reduce competition by minimising overlap in resource use (e.g., Bolnick et al., 2010; Jackson et al., 2016) or increase their niche width in order to maintain access to limited resources (e.g., Svanbäck and Bolnick, 2007). In this study, *A. uranoscopus* and *E. treurensis* shifted their niche and fed at a higher trophic level in invaded areas than in uninvaded areas. The increase in trophic level was likely associated with an increase in the variety of food items utilised by a species in invaded sections relative to uninvaded areas. Both species fed predominately on Ephemeroptera larvae in uninvaded sections of the Blyde River and on Hemiptera and Odonata in the uninvaded Treur River but increased their niche width in invaded sections of the Blyde River to include greater proportions of algae and other aquatic invertebrates such as Diptera, Hemiptera and Odonata. The niche shift by *A. uranoscopus* and *E. treurensis* between invaded and uninvaded river sections may be linked to differences in access to food resources rather than food availability as there were no clear differences in food resources (aquatic invertebrates) available to fishes based on the invasion status. There were however significant differences in fish composition and abundance among sites. Indigenous fish species such as *E. treurensis* occurred at high densities at uninvaded sites relative to invaded sites. It is therefore likely that intraspecific competition for food resources might have constricted the niche width of indigenous fishes at uninvaded sites and conversely, niche expansion occurred at invaded sites because of increased access to food resources that may have been otherwise depleted or monopolised by competitors (Bolnick et al., 2010). This notion is supported by other comparative studies that have observed increases in invertebrate diversity in areas invaded by *O. mykiss* that might be due to *O. mykiss* invasions reducing the abundance of indigenous fish and thereby reducing predation pressure on aquatic invertebrates, with knock-on effect on the structure of these assemblages. For example, in the upper Breede catchment, *O. mykiss* were characterised as weaker regulators of invertebrate abundance than the indigenous river minnows which they replace (Shelton et al., 2015b, 2017). Similar observations were found in mountain headwater streams in eastern Zimbabwe where there was a higher abundance of some invertebrate taxa in areas invaded by trout which could be linked to decreased predation pressure from indigenous invertebrates and fish that occurred in lower abundance in invaded areas (Kadye et al., 2013).

Although not observed in this study, *O. mykiss* has also been observed to selectively consume small river minnows (McIntosh et al., 2010; Shelton et al., 2015a). The processes of competition and predation by alien fish are often linked and interact to determine net effects in invaded systems (e.g., Eby et al., 2006; David et al., 2017). It is, therefore, possible that the decline in populations of indigenous fishes in the upper Blyde River catchment might have been through a combination of predation and competition from *O. mykiss* invasions. The trophic niche concept has been used to assess the impacts of alien species on community structure and function but its use can be hindered by several limitations some of which were evident in this study. These include a lack of long-term monitoring studies to track trends. The sites sampled in this study have been invaded for over a century and there are no monitoring studies that have been done to measure long-term trends in community absolute abundance, predator demands and community compensation mechanisms. Therefore the observed trophic interrelationships may represent a point-in-time snapshot of foodweb dynamics that may shift over time. Second, there are difficulties in determining the food utilised by fish species from sites that are in a continuum along a river profile as upstream river segments often affect nutrient dynamics in downstream segments. In this study, to minimise spatial dependencies of food webs along a river continuum, an isotopic baseline correction was done to allow for comparisons of trophic interactions among fish communities along spatial gradients (see Olsson et al., 2009; Jackson and Britton, 2014). Third, the number of individuals varied significantly among sites, where a species could be abundant at one site but rare at other sites. To avoid data dependency problems among sites, sampling effort was standardised and sites were pooled together based on the invasion status (presence or absence of *O. mykiss*) and location. However, some sites had low sample sizes and the confidence level in some of the estimates of food web matrices could be improved by increasing the sample sizes. Despite these caveats, this study demonstrated that there are spatial differences in trophic functional diversity among the fish communities in the upper Blyde River catchment that are correlated to *O. mykiss* invasions. Fish communities in uninvaded areas had greater trophic redundancy than fish communities in invaded areas. These spatial differences in trophic functional diversity are likely a result of *O. mykiss* invasion through competition for resources with indigenous fishes. We posit *O. mykiss* invasions have reduced the abundance of indigenous fishes and thereby reducing predation pressure on aquatic invertebrates, with a knock-on effect on the trophic interrelationships among fish assemblages. This is consistent with observations elsewhere that have noted that *O. mykiss* invasions can influence the diet and trophic niches of indigenous fish by altering the structure and dynamics of food webs through various mechanisms such as predation and competition.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Ethics statement

The animal study was reviewed and approved by the University of Pretoria Animal Ethics Committee.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The handling editor TD declared a past co-authorship with the author TZ.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2022.949353/full#supplementary-material

SUPPLEMENTARY TABLE S1
Frequency of occurrence (%) of prey items in the diets of indigenous and alien invasive fish species in the catchments of the Blyde and Treur rivers, Mpumalanga Province, South Africa.

SUPPLEMENTARY TABLE S2
Trophic position (TP), corrected Carbon (Ccorr) and values of isotopic niche (SEAc) for indigenous and alien invasive (bold) fish species sampled in uninvaded and invaded catchments of the Blyde and Treur rivers, Mpumalanga Province, South Africa.

SUPPLEMENTARY TABLE S3
Isotopic niche overlap among fish species in river sections invaded by rainbow trout (*Oncorhynchus mykiss*) on the Blyde River, South Africa. Extent of overlap was classified as low (< 40%), intermediate (40 to 60%) and high (> 60%) (Langton, 1982).

SUPPLEMENTARY TABLE S4
Isotopic niche overlap among fish species in the Treur River, South Africa that is free of rainbow trout (*Oncorhynchus mykiss*) invasion. Extent of overlap was classified as low (< 40%), intermediate (40 to 60%) and high (> 60%) (Langton, 1982).

SUPPLEMENTARY TABLE S5
Isotopic matrices of food webs of fish communities sampled in sites invaded by rainbow trout and sites free of invasion on the Blyde and Treur rivers, Mpumalanga Province, South Africa.

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Drivers of water quality in Afromontane-savanna rivers

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Although several studies have investigated the relationships between water quality in rivers and the types of land use within their catchments, many aspects of these relationships remain unclear in Afromontane-savanna rivers, especially the interactions between catchment land use, seasonality and stream size. Afromontane-savanna catchments present a unique situation where headwater regions and lowlands have experienced more dramatic land cover change, but mid-elevation regions remained rather natural. We examined the influence of seasonality, catchment land use and stream size, including their interactions, on water physico-chemistry, nutrients and major ions in the Afromontane-savanna Mara River in Kenya, using data collected from 2010 to 2018 at >150 sampling sites in the Kenyan part of the river. We developed generalized linear mixed models (GLMMs) to explore the influence of seasonality (dry and wet seasons), land use (forest, mixed, agriculture and grasslands), stream size (stream orders 1–7), and their interactions on river water quality. Water quality variables included physico-chemical measures (pH, dissolved oxygen [DO] concentration, temperature, electrical conductivity, total dissolved solids [TDS], turbidity, total suspended solids [TSS] and particulate organic matter [POM]), nutrients (NH_4^+, NO_3^-, total dissolved nitrogen [TDN], total nitrogen [TN], soluble reactive phosphorus [SRP], total phosphorus [TP] and dissolved organic carbon), and major ions (Cl^-, F^-, SO_4^{2-}, Na^+, K^+, Ca^{2+}, Mg^{2+}, Fe^{2+}, HCO_3^- and Si). There were clear differences in average values of most water quality variables among land uses with sites in savanna grasslands having high levels of major ions, ammonium and P, while agricultural sites had higher dissolved fraction of N (except ammonium). Stream order was a poor predictor of water quality, and most parameters did not display any relationship (either linear or non-linear) with stream size. Our results can be used to efficiently enhance water quality by developing strategies for stream restoration and management based on the predomination type of land use in the catchments.

KEYWORDS

linear mixed models, land use, non-linear relationships, savanna rivers, afrotropical rivers, water quality

Introduction

Streams and rivers are sentinels of conditions in the catchments they drain (Karr, 1998; Williamson et al., 2008). Because of the strong influence of catchments on rivers (Hynes, 1975), many studies have sought detailed analysis of water quality to infer biogeochemical and hydrological processes at the catchment scale. Catchment influences on the condition of rivers are assumed to be strongest at the headwaters where the linkage between the terrestrial and the fluvial domain is strongest (Vannote et al., 1980; Lambert et al., 2011). Yet, the contribution of catchments and headwater streams is important to the physical and biogeochemical characteristics of entire river basins, especially in terms of the transfer and transformation of carbon, nitrogen, phosphorus, and associated elements (Cole et al., 2007; Battin et al., 2009; Aufdenkampe et al., 2011).

Land use change from natural vegetation to other uses (e.g., agriculture, urbanization, or grazing) is one of the major causes of water quality degradation and loss of ecological integrity in rivers worldwide (Allan, 2004; Dudgeon, 2010; Vörösmarty et al., 2010; Brauns et al., 2022). Catchment land use change influence the structure and functioning of rivers mainly through changes in runoff and erosional processes, organic matter dynamics, the input of nutrients and major ions, in-stream ecosystem processes such as primary production, organic matter processing and nutrient cycling, and the composition of biological communities (Dudgeon et al., 2006; Bernot et al., 2010; Petrone, 2010; Tank et al., 2010; Woodward et al., 2012; Fugère et al., 2018). The proportion of different land use types in a catchment is closely correlated with many water quality parameters in aquatic systems (Lenat and Crawford, 1994; Tong and Chen, 2002; Hwang et al., 2016). For instance, there is agreement that catchments with high proportions of developed land area (i.e., urban land use) tend to have higher concentrations of water pollutants and nutrients (Roberts and Prince, 2010; Nagy et al., 2012; Hwang et al., 2016). Similarly, levels of electrical conductivity, major ions and suspended sediments in rivers increase with the proportion of agricultural land (Minaya et al., 2013; Kilonzo et al., 2014; Masese et al., 2017). However, some studies have shown that the influence of land use on some water quality parameters in rivers does not only depend on the proportion of different land use types in the catchment area, but also on the size and use of the riparian zone (Minaya et al., 2013; Fierro et al., 2017; de Mello et al., 2018; Hilary et al., 2021; Kadeka et al., 2021).

Apart from spatial variability, surface water quality also varies greatly over time or temporally (Chang, 2008; Guo et al., 2019, 2020). In this regard, seasonality, or the flow regime, which in the tropics is dominated by the difference in the amount of rainfall and runoff generated from the catchments (i.e., wet vs. dry season), can directly or indirectly influence many biogeochemical and ecological processes in rivers, including water quality. Pollutants entering a river system normally depend on rainfall patterns, which drive the rainfall-runoff processes that underpin constituent mobilization and transport (Lintern et al., 2018). During the wet season, increased runoff from farmlands and developed areas can increase the levels of sediments, nutrients and organic matter transported by rivers (Kilonzo et al., 2014), although for most constituents there is often a lag and hysteresis caused by a non-linear relationship between discharge and concentration (Sanderman et al., 2009; Lambert et al., 2011; Bouillon et al., 2012). In intermittent or ephemeral rivers, cessation of flow creates a series of isolated pools that may or may not be connected through interstitial flow. Thus, the spatial and seasonal shifts in flow levels can interact with land use and other human activities to influence the composition and concentrations of materials transported by rivers (Paetzold et al., 2008; Roach et al., 2014; Rodrigues et al., 2018). Therefore, it is crucial to understand patterns and changes in average water quality across broad spatial and temporal scales to develop sustainable management strategies (Thompson et al., 2011; Godsey et al., 2019).

Studies on the spatial and temporal dynamics and determinants of water quality in Afromontane-savanna river systems are very limited. Nevertheless, water quality changes in these systems can occur at short spatial and temporal scales because of the highly heterogenous nature of the landscapes created by rapid changes in elevation, amount of rainfall, geology and vegetation type. Usually, conditions in the uplands are characterized by higher amounts of rainfall and cooler temperature that support broadleaf tropical vegetation while in the lowlands the conditions are much drier, soils shallow and vegetation sparse and dominated by shrublands and grasslands (Tamooh et al., 2012; Abrantes et al., 2013; Aich et al., 2014; Mushi et al., 2019; Englmaier et al., 2020). Unlike other biomes, Afromontane-savanna rivers also present a unique situation where land use features vary both altitudinally and longitudinally from the headwaters. Specifically, while rivers are postulated to exhibit a prominent shift in physical and chemical factors from headwaters to the lower reaches (e.g., water temperature, different fractions of organic matter and their sources (Vannote et al., 1980; Downing et al., 2012; Creed et al., 2015), different patterns have been reported in African savanna rivers (e.g., Masese and McClain, 2012; Masese et al., 2015, 2022). In these landscapes, the abundance and biomass of large mammalian herbivores (both wildlife and livestock) also present a natural gradient characterized by low numbers in the forested uplands and high densities in the savanna grasslands in the lowlands. Rivers that drain these landscapes display a tight coupling with terrestrial ecosystems through the vectoring role of both livestock and large wildlife in transferring large amounts of organic matter and nutrients through egestion and excretion at watering and crossing points along the rivers (Subalusky et al., 2015; Iteba et al., 2021). African savanna landscapes are also emerging as new frontiers of land use change as human

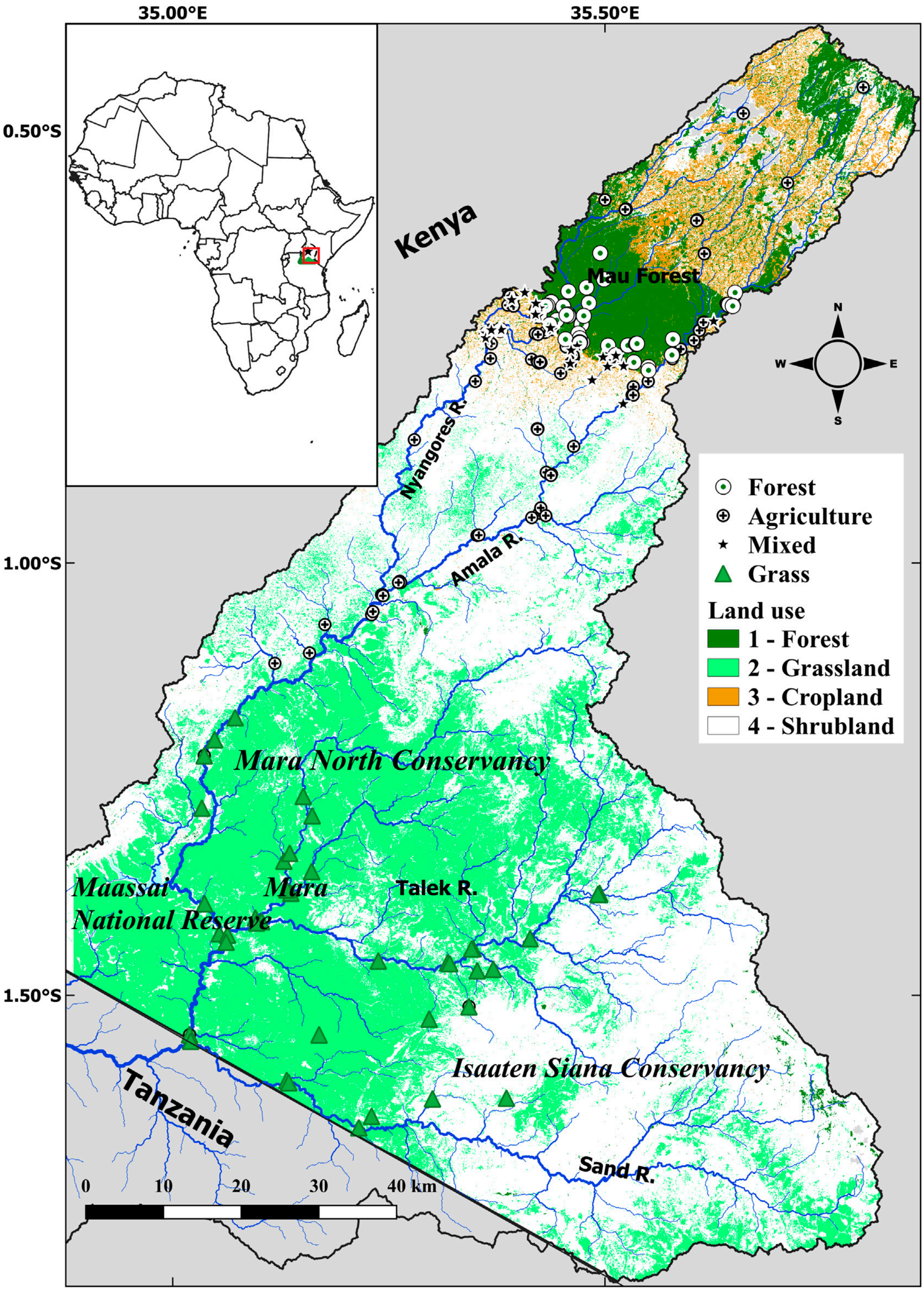

FIGURE 1
Map of the Mara River catchment showing the position of the sampling sites.

populations and their livestock migrate into marginal lands in semi-arid and arid areas to ease pressure on more productive and densely populated humid lands. As a result, the humid headwater regions and drier savanna lowlands have experienced more dramatic landcover changes, but mid-elevation regions remained rather natural. Thus, the postulated upstream-downstream gradients (e.g., Vannote et al., 1980) may not apply to these systems. Moreover, rivers draining savanna landscapes are seasonal with highly variable flow regimes characterized by cessation of flow during the dry season and flash flooding during the rainy season (McClain et al., 2014). These attributes of Afromontane-savanna rivers present intriguing scenarios for understanding the interactions among different drivers of water quality in rivers.

In this study, we investigated the influence of seasonality, land use and stream size/order on several water quality variables (physico-chemical measures, nutrients, and major ions) in Afromontane-savanna rivers using the Mara River, Kenya, as a case study. Our key objectives were to 1) identify the spatial variability in mean water quality variables at the river basin scale and across different land use types and stream sizes/orders; 2) determine the influence of seasonality, land use and stream size and their interactions as drivers of water quality in the river; and 3) determine the influence of stream size/orders on longitudinal trends in water quality variables in the river. We hypothesized that is be strong variability in both mean concentrations and water chemistry across land use types and between seasons, which would influence the role of stream size as a predictor of water quality in the river. The Mara River basin (MRB) is representative of many river basins in Africa that partially drain humid uplands and semi-arid lowlands with wide variations in elevation, rainfall and geology. Therefore, this analysis will contribute to understanding how water quality responds to land use change at the basin scale, which can be used to inform water resources management in the continent.

Materials and methods

Study area

This study was conducted on the Kenyan part of the Mara River basin (MRB, Figure 1). The Mara River is an Afromontane-savanna river that partly drains an Afromontane tropical forest and partly semi-arid grasslands and shrublands hosting large numbers of livestock and wildlife (Ogutu et al., 2016; Veldhuis et al., 2019). The headwaters of its two perennial tributaries, the Nyangores and Amala rivers, drain the larger Mau Forest Complex (MFC) in the Mau Escarpment, which is the most extensive tropical moist broadleaf forest in East Africa (Wass, 1995). In the middle and lower reaches, the Olale Orok, Sand and Talek tributaries drain the semi-arid grasslands and shrublands of the Maasai Mara National Reserve (MMNR) and adjoining conservancies in Kenya and the Serengeti National Park (SNP) in Tanzania (Figure 1).

In the highlands, the climate is relatively cool and seasonal due to the high altitude, and is characterized by distinct rainfall seasons and low ambient temperatures that fall below 10°C during the cold months of January-February. Rainfall varies with altitude with the highlands receiving around 2000 mm of rainfall per annum while the semi-arid lowlands in the MMNR and adjoining areas receiving around 1,000 mm. The altitudes in the basin range from 2,932 m around the sources in the Mau Escarpment to 1,134 m around Lake Victoria. Dry conditions are experienced during January-March and two wet conditions during March-July and October-November, which are periods for the long and short rains, respectively. However, variations sometimes occur at the onset and end of the rainy and dry periods. Potential evapotranspiration varies between 1,400 mm in the highlands to 1800 mm in the lowlands (Jackson and McCarter, 1994).

Until the middle of the past century, the MRB was covered by montane forest in the headwaters and a mixture of shrublands and grasslands throughout its middle and lower reaches in Kenya (Serneels et al., 2001; Mati et al., 2008). However, land use change to agriculture and grazing lands and settlements has reduced coverage of both the broadleaf forest in the headwaters and the grasslands in the middle reaches (Lovett and Wasser 1993; Obati, 2007). With human population growth in the area averaging 3% p. a., land use practices are bound to intensify with remnant forests and grasslands likely to be converted into farmlands, settlements and livestock grazing areas.

Study design

In this study, we used data on water quality variables that were collected from 2010 to 2018 covering both the wet season (April-June and October -December) and dry season (January-March and July-September). These data were collected from more than 150 sites spread out in the entire MRB in Kenya (Figure 1). Some of the data have been published in previous works, including Minaya et al. (2013), Masese et al. (2014a, b, 2017, 2022) and Iteba et al. (2021). Although data were available from 2010 to 2018, most of the data used were collected in 2010, 2012, 2016 and 2018.

To capture spatial and temporal variability in water quality across the entire river basin, sampling sites were in rivers draining a gradient of catchment land use from 100% forestry or grasslands to 100% agriculture. The sampling sites were then classified into four broad land use categories depending on the proportions of forest, grasslands, and agricultural land use in the entire catchment upstream of the sampling sites. Based on the Digital Elevation Model of

FIGURE 2
Sampling sites in rivers in different land uses in the Mara River basin, Kenya; Forest **(A–C)**, Mixed **(D–G)**, Agriculture **(H–J)** and Grasslands **(K–N)**. Upper panel photos show the landscape in each of the four land uses **(A)** forested, **(D)** mixed land use, **(H)** agricultural land use, and **(K)** grasslands. The middle panel photos show the condition of streams in the different land uses during the dry season, **(B)** forested, **(E,F)** mixed land use, **(I)** agricultural land use, and **(L,M)** grasslands. The lower panel photos show the condition of the streams in the different land uses during the wet season, **(C)** forested, **(G)** mixed land use, **(J)** agricultural land use, and **(N)** grasslands. Photo credits: **(A–H,N)**—Frank Masese; **(I,J)**—Elizabeth Wanderi; **(K,L)**—Evans Ole Keshe; **(M)**—Paul Geemi.

Kenya (90 m by 90 m), obtained from the Shuttle Radar Topography Mission, catchments were delineated and the area of each major land use category upstream of each sampling site was calculated. Forest (FOR), grassland (GRAS) and agriculture (AGR) sites drained catchments with the proportion of catchment land use under forestry, grasslands, and agriculture >60%, respectively. Mixed (MIX) sites did not meet the catchment land use criteria for, GRAS and AGR sites, and had an intermediate mixture of two or three of the major land use types.

The forested (FOR) sampling sites were in Mau Forest Complex in the headwaters of the Amala and Nyangores rivers, which are the main and perennial tributaries of the Mara River. Along FOR streams, the riparian corridor was largely intact with a mixture of indigenous vegetation throughout the catchment (Figures 2A,B). Compared to AGR sites, water quality in FOR streams was less turbid even immediately after storm events (Figures 2B,C). The AGR and MIX sampling sites were in agricultural areas in the middle reaches of the MRB. While AGR sites drained catchments that were entirely on agricultural land, MIX sites were in catchments that were partly forested and partly agricultural, with none of the two land uses dominating with >60% (Figure 2D). MIX sampling sites were in agricultural areas (Figures 2E,F). People living in the adjoining areas of the MFC and MMNR in the AGR and MIX catchments are involved in semi-intensive subsistence agriculture of mainly tea, maize, beans and potatoes and animal husbandry. Along many agricultural streams on the highlands, indigenous riparian vegetation has been replaced by exotic *Eucalyptus* species and other exotic trees (Masese et al., 2014b; Kroese et al., 2020). Livestock footpaths that connect grazing areas to the rivers are also a common feature of many AGR and MIX catchments, and overused unpaved tracks are a major cause of erosion and surface runoff into the rivers (Kroese et al., 2020). Cattle entrance points to streams are generally highly disturbed and have degraded riverbanks, and the water is often turbid from livestock access and trampling even during the dry season (Figures I, J).

The GRAS sampling sites were in the seasonal tributaries (the Olare-Orok, Talek and Sand rivers) draining semi-arid areas of the lower MRB basin (Figure 1). These tributaries drain undulating plains that are dominated by grasslands interspersed with shrublands (Figure 2K). Land use here is protected for wildlife in the MMNR and SNP, but the adjoining areas are used as conservancies for livestock grazing while at the same time allowing wildlife protection (Veldhuis et al., 2019). The Serengeti-Maasai Mara ecosystem is host to one of the largest populations of large wildlife in the world (Figure 2K), including >4,000 hippopotami (hippos) in the Mara River and seasonal tributaries in the MMNR and SNP (Kanga et al., 2011). This region hosts large herds of livestock which use the rivers for watering and crossing. Both large wildlife (mainly hippos) and livestock have a strong influence on water quality and ecological integrity of rivers through the input of large amounts of organic matter and nutrients through excretion and egestion (Subalusky et al., 2015; Iteba et al., 2021). Because of the large herds of livestock and the potential for overgrazing, there have been concerns about sheet and gulley erosion and sedimentation of rivers (Dunne, 1979; Dutton et al., 2018).

Water quality variables

The sampling methods and laboratory analysis of the water quality variables used in this study are presented in the Supplementary Materials. The data were divided into three categories or datasets: physico-chemical measures, nutrients and major ions. The data on physico-chemical measures included pH, dissolved oxygen (DO), temperature, electrical conductivity, total dissolved solids (TDS), turbidity, total suspended solids (TSS) and particulate organic matter (POM). The second dataset was on nutrients and included ammonium (NH_4^+), nitrates (NO_3^-), total dissolved nitrogen (TDN), total nitrogen (TN), soluble reactive phosphorus (SRP), total phosphorus (TP) and dissolved organic carbon (DOC). The third dataset was on major ions and include chloride (Cl^-), fluoride (F^-), sulphate (SO_4^{2-}), sodium (Na^+), potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), ferrous iron (Fe^{2+}), bicarbonates (HCO_3^-) and silicon (Si).

These water quality variables were considered in this study because of their exhaustive availability from other studies, where they have been proven to be responsive to catchment scale influences (e.g., agricultural, or urban land use) and local reach-scale disturbances such as wastewater disposal, clearance of riparian zones, livestock disturbance and other stressors (Minaya et al., 2013; Hwang et al., 2016; Iteba et al., 2021; Li et al., 2022). In many countries and monitoring programs, most of these water quality measures, such as dissolved oxygen (DO), pH, dissolved nitrogen and phosphorus, total nitrogen (TN) and total phosphorus (TP) form part of the criteria used for determining the condition of surface waters and for long-term monitoring and management of aquatic ecosystems (e.g., Hwang et al., 2016; Diamantini et al., 2018; Putri et al., 2018). All variables are expressed in mg/L except pH (–), electrical conductivity (μS cm^{-1}) temperature = water temperature (°C). Concentrations below the detection limit were set to the detection limit.

Data analysis

We used principal component analysis (PCA) to reduce the dimensionality of the water quality data by collapsing the variables into individual PCs to avoid multicollinearity. The PCA also allowed us to identify correlated variables and the grouping of sampling sites based on land use and stream order. We included three PCs to describe water quality. The statistical differences in water quality variables between seasons, land use and stream sizes (including interactions), and the statistical significance of the PCAs was assessed using permutational analysis of variance (PERMANOVA) based on z-standardized data (McArdle and Anderson, 2001; Anderson and Walsh, 2013).

We used generalized linear mixed models (GLMMs) to determine the drivers of water quality variables as responses (Zuur et al., 2009; Pinheiro et al., 2017). Given our unbalanced study design (Figure 1), the use of GLMMs allowed us to account for temporal and spatial autocorrelation by including the year of sampling (Lamberti et al., 2010; Strauch, 2012; Chen and Lu, 2016; Wang et al., 2018) and sampling sites in the models. GLMMs were fitted using the lme function in the *nlme* package in the R platform (Pinheiro et al., 2017). For each response variable, GLMMs included season (dry and wet), land use (FOR, MIX, AGR, GRAS), stream order (order 1–7) and the proportion of agricultural land use/cover within the study reach's drainage area (Percent AGR) as fixed effects, including potential interactions, and year and sampling site (nested within a year) as random effects. We included Percent AGR in the models because, in addition to the land use types, agriculture has been found in many studies to have a significant influence on water quality in rivers as an integrated measure of land-use influences on stream condition (Allan, 2004). In addition, Percent AGR was highly negatively correlated with the proportion of forested land use/cover, making it a good indicator of the effects of deforestation on water quality. As a continuous variable, Percent AGR was also better for fitting linear or non-linear relationships with water quality measures in the study area.

For comparison of the GLMM models, we fit an initial "full" model that included seasonality, land use, stream order and Percent AGR as fixed effects, and "year" and "site" as random effects. To identify the most parsimonious GLMM model that included only significant predictors for most water

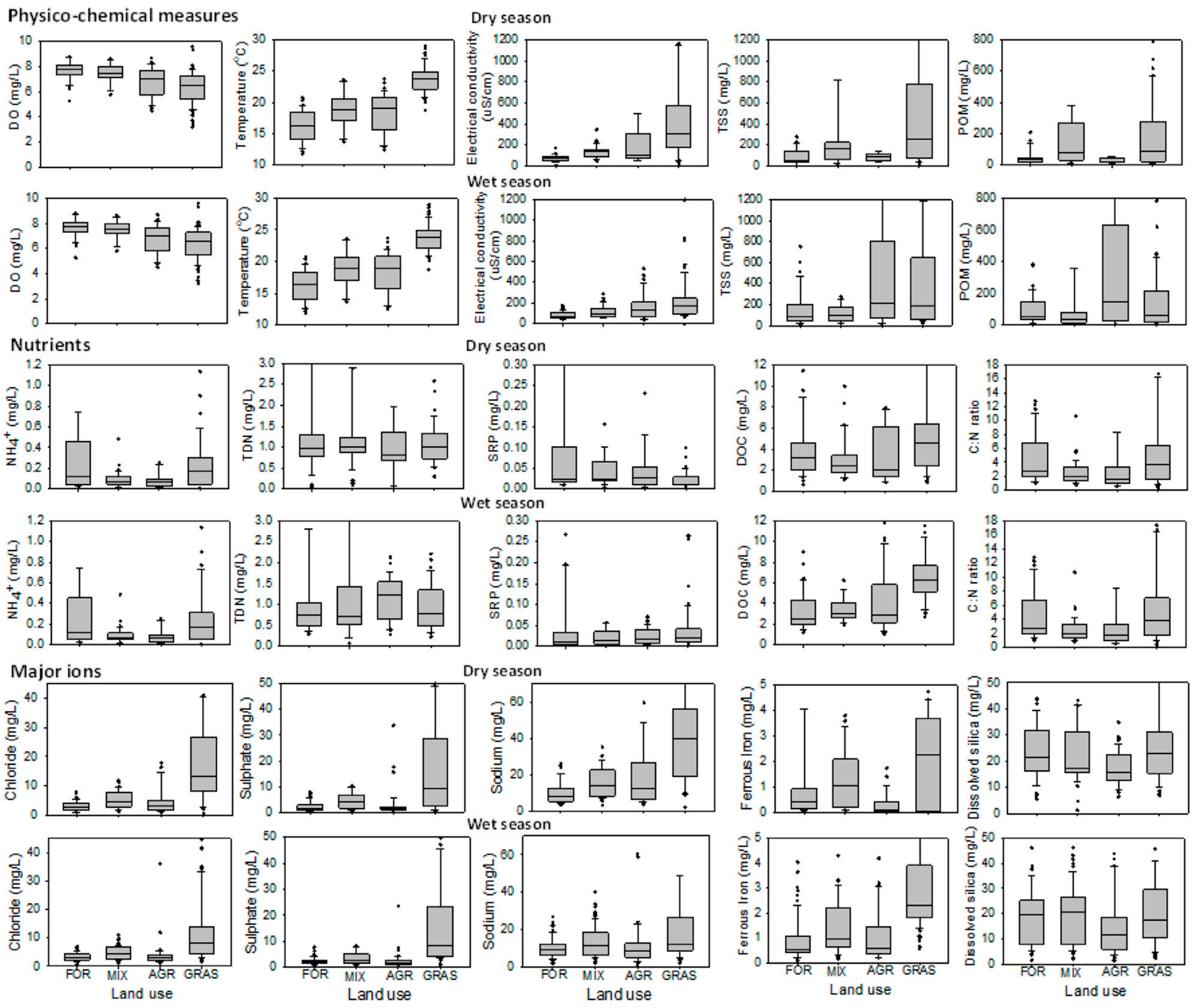

FIGURE 3
Box-and-whisker plots of water quality measures across different land use types in the Mara River basin during the dry and wet seasons. FOR = Forest, AGR = Agriculture, MIX = Mixed and GRAS = Grasslands.

quality variables, we used a step-wise ANOVA approach based on the Akaike Information Criterion (AIC) to achieve an optimal model that explained most variation with the fewest predictors, with the lowest AIC among non-significant models indicating the best model (Burnham and Anderson, 2004). For each GLMM model, we computed marginal R^2 (R^2_m, variance explained by fixed factors) and conditional R^2 (R^2_c, variance explained by the entire model, i.e. by fixed and random factors) coefficients with the "r.squaredGLMM" function in the "MUMIN" package (Barton and Barton, 2015). To compare models, we used Akaike's information criterion (AIC). The best-fit model based on AIC was identified using the *AICcmodavg* package (Mazerolle, 2017) in the R platform.

We used generalized additive models (GAMs) (Wood and Wood, 2015) to explore relationships in catchment characteristics and some select physico-chemical measures in the river. GAMs incorporate smooth functions that are more flexible in modelling nonlinear relationships (Hastie and Tibshirani, 1990). GAMs were fitted using the *mgcv* package (Wood and Wood, 2015) in the R platform (R Core Team, 2020) and built using penalized cubic regression splines with degrees of freedom automatically identified based on the generalized cross-validation score (GCV).

We used GAMs to explore relationships between the river distance from the source (RDS), stream order and the proportion of land under grasslands. We also used GAMs to relate *in situ* turbidity data (NTU) and particulate organic matter (POM, mg/L)

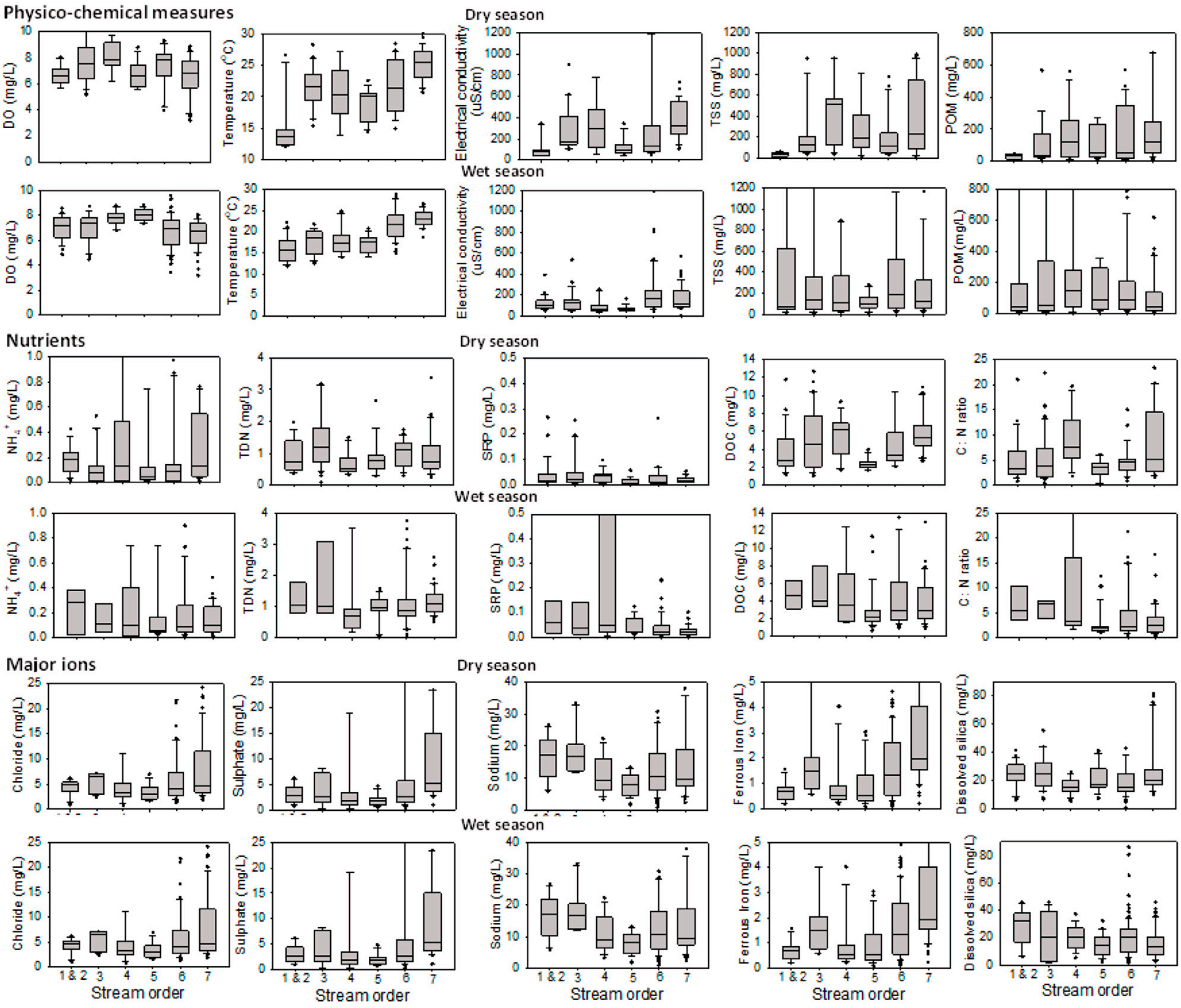

FIGURE 4
Box-and-whisker plots of water quality parameters across different stream orders (stream sizes) in the Mara River basin during the dry and wet seasons. FOR = Forest, AGR = Agriculture, MIX = Mixed and GRAS = Grasslands.

to total suspended solids (TSS) concentration (mg/L). To explore the water quality variables in the study area further, we also evaluated the influence of stream size on longitudinal trends in water quality variables in the Mara River using GAMs. Instead of using stream order (Strahler, 1957), we used the continuous river distance from the source (RDS) as a measure of stream size (Rasmussen et al., 2009; Masese et al., 2015, 2022). For each sampling site, RDS was calculated as the square root of the drainage area in km^2 (Rasmussen et al., 2009) as a general measure reflecting the linear dimension of a watershed. This measure of stream size is based on the understanding that the average length of stream paths leading to a point in the drainage can be expressed as a power function of the drainage area (Gregory and Walling, 1973) with the exponent 0.5 (Smart, 1972).

In all statistical analyses, response variables were log-transformed to meet model assumptions of normality when appropriate, and all analyses were conducted with an alpha of 0.05. Statistical analyses were performed with R version 4.0.2 (R Core Team, 2020) using the packages *vegan* (Oksanen et al., 2020).

Results

Patterns in water quality

There were both seasonal and spatial variations in water quality variables in the study area (Figures 3, 4). For most sites, DOC, TDN, TSS and TP were higher during the wet season

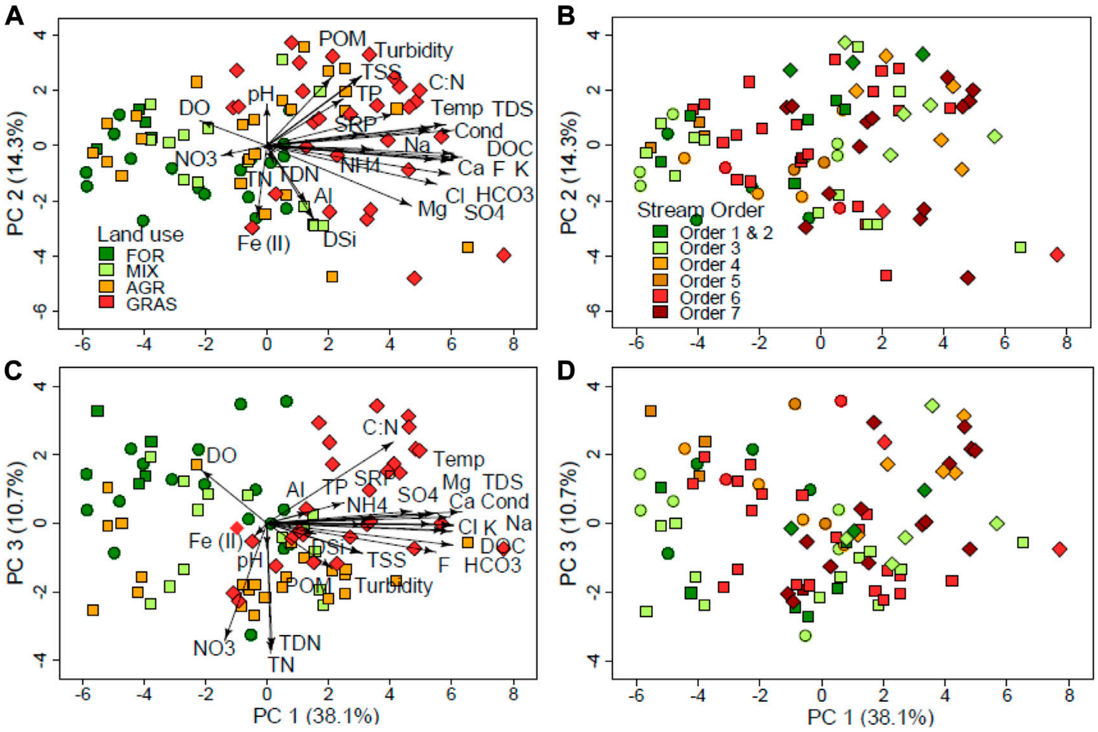

FIGURE 5
PCA biplot for water physico-chemistry variables across five land uses (regions) in the Mara River basin. The left panel **(A,C)** displays loadings for regions, and the right panel **(B,D)** shows loadings for stream size (stream orders). DO = dissolved oxygen, Temp = temperature, Cond = electrical conductivity, HCO_3 = bicarbonate, NO_3 = nitrates, SO_4 = sulphate, DSi = dissolved silica.

compared with the dry season when TN, electrical conductivity and temperature were higher (Figure 3). At AGR and GRAS sites, POM reduced during the wet season indicating input of sediments poor in organic matter.

There were differences in physico-chemical measures, nutrients, and major ions among the land uses (Figure 3), but differences among stream orders were very minimal (Figure 4). There were significant differences in water quality variables between the dry and wet seasons (PERMANOVA F = 6.1, df = 1, p < 0.001), among land use types (PERMANOVA F = 12.3, df = 1, p < 0.001) but not among stream orders (PERMANOVA F = 1., df = 1, p = 0.096). There was a significant interaction of season with land use (PERMANOVA F = 2.6, df = 1, p = 0.026), but not of season with stream order (PERMANOVA F = 1.0, df = 1, p = 0.400). Neither was there an interaction between land use and stream order (PERMANOVA F = 1.4, df = 1, p = 0.198) nor among the three factors–season, land use and stream order (PERMANOVA F = 1.6, df = 1, p = 0.123). This implies that the influence of land use on water quality depended on the season. For variables with significant differences among land uses, GRAS sites had the highest levels of all physico-chemical variables (except DO concentrations) and concentrations of major ions (Figure 3). Although there were seasonal differences in water quality, trends among land use types (Figure 3) and stream orders (Figure 4) were the same during the dry and wet seasons.

Ordinations of water quality variables

In the PCA ordinations all the PCAs were statistically significant (PERMANOVA F = 3.8, df = 7, p = 012). We used three PCA axes (PC1-3) to explain the variance in water quality in the dataset. The first PCA axis (PC 1) accounted for the highest variance in the dataset with 38.1% while PC 2 and PC 3 accounted for 14.3% and 10.7% of the variance, respectively (Figure 5). The PC 1 is considered to be the land use axis or gradient along which sites were separated according to catchment land use with higher levels of electrical conductivity, water temperature and major ions (Cl^-, K^+, Na^+, Ca^{2+} and Mg^{2+}) in AGR and GRAS sites (Figure 5A). FOR sites recorded the highest DO levels and GRAS the lowest levels.

The PC 2 separated sites according to turbidity, TSS, POM and TP with higher levels in AGR and GRAS (Figure 5A). However, the separation of water quality was not complete

TABLE 1 Results of generalized linear mixed models (GLMM) of the effects of season, land use, stream order and percent agricultural land use in the catchments upstream of each sampling site (Percent AGR) on the physico-chemical water quality parameters, nutrients and major ions in the Mara River, Kenya. Physico-chemical measures included dissolved oxygen concentration (DO, mg/L), temperature (^{0}C), electrical conductivity (µS/cm), total suspended solids (TSS, mg/L), particulate organic matter (POM, mg/L), and total dissolved solids (TDS, mg/L). Nutrients included ammonium (NH4, mg/L), nitrates (NO_3^-, mg/L), total dissolved nitrogen (TDN, mg/L), soluble reactive phosphorus (SRP, mg/L), dissolved organic carbon (DOC, mg/L) and the ratio of carbon to nitrogen (C: N). Major ions included chloride (Cl, mg/L), sulphate (SO_4^{2-}, mg/L), potassium (K, mg/L), calcium (Ca, mg/L), ferrous iron (Fe [II], mg/L) and dissolved silica (DSi, mg/L). In all cases, d.f. = 1. Highlighting significant differences: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Fixed effects	β (±SE)t-value	β (±SE)t-value	β (±SE)t-value	β (±SE)t-value	β (±SE)t-value	β (±SE)t-value
Water physico-chemistry	**DO**	**Temperature**	**Conductivity**	**TSS**	**POM**	**TDS**
Intercept	2.01 (±0.07)28.29***	2.67 (±0.04)61.23***	4.75 (±0.23)21.0***	2.99 (±0.56)5.38***	2.24 (±0.65)3.44***	4.43 (±0.19)23.05***
Season	−0.002 (±0.02)−0.09	−0.06 (±0.014)−3.9***	−0.44 (±0.10)−4.5***	0.10 (±0.14)0.70	0.22 (±0.16)1.40	−0.34 (±0.07)−4.73***
Land use	−0.04 (±0.02)−2.36*	0.09 (±0.01)8.88***	0.41 (±0.05)7.91**	0.73 (±0.12)6.29***	0.52 (±0.13)3.87***	0.36 (±0.05)7.99****
Stream order	0.01 (±0.01)1.08	0.04 (±0.01)5.78***	−0.02 (±0.03)−0.6	0.02 (±0.07)0.24	0.08 (±0.08)1.10	−0.02 (±0.03)−0.63
Percent AGR	−0.0002 (±0.001)−0.25	−0.001 (±0.004)−2.3***	−0.003 (±0.002)−1.4	−0.01 (±0.004)−1.65	−0.006 (±0.005)−1.21	−0.001 (±0.002)−0.51
Random effects						
Year (SD)	0.037	1.446	3.611	0.550	0.688	3.115
Site (SD)	0.114	0.104	0.239	0.996	1.197	0.344
Residuals (SD)	0.174	0.095	0.752	0.996	1.043	0.523
AIC	-68.92	-354.6	727.9	982.3	1,043.9	565.7
$R^2_{GLMM(m)}$	0.033	0.595	0.325	0.268	0.149	0.359
$R^2_{GLMM(c)}$	0.344	0.815	0.387	0.693	0.691	0.552
Nutrients	**NH4**	**NO_3^-**	**TDN**	**SRP**	**DOC**	**C: N**
Intercept	−1.67 (±0.38)−4.34***	−1.14 (±0.27)−4.2***	−0.34 (±0.19)−1.83	−4.43 (±0.43)−10.34***	0.94 (±0.21)4.51***	5.29 (±2.11)2.50*
Season	−0.14 (±0.19)−0.75	0.14 (±0.13)1.01	0.13 (±0.10)1.27	0.15 (±0.16)0.94	0.015 (±0.09)0.18	0.01 (±0.86)0.01
Land use	0.23 (±0.08)2.86**	-0.08 (±0.07)-1.29	0.04 (±0.04)0.91	0.29 (±0.08)3.50***	0.27 (±0.04)6.72***	1.89 (±0.37)5.09****
Stream order	−0.10 (±0.05)−1.05	0.003 (±0.04)0.06	−0.04 (±0.03)−1.52	-0.04 (±0.05)-0.88	-0.03 (±0.02)-1.17	-0.30 (±0.29)-1.32
Percent AGR	−0.01 (±0.003)−4.37***	0.01 (±0.003)4.30***	0.004 (±0.002)2.26*	−0.008 (±0.003)−2.48*	−0.005 (±0.002)−3.4***	−0.07 (±0.01)−4.96***
Random effects						
Year (SD)	0.365	0.049	0.055	0.687	0.308	3.725
Site (SD)	0.511	0.496	0.159	0.772	0.343	2.654
Residuals (SD)	1.014	0.836	0.688	0.750	0.412	4.215
AIC	932.9	829.8	666.5	851.8	473.6	1782.3
$R^2_{GLMM(m)}$	0.111	0.078	0.027	0.068	0.203	0.135
$R^2_{GLMM(c)}$	0.357	0.320	0.082	0.679	0.645	0.603
Major ions	**Cl**	**SO_4^{2-}**	**K**	**Ca**	**Fe (II)**	**DSi**
Intercept	1.05 (±0.32)3.23**	0.98 (±0.39)2.52*	2.46 (±0.25)10.0***	1.98 (±0.28)7.13***	-0.54 (±10.75)-0.72	3.76 (±0.24)15.50***
Season	-0.49 (±0.07)-6.96***	-0.47 (±0.09)-5.02***	-0.60 (±0.08)-7.9***	-0.59 (±0.08)-7.03***	0.02 (±0.11)0.20	-0.53 (±0.07)-7.55***
Land use	0.48 (±0.05)8.85***	0.43 (±0.07)6.46***	0.23 (±0.05)4.32***	0.46 (±0.05)8.65***	0.18 (±0.08)2.21*	0.07 (±0.044)1.60
Stream order	0.02 (±0.03)0.47	-0.003 (±0.04)-0.08	-0.04 (±0.03)-1.14	-0.02 (±0.03)-0.57	0.08 (±0.05)1.60	-0.03 (±0.03)-1.26
Percent AGR	-0.005 (±0.002)-2.35*	-0.01 (±0.003)-2.84**	-0.005 (±0.02)-2.51*	-0.01 (±0.002)-3.76***	0.004 (±0.003)1.31	-0.004 (±0.002)-2.45*
Random effects						
Year (SD)	0.540	0.637	0.267	0.377	1.518	0.357
Site (SD)	0.481	0.517	0.438	0.371	0.649	0.292
Residuals (SD)	0.556	0.759	0.611	0.692	0.868	0.585
AIC	931.3	1,172.8	982.3	1,061.5	1,308.8	906.8
$R^2_{GLMM(m)}$	0.319	0.202	0.216	0.342	0.013	0.147
$R^2_{GLMM(c)}$	0.747	0.632	0.541	0.585	0.786	0.474

NB: AIC = Akaike's information criterion. The marginal R^2 (GLMM [m]; fixed effects only) and the conditional R^2 (GLMM [c]; fixed and random effects) represent the proportion variance explained by each model. SE = standard error; SD = standard deviation. For random effects, the "Site" is also the "intercept".

along PC 2, implying that despite the catchment land use influences, local reach-scale conditions also influenced some water quality characteristics. For instance, sites in the semi-arid areas of the MRB (GRAS sites) in the Olare-Orok and Talek tributaries and sites in the Mara River mainstem recorded high levels of electrical conductivity, water temperature and concentrations of major ions, including conservative ions (Cl^-, F^-, SO_4^{2-}).

The PC 3 was a nutrients gradient that separated sites according to nutrient concentrations. Surprisingly, AGR sites and GRAS sites were separated in terms of nutrient concentrations, whereby sites with high densities of livestock and wildlife (GRAS sites) had low concentrations of total and dissolved N (except ammonium) compared with AGR sites, but high concentrations of NH_4^+, SRP and TP concentrations (Figure 5C). Ammonia behaved differently compared to other species of N with high concentrations in GRAS and some AGR sites. The GRAS sites also had high C: N ratios, indicating a predominance of C compared to N in the water.

For all the three ordinations of physico-chemical variables, nutrients and major ions, stream order did not play a major role in the separation of the sites (Figures 5B,D). The only notable separation was on water chemistry (major ions) where stream orders 6 and 7 seemed separated from the rest of the smaller stream orders (Figures 3B,D). However, this is likely coincidental as most of the large river sections of the Mara River are also in semi-arid lowlands where other local influences are potentially playing a major role in influencing water chemistry and quality, such as high temperature due to aridity/increased evaporation, the influence of the large populations of livestock and large wildlife and potential changes in geology and erosion potential of the soils.

Drivers of water quality

A total of 20 model comparisons were made on the effects of different drivers on water quality variables in the Mara River (Supplementary Information). The best model identified for the GLMM model included four drivers without interactions - seasons, land use, stream order and the proportion of agricultural land in the catchments of the sampling sites (Percent AGR) (Tables 1). The model included the year of sampling and sampling site (nested within years) as random effects.

The GLMM identified different responses in physico-chemical measures, nutrients and major ions to seasonality, land use, stream size and percent agricultural land use in the catchments of the sampling sites (Percent AGR) (Table 1). After a series of step-wise evaluation of many GLMM models, land use was identified as the main driver of water quality, followed by Percent AGR, seasonality then stream size. Land use had the strongest influence on water quality by influencing all (eighteen) variables except three (NO_3^-, TDN and Si). Percent AGR was the second most important driver of water quality by having a significant influence on twelve out of the eighteen water quality variables analyzed (Table 1). While land use had a stronger influence on physico-chemical measures, Percent AGR had a very strong influence on all the physical measures and nutrient constituents, respectively. Both land use and Percent AGR had a similar influence on major ions. Seasonality was the third most influential driver of water quality in the river by having a significant influence on five of the six major ions and three physico-chemical measures, but none of the nutrients. Interestingly, stream order played a minor role in influencing water quality in the river by only having a significant influence on water temperature.

Relationships among physico-chemical measures

Several relationships were explored between catchment characteristics and selected physico-chemical water quality variables (Figure 6). The regression converting TSS to turbidity has a slope of 1.051 and an R^2 of 0.98 (Figure 6A), while that for POM has a factor of 0.157 and an R^2 of 0.31 (Figure 6B). The low slope for the relationship between TSS and POM indicates that the proportion of POM in TSS (only 16%) was very low, an indication of large proportions of inorganic particles in TSS. Moreover, the relationship also shows that in large rivers the relationship is non-linear, indicating that small streams have higher amounts of organic matter, while large rivers transport more inorganic materials. The relationship between RDS and the proportion of grasslands in the catchments of the sampling sites (Percent GRAS) was positive (Figure 6C; $r = 0.66$, $p < 0.001$), which is expected given that the Mara River drains large areas of protected semi-arid areas in the MMNR, and conservancies used for livestock grazing.

Longitudinal trends in water quality variables

River distance from the source (RDS), and effectively stream size, had significant influences on many of the water quality variables as explored by GAMs (Figure 7). The stream size influenced dissolved oxygen and water temperature whereby DO concentration decreased with stream size, while water temperature increased as expected (Figure 7). Electrical conductivity had higher values as the distance increased from the sources (Figure 7). Interestingly, TSS and POM did not respond to changes in stream size, implying that other factors played a predominant role. Similarly, none of the nutrients responded to changes in stream size. Small agricultural

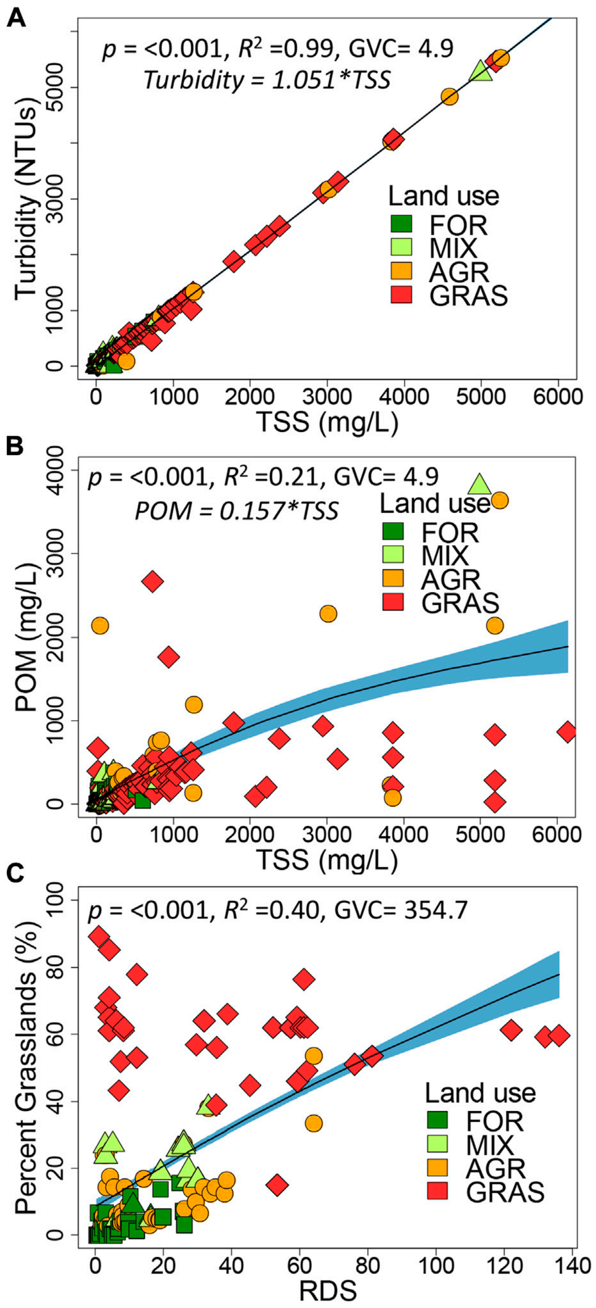

FIGURE 6
Relationships between different characteristics and water quality variables in the Mara River and its basin as explored using generalized additive models (GAMs). The relationships are between **(A)** TSS [mg/l] and turbidity [uS/cm], **(B)** TSS [mg/L] and POM [mg/L], and **(C)** RDS and Percent GRAS. To test the significance of the relationships, we fitted a GAM model with a smoothing function. The black line with light-blue shaded area represents smoother mean and s. e.; smoother significance, R^2 and GCV are supplied in the figures.

streams and large grassland streams had high nutrient concentrations which diminished the influence of stream size on nutrient concentrations.

Major ions showed the strongest positive response to increasing stream size. Four solutes Cl$^-$, SO$_4^{2-}$, Na$^+$ and Fe^{2+} showed a positive response while dissolved silica (DSi) did not. These relationships were driven by high concentrations in large river sections that were in grassland areas that are semi-arid and host large populations of livestock and large wildlife.

Discussion

In this study, we explored the influence of land use, seasonality and stream size or order as drivers of water quality in the Mara River using a dataset collected from 2010 to 2018. The results show that land use has the greatest influence on water quality and diminishes the influence of stream size as a predictor of water quality in the rivers. Specifically, agriculture and grazing were identified as the main drivers of water quality in the river with suspended sediments, turbidity, nutrients, and major ions (including conservative ions) having the highest concentrations in AGR and GRAS sites compared with FOR and MIX sites. Although seasonality played a major role in influencing water quality in the rivers, this influence is a likely amplification of land use effects through runoff, erosion and leaching of solutes. Despite the predicted influence of stream size on some physical and chemical characteristics of rivers, including different fractions of organic matter (Vannote et al., 1980), the influence of stream order on water quality was rather small in this study.

Influence of land use on water quality

The AGR and GRAS sites had lower levels of dissolved oxygen (DO) concentration, and higher levels of electrical conductivity, dissolved organic carbon and the ratio of C: N. These patterns have previously been reported in other Afrotropical rivers (Kroese et al., 2020; Dalu et al., 2022), but limited comparative data exist for Afromontane-savanna rivers. Specifically, the influence of land use and livestock grazing on water quality in rivers in semi-arid landscapes has been done anecdotally without basin-wide comparisons. This is of significance because agricultural land use and other human activities are likely to intensify as human populations in many African countries (averaging 2–3% p. a.) continue to grow. The most notable development over the years has been an expansion of agriculture and human settlements to

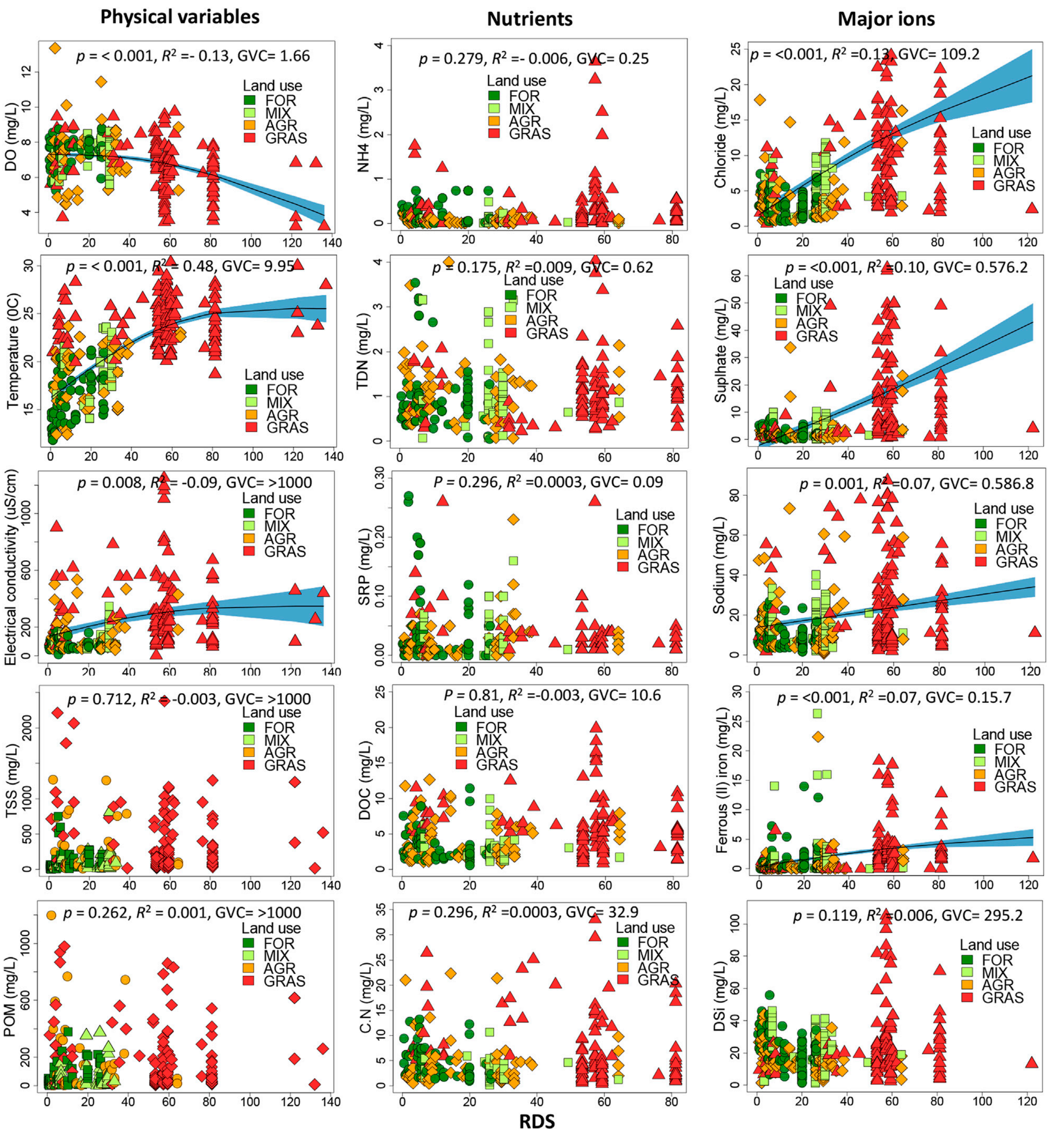

FIGURE 7
Longitudinal trends (changes with stream size) in water quality variables in the Mara River, Kenya as explored using generalized additive models (GAMs). RDS = river distance from source, determined as the square-root of catchment area in km^2. To test the significance of the relationships, we fitted a GAM model with a smoothing function. The black line with light-blue shaded area represents smoother mean and s. e.; smoother significance, *R2* and GCV are supplied in the figures that show significant relationships.

marginal (semi-arid) areas of the basin; while natural forests reduced significantly between 1986 and 2000, grasslands reduced even at a higher rate (Serneels et al., 2001; Mati et al., 2008).

The land use influence on water quality was clearly expressed in total suspended sediments, turbidity, electrical conductivity, nutrients and major ions (including conservative ions), which were higher in AGR, and GRAS sites compared with FOR and

MIX sites. These findings indicate that these water quality variables, especially electrical conductivity and major ions, respond to changes in catchment biogeochemical processes, such as runoff, mineralization and leaching of elements, that are brought about by land use change (Neill et al., 2006). Previous studies in Afrotropical rivers have also reported higher values of these measures for agricultural streams (Kibichii et al., 2007; Kasangaki et al., 2008).

Although AGR and GRAS sites had high concentrations of nutrients, the specific constituents differed between the two land uses. AGR sites had high amounts of NO_3^- and TDN while GRAS sites had high amounts of ammonia, SRP and TP. For AGR sites, high concentrations is linked to the use of nitrogenous fertilizers on farmlands by subsistence farmers. Studies have shown that the amount and timing of fertilizer application on farmlands have strong consequences on surface water quality (e.g., Potter et al., 2010). As in other highlands of the Rift Valley, Kenya, the headwaters of the Mara River basin experience a lot of use of nitrogenous and other types of fertilizers on horticultural farms and tea plantations (Maghanga et al., 2013; Jacobs et al., 2017). Kenya is one of the African countries with high usage of fertilizers (FAO, 2019) that can get into streams and rivers as part of runoff from agricultural lands. Previous studies in streams and rivers draining high potential agricultural lands have reported high levels of dissolved nitrogenous fractions potentially arising from fertilizer use on farmlands (Mokaya et al., 2004; Maghanga et al., 2013; Kilonzo et al., 2014; Jacobs et al., 2017; Masese et al., 2017).

Conversion of native forest cover to farmlands is also accompanied by changes in the composition of vegetation in the riparian zones of streams and rivers. One such common change in highland streams in Kenya is plantation forestry of exotic tree species, which has significant negative effects on aquatic ecosystems (Reinhart and VandeVoort, 2006). Most riparian zones of agricultural streams are often lined with non-native trees, such as *Eucalyptus*, which have been linked to changes in organic matter standing stocks, modified food webs and flow regimes, reduced leaf processing by invertebrates and changes in the composition of aquatic communities in streams (Cooper et al., 2013; Masese et al., 2014a; Silva-Junior et al., 2014). Alteration of riparian vegetation can also influence water quality and nutrient cycling in streams. For instance, the clearance of riparian vegetation can increase mean water temperature in streams and lead to the entry of sediments and nutrients from agricultural lands through runoff (Minaya et al., 2013; Fugère et al., 2018; Kroese et al., 2020).

The high concentrations of ammonia and phosphorous in GRAS sites are likely linked to the high numbers of livestock and large wildlife in these areas that deposit large amounts of organic matter and nutrients in water through egestion and excretion during watering or crossings (Kanga et al., 2011; Subalusky et al., 2015; Iteba et al., 2021). That ammonia behaved differently from the other N species suggests that other factors were involved in its occurrence in the AGR sites. Previous studies in the river reported low DO levels at sites inhabited by hippos, especially during the dry season when an accumulation of organic matter in pools can drive DO to hypoxic or anoxic levels, which leads to the accumulation of ammonia from hippo excretion (Dutton et al., 2018; Dutton et al., 2021). High amounts of ammonia in some of the GRAS sites influenced by livestock are also linked to excretion in water by the livestock during watering and crossings (Iteba et al., 2021). Livestock access to stream can mobilize sediments leading to increased levels of turbidity, and their excretion and egestion in water can increase nutrients and organic matter.

Both AGR and GRAS sites recorded high levels of turbidity and TSS. An increase in turbidity or TSS is one of the most reported negative influences of livestock on water quality (Meehan and Platts, 1978; Belsky et al., 1999; Kroese et al., 2020). The GRAS sites are in savanna grasslands that host high populations of livestock, which can mobilize soils and render them susceptible to erosion. Previous studies on erosion have shown that grazing lands generate the highest sediment yield (Dunne, 1979), and increased erosion in the Talek catchment has previously been attributed to cattle grazing (Glover and Wateridge, 1968). Similarly, large numbers of wildlife, especially hippos that spend 50% of their time in the river (Subalusky et al., 2015), can mobilize sediments through bioturbation, impacting downstream river reaches (Dutton et al., 2018; Iteba et al., 2021).

Compared to AGR sites GRAS sites had higher levels of POM, DOC and carbon to nitrogen ratio (C: N) (Figure 5). An increase in POM relative to TSS in GRAS sites is interesting. It points to the input of large amounts of organic matter by livestock and hippos, which can increase the levels of C in the water column relative to other suspended materials. A previous study has shown that CPOM in the Mara River is mainly composed of hippo faeces (Subalusky et al., 2017). Similarly, livestock and hippo subsidies in the form of dung are very high in C relative to N and P (Subalusky et al., 2015; Iteba et al., 2021; Masese et al., 2022). During decomposition, hippo and cattle dung can be major sources of C in water which would elevate the amount of DOC and C in water relative to N and P (Masese et al., 2018; Subalusky et al., 2018). In the Mara River, studies have shown that hippo egestion accounts for 88% of total carbon (C) inputs from hippos (Subalusky et al., 2018), and such levels of inputs can explain the high C to N ratio at GRAS sites.

Despite the overwhelming influence of land use on water quality in streams and rivers in our study area, there is evidence that some of the dissolved constituents (especially the major ions) are likely driven by climate and geology. While the concentrations of biogenic solutes that are involved in biological processes (e.g., nutrients and carbon) are largely driven by land use (or land cover) and anthropogenic activities (Worrall and Burt, 2007; Van Meter et al., 2018; Kim et al., 2020), the concentrations of elements released from rock weathering (e.g., the major ions in this study) are thought to be driven by geology (Gaillardet et al., 1999; Ibarra et al., 2016). GRAS sites differ from the rest of the sites in terms of climate (the area is largely semi-arid with seasonal flows) and geology, and this can partly explain the elevated levels of solutes in these sites. The humid uplands in the Nyangores and Amala sub-catchments are underlain by quaternary volcanic deposits and Cambisols, while tertiary deposits and Vertisols dominate in the semi-arid lowlands (Sombroek et al., 1982; Jackson and McCarter, 1994). In addition to these differences, grassland streams are also characterized by reduced canopy cover, which, together with the semi-arid conditions and high evaporation rates, can concentrate solutes leading to elevated levels of major ions in streams, especially during the dry season.

The role of seasonality

Considering the wide variability in climatic conditions across the MRB and similar Afromontane-savanna rivers, seasonality is bound to play a major role in determining water quality and availability, especially during the dry season. Apart from differences in discharge levels that can influence solute concentrations, seasonality also influences the interaction with and use of streams and rivers by both people and their livestock. It has been noted that during the dry season, the dependence on streams and rivers as a source of water for domestic use and livestock watering is very high (Yillia et al., 2008). This increased traffic in rivers by people and livestock can have negative consequences on water quality. Previous studies have shown that seasonality can increase inter-site differences during the dry season due to a predominance of reach-scale influences (e.g., daily animal and human disturbance (Mathooko, 2001; Yillia et al., 2008; Minaya et al., 2013; Masese et al., 2014b). In contrast, during the wet season, rainfall events can make conditions in rivers uniform and increase longitudinal connectivity that reduces inter-site differences (Leung et al., 2012).

TSS, POM and DOC are some physico-chemical variables that showed strong responses to seasonality with higher concentrations during the wet season. This is linked to runoff and erosional processes that deliver large amounts of terrestrial organic matter and sediments from terrestrial to aquatic ecosystems. During the wet season, the strength of association between rivers and their catchments is increased when hydrologic flowpaths are activated, leading to increased delivery of solutes and organic matter, including sediments and nutrients, into rivers (Elsenbeer, 2001; Saunders et al., 2006). The amount of sediments and organic matter increased significantly in AGR and GRAS sites. This can be attributed to increased rates of sheet and gully erosion caused by livestock, bioturbation by hippos and loading of organic by both livestock and hippos (Glover & Wateridge, 1968; Dunne, 1979; Dutton et al., 2018).

It is expected that major ions, especially the elements driven by geology (Gaillardet et al., 1999; Ibarra et al., 2016), would show a geologic signal and have high concentrations during the dry season and low concentrations during the wet season because of dilution by rainwater. Some ions, such as Cl^-, SO_4^{2-} and Na^+ displayed this response with higher concentrations during the dry season while, surprisingly, DSi and other major ions did not display significant differences between the dry and wet seasons (Figures 3, 4). In most surface waters, Na^+ usually originates from weathering of silicate minerals (Meybeck, 1987), implying that Na^+ and DSi would be highly correlated. This shows a disconnect in terms of the drivers of both Na^+ and DSi. In the Mara River, DSi cycling is strongly influenced by large wildlife through grazing silica-rich savanna grasses and depositing them into the river in the form of dung through defecation (Subalusky et al., 2015; Schoelynck et al., 2018). Through leaching and mineralization, the grass-rich dung becomes a major source of silica, nutrients and dissolved organic carbon in the river (Schoelynck et al., 2017; Masese et al., 2022). Indeed, grasses generally accumulate more silica than forest or agricultural vegetation (Schoelynck et al., 2014), and the rate of mineral weathering in grassland vegetation is higher than in forest ecosystems (Blecker et al., 2006). The daily defecation and excretion of cattle and hippos on the entire gradient of the Mara River (Iteba et al., 2021) is a continuous source of DSi and other nutrients that would diminish any seasonal patterns driven by geology, hence the limited seasonal responses in the concentrations of these variables in the river.

The higher concentrations of Cl^- and $SO_4^{2-}$and EC during the dry season is an indication of increased salinity of groundwater in the basin. An increase in groundwater salinity could be attributed to a slight increase in ionic concentration because of evaporation of water recharging the groundwater as well as the interaction of groundwater with the rock forming the geology of the area. Most of the major ions also recorded very high values in GRAS sites during the dry and wet seasons. In the GRAS sites in the semi-arid and seasonal tributaries of the Mara River, the flows are highly variable and flashy, with frequent flooding (Dutton et al., 2018). Reduced flows during the dry season can concentrate solutes and cause concentrations to be very high, as reported in this study.

Influence of stream size and longitudinal trends in water quality

The hypothesized influence of stream size or stream order on physico-chemical variables in the Mara River was not met. Only water temperature increased with an increase in stream size, as predicted from the opening of the canopy cover as rivers widen (Vannote et al., 1980) and the increase in ambient temperature occurring with lower elevation. The diminished influence of stream size on water quality was unexpected, but previous studies in this river have shown that land use change and human activities can alter biogeochemical processes in both low and high-order streams (Minaya et al., 2013; Mwanake et al., 2019). Similarly, reach-scale influences, such as watering of livestock, laundry washing and water abstractions by people and related disturbances abound along many rivers in the region leading to localized effects that can have a strong influence on water quality at the local scale (Mathooko, 2001; Yillia et al., 2008; Masese et al., 2009; Kroese et al., 2020).

Many attributes are predicted to have a linear or nonlinear response from low order to high order streams, e.g., water temperature, suspended sediments and the different fractions of organic matter (Vannote et al., 1980). However, in agreement with the diminished role of stream size on water quality variables in this study, several parameters did not respond to changes in stream size (Figure 4). As expected, there was a strong increase in water temperature, concomitant with a decrease in dissolved oxygen concentrations along the river (Figure 7). The electrical conductivity also increased downstream, probably linked to increased evaporation and accumulation of solutes in the semi-arid lower reaches of the river.

Expectedly, TSS and POM should increase with stream size because of the cumulative effects as low-order streams join to form large rivers. However, erosion from agricultural lands drained by the small streams and high livestock and wildlife (hippo) population in the lower reaches of the rivers can explain a lack of longitudinal trends in these two parameters, especially for TSS, as high levels were reported along the entire size gradient of the rivers (Figure 7). Similarly, there were no longitudinal trends in the concentrations of nutrients along the river. Similar levels were in small streams in agricultural areas and large river sections influenced by livestock and wildlife (hippos). Thus, the longitudinal patterns in TSS and nutrients observed in this study were confounded by changes in catchment land use and by the activities of livestock and large wildlife (Mwanake et al., 2019; Masese et al., 2022).

Among the water quality variables considered in this study, major ions showed the greatest response to changes in stream size. These patterns are typical of many other parameters that respond to changes in stream order. In the Lake Victoria basin, temperature, CPOM, FPOM and algal standing stocks (both benthic and water column) change with stream size, although expected patterns have been obscured by human activities that have modified riparian vegetation and in-stream conditions in rivers (Masese and McClain 2012).

Conclusion

The negative impacts of agriculture or land use in general on water quality in streams and rivers are a key concern for the catchment restoration and management of rivers in many regions around the world. Studies have shown that the influence of agriculture on water quality in streams does not only depend on the proportion of agricultural land in the catchment area, but also on the size and use of the riparian zone (Minaya et al., 2013; Kadeka et al., 2021). Similarly, low concentrations of suspended sediments and nutrients have been reported in agricultural streams that have a well-protected riparian zone (Kroese et al., 2020). These findings suggest that factors operating both at the catchment scale (e.g., the proportion of agricultural or urban land use) and local scale (e.g., the width and length of the riparian zone and its use) all have a role to play in explaining the quality of water in rivers (Schwarzenbach et al., 2010; Michalak, 2016). For water quality improvement, management efforts should focus to improve the conditions at both the catchment and local scales.

Our findings underline the role of land use, and most importantly, agriculture and grasslands, as drivers of water quality in Afrotropical rivers. Potential differences in catchment characteristics and, in our case, aridity and use (crop farming vs. grazing) need to be addressed in future studies to better constrain the effect of different land use practices and intensities on water quality in rivers. The results of this study are useful for stream restoration and management because they highlight the differences in water quality arising from agriculture and grazing. Furthermore, the findings show that responses in water quality to land uses might differ between regions of the same catchment that differ in climatic conditions (e.g., humid vs. semi-arid conditions). Thus, improved water quality in Afromontane-savanna rivers can only be improved by crafting management plans according to a region's specific climatic characteristics. In this regard, the potential role of climate and climate change on water chemistry and water quality in Afromontane-savanna needs to be explored, especially the linkage between discharge and water quality variables to better understand fluxes and other attributes of solute concentrations and transport in these rivers.

Data availability statement

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

Author contributions

EW, FM, GM, and GS: conceptualization, experimental design, sampling; EW and FM: data compilation and data analysis; EW: drafting and editing of the manuscript; All authors commented on the manuscript and approved it for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fenvs.2022.972153/full#supplementary-material

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Bioassessment of multiple stressors in Afrotropical rivers: Evaluating the performance of a macroinvertebrate-based index of biotic integrity, diversity, and regional biotic indices

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Many streams and rivers outside conservation areas across the Afrotropics face multiple stressors from land use change, urbanization, and excessive water withdrawals. Thus, there is a need to develop cost-effective tools for assessing and monitoring ecological changes to inform management decisions. Studies utilizing macroinvertebrate communities as indicators of the ecological condition of streams and rivers in the Afrotropics use diverse methods, including diversity, richness, biotic and multimetric indices. However, some of these indices are region- or country-specific, which limits their general use across multiple regions or countries. In this study, we address this challenge by testing and comparing the performance of diversity and richness indices (e.g., Shannon-Wiener and Simpson), regional biotic indices (the African Scoring System Version 5 [SASS5], Tanzanian River Scoring System [TARISS] and a biotic index developed for the Ethiopian highlands [ETHbios]), and a macroinvertebrate-based index of biotic integrity (M-IBI) in assessing the ecological condition of Afrotropical rivers with the transboundary Mara River, Kenya and Tanzania, as a case study. In this study, we analyzed water and habitat quality degradation caused by multiple stressors such as land use change, organic pollution and flow alteration and the corresponding responses in macroinvertebrate communities. We utilized macroinvertebrates data collected from 143 sites covering the entire gradient of the river and its major tributaries in Kenya and Tanzania. To develop the M-IBI, we used 12 metrics that describe macroinvertebrate community richness, composition, tolerance to disturbances (indicator taxa), and the composition of functional feeding groups. Although all the biotic indices were sensitive to poor water quality and human disturbance of the river, the M-IBI performed better than biotic indices (SASS5, Tanzanian River Scoring System, and Ethiopian highlands), diversity and richness indices by having a higher discriminatory ability of site categories according to different levels and types of disturbance. Diversity and richness indices performed poorly and failed to discriminate between stressor gradients in the river. This study demonstrates a need for testing and evaluating indices or protocols before adoption and use in biomonitoring streams and rivers in other countries and regions. There is an even greater need to assess the tolerance of macroinvertebrate taxa before inclusion in biotic indices for improved performance as discriminators of multiple stressors.

KEYWORDS

Afrotropics, biomonitoring, diversity, flow alteration effects, land use change, savanna rivers, water quality

1 Introduction

As frontiers and enablers of human civilization and development, freshwater ecosystems bear the most visible imprints of the Anthropocene (Dudgeon et al., 2006; Strayer and Dudgeon, 2010; Vörösmarty et al., 2010; Reid et al., 2019). Although inland waters in the Afrotropics are rich in biodiversity and endemism, they are some of the most threatened freshwater ecosystems in the world (Darwall and Freyhof, 2016; Barlow et al., 2018; Sayer et al., 2018). Streams and rivers are increasingly threatened by deforestation, habitat fragmentation, sedimentation, flow regulation (dam construction), urbanization, excessive water withdrawals, water pollution and biological invasions (Getahun and Stiassny, 1998; McClain et al., 2014; Fugère et al., 2016; Grill et al., 2019; Seeteram et al., 2019).

Identifying the major anthropogenic threats in aquatic ecosystems and understanding how biological communities respond to the changes is essential in developing bioassessment protocols for streams and rivers. Monitoring programs prefer biological indicators because they integrate both spatial (small- and large-scale) and temporal (short- and long-term) human disturbances (Karr and Dudley, 1981; Davies and Jackson, 2006; Hughes, 2019). Development of bioassessment or biomonitoring protocols involves determining the effects of pollution and other disturbances, whether natural or human-induced, on the presence or absence of species from a site or changes in species composition following disturbance (Barbour et al., 1999). The presence or absence data are used to assign tolerance scores or sensitivity weightings to the various species or taxa, with highly sensitive taxa assigned a high weighting. In contrast, highly tolerant taxa are assigned a low weighting. Biomonitoring assists in identifying regional biotic attributes and patterns, as well as potential sources and causes of degradation. It can also be used to detect and assess cumulative impacts and evaluate the effectiveness of pollution control and remediation activities (Barbour et al., 1999; Hering et al., 2006). Biomonitoring protocols and programs form part of the regular monitoring and managing of water quality and river health in riverine ecosystems worldwide (Buss et al., 2015). Compared to biological indicators, assessments of water chemistry, physical habitat structure, and landscape or riverscape condition usually explain less than half the variability in biological conditions and are extremely sensitive to sampling efforts and natural variability (USEPA, 2016; Hughes, 2019).

Biological communities have been used to assess the ecological status of many ecosystem types globally (Mangadze et al., 2016; Vadas et al., 2022; Ofogh et al., 2023). Although limited in comparison to other continents (e.g., Herman and Nejadhashemi, 2015; Ruaro et al., 2020; Feio et al., 2021; Vadas et al., 2022), several studies have used biological communities to study the condition of streams and rivers in Africa. These studies use heterogeneity (richness) and diversity indices (Odume and Muller, 2011; Olawusi-Peters and Ajibare, 2014; Soko and Gyedu-Ababio, 2015; Arimoro and Keke, 2017; Masese et al., 2020), regional or country-specific biotic indices (Dickens and Graham, 2002; Aschalew and Moog, 2015; Kaaya et al., 2015; Dallas et al., 2018; Musonge et al., 2020) and multimetric indices (Odume et al., 2012; Mereta et al., 2013; Lakew and Moog, 2015; Edegbene 2021; Edegbene et al., 2019; Tampo et al., 2020; Edegbene et al., 2022; Kaboré et al., 2022). Diversity indices provide a numerical measure of species diversity in a community based on composition and structure other than the number of species, while richness or heterogeneity indices are an indicator of the relative diversity of species in a community (Mouchet et al., 2010; Magurran, 2013). The use of diversity and richness indices as a measure of ecological condition assumes that their values decrease with environmental degradation and can reveal community compositional differences among sites or over time in response to disturbances (Lake, 2000; Ives and Carpenter, 2007; Sundstrom et al., 2017). In contrast, biotic indices employ the sensitivity values of different taxa in a community to develop an overall score of the ecological condition depending on the level of disturbance (Washington, 1984; Chessman and McEvoy, 1997; Dallas, 2021). The basis of biotic indices is the presence or absence of taxa (species, genus, family) in response to the severity of a particular stressor (Junior et al., 2015). Examples of macroinvertebrate-based biotic indices in Africa include the South African Scoring System Version 5 (SASS5; Dickens and Graham, 2002), the Tanzania River Scoring System (TARISS; Kaaya et al., 2015) and the biotic score (ETHbios) developed for the Ethiopian highlands (Aschalew and Moog, 2015).

Compared to species diversity, richness, and biotic indices, multimeric indices (MMIs) integrate a community's biological attributes or metrics into a single index that is responsive to different forms of disturbance (Karr, 1981; Hughes et al., 1998; USEPA, 2016). In this regard, a community attribute or metric is defined as a calculated term or enumeration representing some aspect of biological assemblage structure, function or other measurable characteristics that changes predictably with human influence (Barbour et al., 1995). MMIs are effective for biomonitoring because they are better at identifying and discriminating the effects of different stressors on ecological conditions (Hering et al., 2006; Lunde and Resh, 2012). They are considered one of the best cost-effective methods for biomonitoring aquatic ecosystems (Bonada et al., 2006; Ruaro and Gubiani, 2013). Because of their popularity, published literature reviews have shown that MMIs have been used to evaluate water quality and the overall ecological integrity of aquatic ecosystems globally (Resh and

Jackson, 1993; Buss et al., 2015; Herman and Nejadhashemi, 2015; Ruaro et al., 2020; Eriksen et al., 2021). In Africa, macroinvertebrate- and fish-based MMIs have been developed and used in different regions, including eastern Africa (e.g., Masese et al., 2009a; Aura et al., 2010; Raburu and Masese, 2012; Achieng et al., 2021), southern Africa (e.g., Hocutt et al., 1994; Kleynhans, 1999; Odume et al., 2012) and western Africa (e.g., Hugueny et al., 1996; Thorne and Williams, 1997; Toham and Teugels, 1999; Tampo et al., 2020; Kaboré et al., 2022).

Despite the broad use of diversity, richness, biotic and multimetric indices in the African continent, studies that compare the efficacy and applicability of these different indices in assessing water quality and the overall ecological condition of streams and rivers are limited. Specifically, most biotic and multimeric indices have not been standardized or tested for evaluating aquatic ecosystems beyond regions or countries where they were first developed (Masese et al., 2013; Mangadze et al., 2019; Dallas, 2021). Additionally, most countries in Africa lack a legal provision for the use of biological criteria as a basis for assessing the condition of surface waters at the national level, which would require defining and standardizing biomonitoring protocols or tools used for this purpose (Dallas, 2021; Masese et al., 2022). Nevertheless, biotic indices and MMIs based on benthic macroinvertebrates are growing across the continent, with some being used without testing and validation. For instance, while SASS was developed for assessing water quality and the ecological condition of South African rivers (Dallas, 1997; Chutter, 1998; Dickens and Graham, 2002), version five of SASS (SASS5) has been applied without modification in other countries in southern and eastern Africa, including Zimbabwe (Bere and Nyamupingidza, 2014; Mwedzi et al., 2016), Swaziland (Mthimkhulu et al., 2004) and Kenya (Oigara and Masese, 2017; Mbaka et al., 2014; M'Erimba et al., 2014). Similarly, TARISS, which is a modified version of SASS5 developed for assessing streams and rivers in Tanzania, has recently been applied in Rwanda (Dusabe et al., 2019) and Uganda (Tumusiime et al., 2019; Turibamwe and Wangalwa, 2020; Ochieng et al., 2021) without modifications. Similarly, while some MMIs have been tested and validated for monitoring of some of the African aquatic ecosystems (e.g., Raburu and Masese, 2012; Moges et al., 2016; Tampo et al., 2020; Achieng et al., 2021; Kaboré et al., 2022), some have been used without validation, including Toham and Teugels (1999), Masese et al. (2009a), Aura et al. (2010), Alemu et al. (2018) and Aura et al. (2021). This means that these biotic indices and MMIs must be tested for performance and validated before being used widely to assess the ecological conditions of streams and rivers across the African continent (e.g., Bere and Nyamupingidza, 2014; Musonge et al., 2020).

By offering various responses to multiple stressors, macroinvertebrates are among the most widely used bioindicator organisms in streams and rivers (Barbour et al., 1999; Raburu, 2003; Birk et al., 2012; Carter et al., 2017; Ruaro et al., 2020). In addition, macroinvertebrates have many practical advantages for bioassessments, including a wide distribution in most streams and rivers, ease of sampling and identification at the family level, and the sampling equipment is relatively inexpensive (Rosenberg and Resh, 1993; Barbour et al., 1999; Bonada et al., 2006). However, in the Afrotropics, high-elevation, low-order streams have a very low diversity of fishes (Kleynhans, 1999; Mangadze et al., 2016; Raburu et al., 2022), which is the alternative bioindicator group for use in river ecosystems (Barbour et al., 1999). This low diversity of fishes makes macroinvertebrates the only best alternative available biotic group for biomonitoring headwater or high-elevation streams.

In this study, we tested the applicability and performance of diversity and richness indices, regional biotic indices, and a macroinvertebrates-based index of biotic integrity (M-IBI) to assess the influence of multiple stressors on the ecological condition of the Afromontane-savanna Mara River in Kenya/Tanzania. We focused on the Mara River as a case study because it is transboundary between Kenya and Tanzania. The river also experiences multiple stressors arising from land use change, soil erosion and nutrient runoff from farmlands and grazing areas, excessive water withdrawals, organic matter and nutrient input by livestock and large wildlife, human waste from urban settlements and tourism establishments, and mercury from artisanal gold mining in the lower basin. The specific objectives were to; 1) determine structural responses in macroinvertebrates to changes in water quality across different land uses and stressors in the river, 2) use regional indices (ETHbios, SASS5 and TARISS) and macroinvertebrate index of biotic integrity (M-IBI) to assess the ecological condition of different sites in the river, and 3) compare the sensitivity of the different groups of indices (diversity and richness indices, biotic indices and the M-IBI or MMI) as indicators of water quality and ecological condition of the river.

2 Materials and methods

2.1 Study area

This study was conducted in the Mara River basin (Figure 1). The river originates from the Mau Escarpment and Loita Hills in Kenya and drains into Lake Victoria in Tanzania. In the headwater, the Mara River drain remnants of tropical broadleaf forests interspersed with plantations of tea and mixed agriculture (crop farming and livestock rearing). Until the middle of the past century, the river basin was covered by extensive Afromontane forests in its headwaters and a mixture of shrublands and grasslands throughout its middle and lower reaches (Mati et al., 2008). However, extensive loss of the native forests, grasslands and shrublands to human settlements, agricultural and grazing lands has occurred over the years resulting in reduced coverage of natural vegetation in the basin (Serneels et al., 2001; Mati et al., 2008; Mango et al., 2011). The land use changes have resulted in 27% reduction in grasslands and shrublands, 32% reduction in forest cover, and 203% increase in agriculture (Mati et al., 2008).

The Mara River basin transcends varying climatic conditions that range from the humid uplands, and the sub-humid upper middle reaches on the slopes and base of the Mau Escarpment, and the semi-arid middle and lower reaches in Kenya and Tanzania. Two perennial tributaries, the Nyangores and Amala, drain the humid and sub-humid uplands and join to form the Mara River mainstem (Figure 1). In the middle and lower reaches, all tributaries draining the grasslands and shrublands are ephemeral, including the Talek and Sand Rivers, despite their extensive drainage basins. The Talek and Sand Rivers drain the Kenyan part before the Mara River crosses into Tanzania. In Tanzania, most tributaries draining the

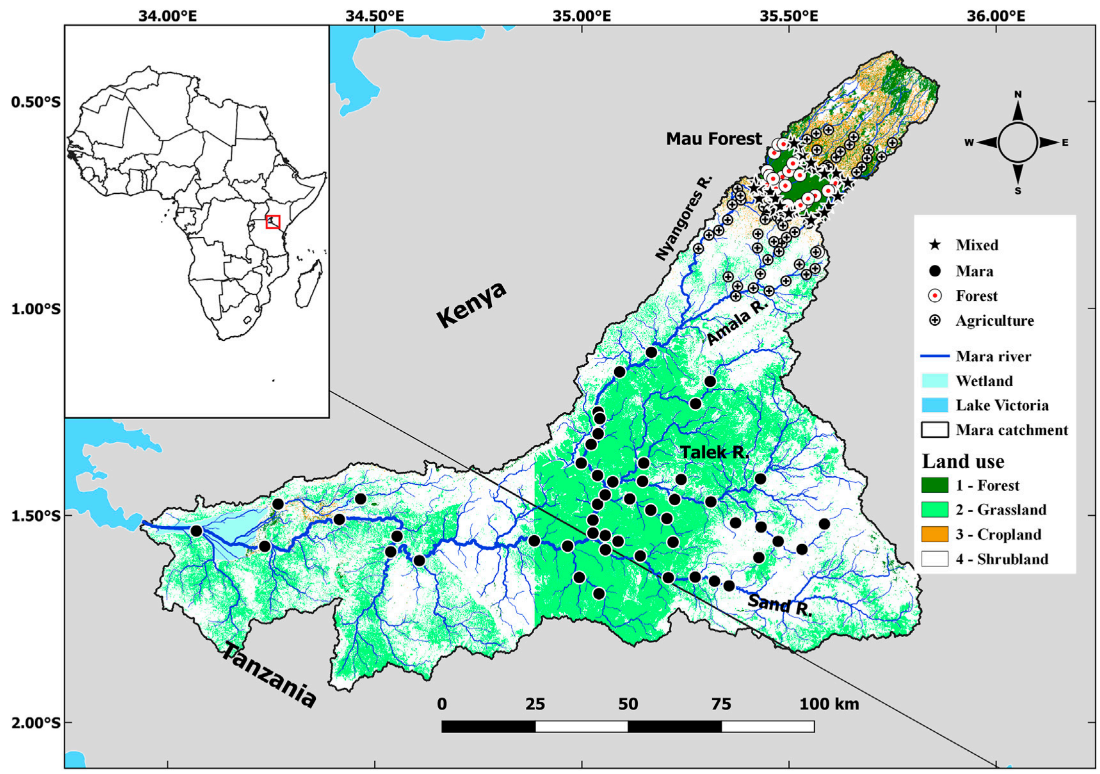

FIGURE 1
Map of the Mara River catchment showing the position of the sampling sites.

Serengeti grasslands and adjoining areas are seasonal, including the Gurubi, Nyarwera, Nyarusondobiro, Tobora, Somoche and Tigithe. The Tigithe River is the last of the major tributaries in the lower reaches that join the Mara River before entering the extensive rivermouth wetland and is one of the most impacted by gold mining activities.

On the Mau Escarpment, the climate is cooler and seasonal due to the high altitude, characterized by two dry and wet seasons. The amount of rainfall varies across the basin depending on altitude, with the highlands receiving around 2,000 mm of rain per annum (pa) while the lowlands receive around 1,000 mm pa (Camberlin et al., 2009). Dry conditions are experienced during January-March and wet conditions during March-July and October-November, which are periods for the “long rains” and “short rains,” respectively. However, variations sometimes occur at the onset and end of the rainy and dry periods. Potential evapotranspiration varies between 1,400 mm in the highlands to 1,800 mm in the lowlands (Jackson and McCarter, 1994).

The Mara River is one of the most important rivers in east Africa as the only permanent water source for large wildlife in the Serengeti - Mara Ecosystem in northern Tanzania and southern Kenya. It acts as a signpost for wildebeests that cross the river multiple times during their annual migrations (Gereta et al., 2009; Holdo et al., 2009). The Mara River basin also supports over one million people, with over 60% directly reliant on the river for watering their livestock and domestic water supply (Hoffman et al., 2011). The basin is also ecologically diverse, with the wetter upper reaches supporting Afromontane forests and mixed but intensive agriculture of both livestock and subsistence crops. In contrast, the drier middle reaches outside conservation areas support both small- and large-scale cultivation of subsistence and cash crops and livestock grazing. However, pressures on the conservation areas both in the headwater and middle reaches are high, and there is evidence that the changing land use has had significant effects on water quality and the natural flow regime of the river and its tributaries (Melesse et al., 2008; Kilonzo, 2014; McClain et al., 2014).

2.2 Study design

A total of 143 sites were sampled in this study. The sites cover the Mara River and its tributaries in Kenya and Tanzania. Most of the sites have been sampled before for other ecological studies, and these provided some of the historical data used in this study (Minaya et al., 2013; Masese et al., 2014a; Masese et al., 2014b; Kilonzo, 2014; McClain et al., 2014). Additional sites were selected along the Talek and Sand Rivers in Kenya, and other tributaries in Tanzania for sampling to represent the basin’s highly hydrologically variable and modified streams and river reaches.

Sites were grouped into four site categories depending on the percentages of the forest, and agricultural land uses and levels of stressors they were experiencing. The percentages of forest, grasslands, and agricultural land use in the entire catchment upstream of the sampling sites were calculated based on the Digital Elevation Model of Kenya (90 m by 90 m), obtained from the Shuttle Radar Topography Mission (Masese et al., 2017; Wanderi et al., 2022). Sub-catchments were delineated, and the area of each major land use category upstream of each sampling site was calculated. Forest (FOR) and agriculture (AGR) sites drained catchments with the proportion of catchment land use under forestry and agriculture >60%, respectively. Mixed (MIX) sites did not meet the catchment land use criteria for GRAS and AGR sites and had an intermediate mixture of two or three major land use types. The sites were grouped into four site categories that were; 1) Forested sites (FOR), which represented minimally disturbed sites in forested areas that acted as a reference for the rest of the sites, 2) Agricultural sites (AGR) that were located in agricultural areas, 3) Mixed sites (MIX) were located in catchments draining forested and agricultural land uses, and 4) Mara sites (MARA) were located in the Mara River mainstem and tributaries where the stressors were flow variation, loading of organic matter and nutrients and trampling by livestock and large wildlife (mainly hippopotami). FOR sites in the Mau Forest Complex in the headwaters of the Amala and Nyangores rivers were used as reference sites. Along FOR streams, the riparian corridor was largely intact, with a mixture of indigenous vegetation throughout the catchment. Compared to AGR sites, water quality in FOR streams was less turbid even immediately after storm events.

2.3 Sampling methods

We sampled sites during the dry season in January-March and July-August and the wet season in April-June and October-December from 2012 to 2018. During the dry season, water flow levels were significantly low, with low turbidity, and there was more excellent bed stability. Sampling habitats were also more distinguishable and accessible, and macroinvertebrates were most abundant but not necessarily in terms of taxa richness (Melo and Froehlich, 2001; Hughes and Peck, 2008). As opposed to the dry season, the wet season flows were fast and deep, and the water was more turbid. The deep water and fast flows made it challenging to access some of the permanent habitats in the middle of the large rivers for macroinvertebrates sampling. This meant that in some cases, the biotopes sampled were recently inundated, resulting in low abundances of macroinvertebrates.

2.3.1 Environmental variables

The water chemistry and physical variables measured included physical measures, nutrients, and major ions. The data on physical measures had river width, depth, discharge, pH, dissolved oxygen (DO), temperature, electrical conductivity, turbidity, and total suspended solids (TSS). *In situ*, water quality physico-chemical parameters were measured using portable probes (YSI multi-probe water quality meter (556 MPS, Yellow Springs Instruments, Ohio, United States). They include pH, dissolved oxygen (DO) concentration, temperature, and electrical conductivity. Turbidity was measured using a portable Hach turbidity meter (Hach Company, 2100P ISO Turbidimeter, United States). Water samples were collected from the thalweg using acid-washed HDP bottles to analyse nutrients, dissolved organic carbon (DOC), and major ions. For total suspended solids (TSS), water samples were filtered immediately through pre-weighed and pre-combusted glass-fibre filters (Whatman GF/F, pre-combusted at 450°C, 4 h). GF/F filters holding suspended matter were carefully folded and wrapped in aluminium foils before transport in a cooler box at 4°C to the laboratory. The filtered and unfiltered water samples were stored and transported in a cooler box and kept frozen until analysis.

The nutrients measured in the laboratory included TSS, DOC, ammonium (NH_4^+), nitrates (NO_3^-), total dissolved nitrogen (TDN), total nitrogen (TN), soluble reactive phosphorus (SRP), total phosphorus (TP) and dissolved organic carbon (DOC). The major ions analysed included sodium (Na^+), potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), aluminium (Al^{3+}), dissolved silica (DSi), chloride (Cl^-), fluoride (F^-), and sulphate (SO_4^{2-}). GF/F filters holding suspended matter were dried (95°C) to constant weight, and TSS was determined by re-weighing on an analytical balance and subtracting the filter weight. DOC and TDN concentrations were determined using a Shimadzu TOC-V-CPN with a coupled total nitrogen analyzer unit (TNM-1). TN, SRP, TP were determined using standard colorimetric methods (APHA, 1998). Major anions, including nitrate (NO_3^-), chloride (Cl^-), fluoride (F^-) and sulphate (SO_4^{2-}) were determined using a Dionex ICS-1000 ion chromatographer. The major cations sodium (Na^+), potassium (K^+), ferrous iron (Fe [II]), calcium (Ca^{2+}), magnesium (Mg^{2+}), dissolved silica (DSi) and NH_4^+ were analyzed using an ICP-MS. All variables are expressed in mg/L except pH (–), electrical conductivity ($\mu S\ cm^{-1}$), water and air temperatures (°C) and turbidity (NTUs). Concentrations below the detection limit were set to the detection limit.

2.3.2 Sampling of macroinvertebrates

Within each sampling site, representative river reaches were selected for macroinvertebrates sampling. Each sampling site provided a range of biotopes (riffles, pools, marginal vegetation) with varying velocities, depths and substrate types that ranged from gravel, sand, and mud (GSM) to boulders and bedrock. Sampled biotopes ranged from GSM, stones-in-current and stones-out-of-current (STONES) and aquatic and marginal vegetation (VEG) (Dickens and Graham, 2002; Dallas, 2007).

We used sampling methods in the South African Scoring System Version 5 (SASS5; Dickens and Graham, 2002) and Tanzanian River Scoring System (TARISS; Kaaya et al., 2015). The sampling methods in these two biotic indices are similar and use the SASS net, a modified kick net measuring 300 mm by 300 mm with a mesh size of 1,000 μm. Several biotopes were sampled within a prescribed time limit and/or areal coverage. The stones-inside-current (SIC) and bedrock were searched ("kicked") for 2–5 min. Similarly, stones-out-of-current (SOOC) and bedrock were searched for 1 min. The SIC and SOOC samples were combined into a "Stones" (STONES) sample. Suitable stretches covering 2 m^2 of marginal vegetation (SIC and OOC) were swept as well as aquatic vegetation covering 1 m^2. This represented the VEG sample. Gravel, sand, and mud (GSM sample) were stirred and swept for 1 min and checked for macroinvertebrates. Hand-picking and visual observation was also employed for 1 min, and biotopes with macroinvertebrates were recorded. Scoring was done for 15 min per biotope but stopped when no new taxa were seen after 5 min. As a modification to the SASS procedure, all samples from the three habitats, Stones, GSM and Vegetation, were preserved in formalin in separate containers and taken to the laboratory for further processing and enumeration of individuals of the various taxa.

These data were needed for statistical analyses of the macroinvertebrate samples.

In the laboratory, macroinvertebrate samples were sorted, enumerated, and identified to the lowest taxon level possible, mainly family level. Many taxonomic keys were used, such as Gerber and Gabriel (2002), Day and de Moor (2002a), Day and de Moor (2002b), de Moor et al. (2003a), de Moor et al. (2003b) and Merritt and Cummins (2008).

2.4 Analysis of macroinvertebrates data

2.4.1 Taxa richness and diversity indices

Data on macroinvertebrates samples collected were pooled for each sampling site per season to analyze various indices. Community composition was summarized for each sampling site using the number of species (S), the total number of individuals (N), richness and diversity indices, and the relative abundance of each taxon. Several site-level diversity and richness indices were calculated by separately summing species-specific abundances across biotopes for each season. The Shannon index (H') was used as a measure of diversity (Shannon, 1948), and an associated $H'/H'_{\max}$ index (Pielou, 1975) was used for evenness. The reciprocal form of the Simpson Index ($1/Ds$) (Simpson, 1949) was used as a measure of community richness. We used Hill's number (i.e., gamma diversity; Hill, 1973) and Fisher's alpha (Fisher et al., 1943) as extra measures of community diversity. Hill's number was calculated as the ratio between H' and $1/Ds$. Other diversity indices calculated included Brillouin's (Brillouin, 1956), Menhinick's (Menhinick, 1964), Margalef's (Margalef, 1958), Equitability (Lloyd and Ghelardi, 1964), Berger-Parker (Berger and Parker, 1970), and Chao-1 (Chao, 1984).

These indices were used to measure different aspects of macroinvertebrate diversity in the river. The first group of indices consists of dominance and evenness indices, which are sensitive to the abundance of the most common species rather than species richness (Simpson, Berger-Parker, Chao1, evenness and equitability). The second group consists of diversity and richness indices, focusing on the number of species in relation to the number of individuals (Fisher, Hill, Margalef and Menhinick); and lastly, indices based on information theory, which takes into consideration evenness and species richness components of diversity (Shannon and Brillouin).

2.4.2 Biotic indices

We used SASS5 (Dickens and Graham, 2002), TARISS (Kaaya et al., 2015) and the ETHbios biotic index, which was developed to assess ecological conditions in the highlands of Ethiopia (Aschalew and Moog, 2015), to evaluate the ecological conditions of the sampled study sites. For SASS5 and TARISS, we identified macroinvertebrate specimens in the field to the family or higher levels per the protocols. Taxa that were identified in the field and recorded in the SASS5 Score sheet, and the total abundances estimated for each taxa (Dickens and Graham, 2002; Kaaya et al., 2015). The SASS5 and TARISS Score and their associated average score per taxon (ASPT) were used to characterize macroinvertebrates at each site (Dickens and Graham, 2002). For ETHbios, the list of taxa encountered at each site was subjected to the sensitivity scores developed for Ethiopian highlands to obtain the total ETHbios score and its related ASPT.

2.4.3 Macroinvertebrate index of biotic integrity (M-IBI)

Community composition data at the site level were also used to develop a macroinvertebrate index of biotic integrity (M-IBI) for the Mara River. Metrics for the index were selected following the performance on range, responses to changes in water quality, redundancy with other metrics, signal-to-noise ratio (repeatability), seasonal stability and ecological significance (Kurtz et al., 2001; Klemm et al., 2003). We eliminated candidate metrics that failed a test from additional consideration and testing. In the range test, we required that the difference between the value of a metric was >10 for percentage or proportion metrics and >5 for taxa richness or diversity metrics. Regarding the response to water quality changes, metrics were required to correlate with at least one of the water quality physico-chemical variables. Redundancy in the remaining metrics was evaluated using Spearman correlation coefficients and visual inspection of scatter plots. Metrics with a correlation coefficient $r \geq 0.85$ were considered redundant, and only one was selected for inclusion in the final M-IBI (Hughes et al., 1998).

We used the signal-to-noise ratio (S/N) to assess the reproducibility of metrics, i.e., to ensure that between-site differences in metrics are caused by differences in site conditions rather than by sampling variation within a site (Stoddard et al., 2008). The S/N is the ratio of variance among sites (signal) to the variance of repeated visits at the same site (noise; repeated seasonal sampling of the same site in our case) (Kaufmann et al., 1999; Stoddard et al., 2008; Stevenson et al., 2013). To represent the S/N ratio, we used the F-ratios in an analysis of variance to evaluate a metric's ability to discriminate differences among sampling sites over the "noise" of measurement variation (Kaufmann et al., 1999). We used one-way ANOVA to calculate F-ratio and to calculate S/N as follows:

$$S/N = \frac{F-1}{C_1}$$

Where F is the ratio between the F in one-way ANOVA among sites and F in one-way ANOVA within sites, and C_1 is a constant varying between one and r, the number of times the repeat-sample sites are visited (Neter and Wasserman, 1974). Metrics with higher S/N have greater reproducibility and serve as reliable indicators of differences in condition among sites (Esselman et al., 2013). Metrics with S/N higher than two (2) were retained for further consideration (Stoddard et al., 2008). A low S/N ratio indicated that a metric didn't distinguish well among sites.

Seasonal stability in metrics was evaluated by box-and-whisker plots, whereby those metrics that failed to discriminate the reference and disturbed site categories during both the dry and wet seasons in different years were eliminated from further consideration. Metrics were also included in the final M-IBI following their proven utility in previous multimetric indices developed in the region (e.g., Masese et al., 2009a; Raburu et al., 2009a; Aura et al., 2010). The metrics fall into five categories that describe taxa richness, community composition, tolerance of the various taxa to disturbances (indicator individuals or taxa), and functional feeding groups.

Metrics sensitive to changes in substrate type or quality, channel stability and flow variation were also selected for inclusion in the M-IBI for the Mara River.

2.5 Data analysis

Differences in water quality physico-chemical variables among the four condition categories were explored separately for each season using One-way Kruskal-Wallis ANOVA. The ability of the different macroinvertebrate indices (diversity, richness, biotic and multimetric) to discriminate among the four site or condition categories was explored using box-and-whisker (box) plots. We defined separation power as the degree of overlap between boxes (i.e., 25th and 75th quartiles) in box plots of the values of the indices for all condition categories (Barbour et al., 1996; Barbour et al., 1999). The derivatives of the biotic indices tested included the total number of taxa, total site scores (SASS5, TARISS and ETHbios) and their associated average score per taxon (SASS5-ASPT, TARISS-ASPT, and ETHbios-ASPT).

We used Spearman correlations to test for relationships among diversity and biotic indices and the M-IBI. Before analyses, macroinvertebrates abundance (count) data and water quality physico-chemistry data were log $(x + 1)$ and square-root transformed, respectively. Metrics calculated as proportions were normalized by Arcsine-square-root transformations before analysis. Analyses were performed using SigmaPlot Version 12. Statistical tests were considered significant at $p < 0.05$.

We used the continuous method to score individual metrics. Positive scoring metrics (which declined with increasing water quality degradation or disturbance) were scored 0–10 points: 0 points for values less than the 5th percentile of impacted sites and 10 points for values greater than the 50th percentile of high-quality reference sites. Negative scoring metrics (which increased with increasing water degradation or disturbance) were scored 0–10 points: 0 points for values greater than the 90th percentile of impacted sites and 10 points for values less than the 50th percentile of reference sites. These percentiles were chosen to maximize the discrimination among sites for each metric. Metric scores were linearly interpolated between 0 and 10. We felt continuous scoring over the individual metric ranges of 0–10 led to a more precise index than traditional methods (Hughes et al., 1998). To scale the index from 0 to 100 points, we summed the M-IBI score of the individual metric scores for each of the twelve metrics times 0.83.

Four condition categories of excellent, good, fair, and poor, using percentiles of frequency distributions of M-IBI scores at reference and impaired sites, were established to distinguish the different environmental conditions of the sampling sites (Table 8). The M-IBI scores exceeding the 75th percentile for reference sites (M-IBI >81) were classified as having "excellent" biotic integrity, and scores between the 75th and 25th percentiles (66 < M-IBI ≤81) were identified as having "good" biotic integrity. M-IBI scores between the 5th and 25th percentile for reference sites (M-IBI = 52–66) were identified as being in a "'fair" condition, and scores below the 5th percentile for reference sites (M-IBI <2) were defined as in a "poor" condition.

3 Results

3.1 Environmental variables

There were seasonal and spatial variations in water quality variables and solutes in the study area (Table 1). For most site categories, levels of *in situ* physico-chemical variables (DO, electrical conductivity and temperature) and major solutes (Al, Ca, Mg, K, Na, F, Cl, SO_4, and DSi) were higher during the dry season compared with the wet season (Table 1). In contrast, variables related to water clarity (turbidity and TSS) had elevated levels during the wet season compared to the dry season. Non-significant differences were found in nutrient concentration temporally.

There were no significant differences in stream/river width and depth between the site categories (Table 1). Discharge only differed between the sites during the wet season, with sites in the Mara River mainstem recording significantly higher discharge. Apparent differences in water physico-chemistry were observed between the site categories (Table 1). While forest (FOR) and Mara sites (MARA) showed differences in most physico-chemical variables, for some variables, differences with agricultural (AGR) and mixed (MIX) sites were not clearcut, and levels lay between the FOR and MARA sites. For nutrients, the lowest levels were recorded in FOR sites, while AGR and MARA sites had the highest concentrations of ammonium, SRP, TN, and TDN. Similarly, AGR and MARA sites recorded the highest concentration of dissolved organic carbon (DOC), and FOR sited recorded the lowest concentration.

3.2 Diversity indices

There were significant correlations between water quality variables and the total number of taxa (S), total number of individuals (N) and diversity indices (Table 2). The total number of species and abundance displayed the largest number of correlations, 9 and 11, respectively, out of the 22 water quality variables tested in this study. Among the diversity indices, Menhinick's index had the highest discriminant ability by having strong correlations with nine (9) water quality variables. Evenness and equitability indices had the second-highest discriminant ability with eight (7) correlations. In contrast, Simpson's, Shannon's, Hill's, Brillouin's, Margalef's, and Berger-Parker's indices had six (6) correlations with water quality variables each. Finally, Fisher's diversity index had the lowest number of correlations (4) with water quality variables (Table 2).

3.2.1 Performance of diversity indices

The ability of the diversity indices and the total number of taxa and families to discriminate the different levels of disturbance among the four site categories were explored using box-and-whisker plots (Figures 2, 3). During the dry season, the number of taxa and the number of families were highest in MARA sites, but this was not different from the FOR sites (Figure 2). However, the FOR sites weren't separated from the MIX and AGR sites. Although most diversity indices showed a decline from FOR sites which had the highest values, to MIX and AGR sites, which recorded the lowest values, they weren't able to discriminate between FOR and MARA

TABLE 1 Variability in mean (±SD) water quality physico-chemical variables across site categories in the Mara River Basin during the dry and wet seasons. *p*-values in boldface are significant at $p < 0.05$.

Variables		Site category				K-W ANOVA	
	Season	For	MIX	AGR	Mara	H-value	*p*-value
Width (m)	Dry	2.1 ± 2.6^{b}	2.4 ± 0.7^{ab}	7.2 ± 7.3^{a}	2.1 ± 1.9^{b}	7.7	0.054
	Wet	1.9 ± 1.82^{a}	2.2 ± 2.7^{a}	2.4 ± 3.5^{a}	5.8 ± 7.6^{a}	1.6	0.656
Depth (m)	Dry	0.1 ± 0.06^{a}	0.09 ± 0.03^{a}	0.13 ± 0.1^{a}	0.15 ± 0.1^{a}	2.0	0.579
	Wet	0.12 ± 0.11^{a}	0.10 ± 0.06^{a}	0.12 ± 0.08^{a}	0.20 ± 0.10^{a}	3.7	0.298
Discharge (m^3/s)	Dry	0.8 ± 1.6^{a}	2.4 ± 3.9^{a}	1.6 ± 0.7^{a}	3.8 ± 4.1^{a}	5.4	0.148
	Wet	1.2 ± 2.4^{b}	11.2 ± 18.9^{a}	9.3 ± 19.0^{a}	0.53 ± 0.64^{b}	8.3	**0.041**
pH (units)	Dry	7.3 ± 0.2^{a}	7.84 ± 1.0^{a}	7.29 ± 0.4^{a}	7.4 ± 0.6^{a}	2.3	0.522
	Wet	7.7 ± 0.4^{a}	7.4 ± 0.4^{ab}	7.2 ± 0.6^{ab}	7.2 ± 0. 5^{b}	9.3	**0.026**
Conductivity (μS/cm)	Dry	100.7 ± 58.1^{b}	237.7 ± 210.5^{ab}	165.7 ± 157.6^{ab}	371.7 ± 217.8^{a}	9.8	**0.022**
	Wet	90.0 ± 32.2^{b}	95.5 ± 39.2^{ab}	163.5 ± 111.2^{a}	121.6 ± 65.4^{ab}	4.1	**0.024**
Dissolved oxygen (mg/L)	Dry	7.0 ± 1.1^{a}	7.3 ± 0.8^{a}	5.7 ± 2.4^{ab}	4.6 ± 1.0^{b}	7.9	**0.047**
	Wet	6.6 ± 0.9^{a}	6.7 ± 0.9^{a}	3.4 ± 3.8^{b}	7.1 ± 1.0^{a}	5.3	0.153
Temperature (°C)	Dry	15.1 ± 4.1^{b}	22.7 ± 3.8^{a}	20.7 ± 3.0^{a}	24.5 ± 3.6^{a}	17.2	**<0.001**
	Wet	15.3 ± 1.90^{a}	15.6 ± 1.9^{a}	15.8 ± 6.7^{a}	18.9 ± 3.4^{a}	6.4	0.092
Turbidity (NTUs)	Dry	39.2 ± 62.8^{b}	458.3 ± 244.1^{a}	342.4 ± 404.6^{ab}	202.1 ± 228.4^{ab}	14.3	**0.003**
	Wet	41.2 ± 45.4^{b}	198.8 ± 243.5^{ab}	275.4 ± 328.1^{a}	302.3 ± 288.2^{a}	12.1	**0.007**
Total suspended solids (mg/L)	Dry	22.8 ± 20.3^{b}	28.3 ± 15.2^{b}	296.5 ± 427.3^{a}	203.8 ± 303.7^{a}	11.4	**0.012**
	Wet	46.0 ± 48.61^{b}	48.9 ± 34.4^{ab}	295.3 ± 300.2^{a}	284.2 ± 360.1^{ab}	8.6	**0.035**
Ammonium (mg/L)	Dry	0.10 ± 0.08^{a}	0.21 ± 0.3^{a}	1.2 ± 2.6^{b}	1.7 ± 0.2^{b}	9.0	**0.031**
	Wet	0.23 ± 0.09^{b}	0.26 ± 0.24^{ab}	1.6 ± 2.5^{a}	0.28 ± 0.28^{ab}	3.5	0.317
Nitrates (mg/L)	Dry	0.48 ± 0.5^{a}	1.24 ± 1.1^{a}	0.97 ± 1.1^{a}	2.4 ± 2.8^{a}	4.1	0.253
	Wet	0.83 ± 1.11^{a}	0.93 ± 1.30^{a}	0.55 ± 0.51^{a}	1.3 ± 1.5^{a}	1.8	0.611
Total dissolved nitrogen (mg/L)	Dry	0.99 ± 1.6^{b}	1.3 ± 0.9^{ab}	1.88 ± 1.3^{a}	1.4 ± 0.9^{ab}	13.1	**0.004**
	Wet	1.3 ± 1.11^{a}	1.5 ± 0.95^{a}	1.62 ± 1.4^{a}	1.2 ± 0.53^{a}	3.1	0.379
Total nitrogen (mg/L)	Dry	0.73 ± 0.2^{c}	1.7 ± 0.7^{ab}	1.05 ± 0.5^{bc}	1.83 ± 0.9^{a}	15.5	**<0.001**
	Wet	1.0 ± 0.4^{a}	1.3 ± 0.4^{a}	1.3 ± 0.5^{a}	1.5 ± 0.82^{a}	3.4	0.335
Soluble reactive phosphorus (mg/L)	Dry	0.15 ± 12.1^{a}	0.59 ± 0.36^{a}	1.1 ± 2.7^{a}	1.1 ± 0.62^{a}	4.35	0.227
	Wet	0.64 ± 1.26^{b}	0.72 ± 1.51^{ab}	2.9 ± 3.1^{a}	2.8 ± 2.76^{ab}	0.8	**0.029**
Total phosphorus (mg/L)	Dry	0.17 ± 0.04^{a}	0.37 ± 0.3^{a}	0.27 ± 0.2^{a}	0.39 ± 0.3^{a}	2.9	0.403
	Wet	0.23 ± 0.18^{a}	0.35 ± 0.27^{a}	0.35 ± 0.27^{a}	0.28 ± 0.21^{a}	1.2	0.744
Dissolved organic carbon (mg/L)	Dry	2.8 ± 1.0^{b}	5.1 ± 2.2^{b}	4.9 ± 2.7^{b}	12.5 ± 6.3^{a}	19.6	**<0.001**
	Wet	5.5 ± 3.22^{a}	6.8 ± 2.60^{a}	6.0 ± 2.9^{a}	4.1 ± 2.6^{a}	4.0	0.259
Sodium (mg/L)	Dry	11.0 ± 6.0^{a}	12.1 ± 5.0^{a}	23.1 ± 17.8^{a}	15.3 ± 6.6a	4.5	0.261
	Wet	12.4 ± 6.61^{a}	13.8 ± 7.6^{a}	15.3 ± 8.3^{a}	12.1 ± 6.1a	0.8	0.856
Potassium (mg/L)	Dry	5.8 ± 2.4^{a}	9.0 ± 4.0^{a}	8.3 ± 4.3^{a}	9.0 ± 3.9a	3.9	0.274
	Wet	5.7 ± 2.91^{a}	7.3 ± 3.8^{a}	8.1 ± 4.3^{a}	6.5 ± 3.4a	1.6	0.661
Calcium (mg/L)	Dry	6.2 ± 3.3^{a}	8.4 ± 3.3^{a}	15.8 ± 14.1^{a}	14.4 ± 13.8a	4.8	0.189

(Continued on following page)

TABLE 1 (*Continued*) Variability in mean (±SD) water quality physico-chemical variables across site categories in the Mara River Basin during the dry and wet seasons. *p*-values in boldface are significant at $p < 0.05$.

Variables		Site category				K-W ANOVA	
	Season	For	MIX	AGR	Mara	H-value	*p*-value
	Wet	6.9 ± 5.21^{a}	6.4 ± 4.5^{a}	10.5 ± 9.3^{a}	6.2 ± 3.1a	1.4	0.717
Magnesium (mg/L)	Dry	1.3 ± 0.5^{a}	2.3 ± 1.2^{a}	2.8 ± 1.8^{a}	2.8 ± 2.0a	7.7	0.053
	Wet	1.5 ± 0.8^{a}	1.9 ± 1.0^{a}	2.2 ± 1.4^{a}	1.7 ± 0.9a	1.1	0.785
Aluminium (mg/L)	Dry	1.4 ± 1.9^{ab}	0.29 ± 0.4^{b}	2.7 ± 2.7^{a}	2.4 ± 2.5a	12.7	**0.005**
	Wet	2.3 ± 2.1^{a}	1.2 ± 1.8^{a}	2.1 ± 2.7^{a}	2.0 ± 2.3a	3.9	0.276
Dissolved silica (mg/L)	Dry	28.1 ± 11.2^{a}	30.0 ± 7.7^{a}	28.0 ± 11.5^{a}	30.3 ± 15.1^{a}	0.6	0.894
	Wet	24.3 ± 12.0^{a}	23.6 ± 12.4^{a}	24.7 ± 11.4^{a}	26.4 ± 13.1^{a}	0.4	0.952
Chloride (mg/L)	Dry	4.9 ± 2.9^{a}	3.8 ± 1.4^{a}	11.4 ± 12.1^{a}	12.7 ± 11.3^{a}	5.2	0.159
	Wet	5.2 ± 3.7^{a}	4.8 ± 3.7^{a}	5.6 ± 3.1^{a}	4.6 ± 2.3^{a}	1.1	0.772
Fluoride (mg/L)	Dry	0.37 ± 0.31^{a}	0.69 ± 0.4^{b}	5.5 ± 7.9^{a}	7.5 ± 6. 8^{a}	10.6	**0.014**
	Wet	0.31 ± 0.8^{a}	0.4 ± 1.3^{a}	1.9 ± 2.1^{a}	1.9 ± 2.3^{a}	2.5	0.485
Sulphates (mg/L)	Dry	3.7 ± 2.5^{a}	3.4 ± 2.5^{a}	13.3 ± 8.7^{b}	25.7 ± 13.6^{b}	9.3	**0.031**
	Wet	6.5 ± 12.7^{a}	5.7 ± 9.7^{a}	5.7 ± 6.3^{a}	3.1 ± 2.9^{a}	1.1	0.782

sites. Only Menhinick's index separated FOR, MIX, and AGR sites, but MIX and MARA sites were inseparable (Figure 2). During the wet season, the performance and separation power of all diversity indices was much reduced as none separated the different site categories (Figure 3). Overall, FOR sites recorded the highest number of taxa and families and the highest diversity compared to other site categories.

3.3 Biotic indices

In total, 96 macroinvertebrate taxa were collected in the Mara River, belonging to 19 major taxonomic groups identified to order level or higher (Table 3). Although some taxa were identified at a lower than the family level, we used sensitivity weightings or scores at the family level, which is the level used in most of the biotic indices currently used in the region (Table 3). Of the 96 taxa, the SASS5 score had the highest taxa represented in the Mara River (78), closely followed by TARISS (77). ETHbios had the lowest number of taxa (40) represented in the Mara River.

A total of 16 taxa identified mainly to the family level did not occur or have sensitivity weightings in any of the three biotic indices (Table 3, Table A1). SASS5 and TARISS had a similar number of taxa (16) missing from the Mara River list, while ETHbios had 40. The taxa in the Mara River included the most intolerant to poor water quality or pollution, having a sensitive weighting of 15 in the SASS5 and TARISS scores (Propistomatidae and Oligoneuridae). Other sensitive taxa collected in the river included Heptageniidae (13), Perlidae, Crambidae (Pylaridae), Baetidae (>2 spp.), Hydropsychidae (>2 spp.), Polycentropodidae and Scirtidae/Helodidae (12). The most tolerant taxa to pollution encountered in the river included Coelenterata (Cnidaria), Oligochaeta, Culicidae, Psychodidae and Muscidae (1), and Chironomidae (2).

3.3.1 Performance of biotic indices

The sensitivity of the biotic indices to discriminate between levels of disturbance of the four site categories was evaluated using whisker-and-box plots (Figure 4). The total score derivatives of the three biotic indices (SASS5, TARISS and ETHbios) had poor discriminatory ability among disturbance categories during the dry and wet seasons. However, the ASPT derivatives of the indices had a better discriminatory power among site categories during the dry season. During the wet season, the ASPT derivatives, like the total scores, did poorly in discriminating the levels of disturbance among the sites.

There was discordance between the number of taxa and sensitivity scores. The MARA sites recorded the highest number of taxa but the lowest ASPT scores for both SASS5 and TARISS during the dry and wet seasons (Figure 4). Similarly, there was close agreement in the performance of SASS5 and TARISS by showing similar trends in both total and ASPT scores. ETHbios showed little variability among the site categories, except for a drop in ETHbios-ASPT score in the MARA sites during the dry season.

3.4 Macroinvertebrate index of biotic integrity (M-IBI)

3.4.1 Metrics selection, testing and scoring

In total, 12 metrics falling into five categories that describe richness, composition, tolerance to disturbances (indicator individuals or taxa), functional feeding groups and effects of channel stability and flow variation were selected for inclusion in the M-IBI for the Mara River (Table 4). The final metrics in the Mara M-IBI had sufficient variability in data values among sites (data range), were reproducible (temporal stability), and were independent of

TABLE 2 Spearman correlations between macroinvertebrate indices and physico-chemical variables. (S, total number of species/taxa; N, total number of individuals; Simpson, Simpson's index (1/Dominance); Shannon, Shannon's index; Hill, Hill's index; Evenness, Evenness index; Brillouin, Brillouin's index; Menhinick, Menhinick's index; Margalef, Margalef's index; Equitability, Equitability index; Fisher, Fisher's index; Berger-Parker, Berger-Parker's index; Chao-1, Chao's index; EC, Electrical conductivity; DO, dissolved oxygen; HCO3, Bicarbonate; NH4, Ammonium; SRP, Soluble reactive phosphorus; TSS, Total suspended solids; Ca, Calcium; Cl, Chloride; F, fluoride; K, Potassium; Mg, Magnesium; Na, Sodium; NO3, nitrates; SO4, Sulphate; Al, Aluminium; DOC, Dissolved organic carbon; DSi, Dissolved silica; TN, Total nitrogen; TDN, Total dissolved nitrogen. Spearman correlation coefficients in boldface are significant at $p < 0.05$.

	S	N	Simpson	Shannon	Hill	Evenness	Brillouin	Menhinick	Margalef	Equitability	Fisher	Berger-parker	Chao-1
pH	**−0.25***	−0.03	−0.19	**−0.22***	−0.14	−0.02	**−0.23***	**−0.22***	**−0.29****	−0.09	**−0.26***	0.17	**−0.22***
EC	**0.26***	**0.36****	−0.07	−0.02	−0.10	**−0.28***	0.05	−0.20	0.15	**−0.22***	0.09	0.05	**0.26***
DO	0.12	0.13	**0.22***	0.19	**0.23***	0.06	0.17	−0.03	0.07	0.14	0.05	**−0.22***	0.07
Temperature	0.20	**0.37****	−0.04	0.01	−0.05	−0.19	0.08	**−0.31****	0.08	−0.17	.001	0.01	0.18
HCO3	**0.67****	**0.54****	0.19	**0.31***	0.10	**−0.29***	**0.40****	−0.10	**0.56****	−0.12	**0.41****	−0.16	**0.59****
NH4	**−0.26***	**−0.28***	**−0.38***	**−0.39***	**−0.38***	−0.14	**−0.42****	**0.28***	−0.16	**−0.22***	−0.05	**0.37***	−0.11
SRP	**−0.29***	−0.14	−0.12	−0.17	−0.09	0.02	−0.17	−0.09	**−0.24***	−0.05	−0.19	0.03	−0.21
TSS	−0.06	0.11	**−0.24***	−0.19	**−0.27***	−0.21	−0.16	−0.13	−0.15	**−0.25***	−0.16	**0.26***	−0.02
Ca	**−0.24***	−0.02	−0.03	−0.04	0.01	0.11	−0.04	−0.12	**−0.23***	0.06	−0.19	−0.05	−0.21
Cl	0.07	**0.27***	0.05	0.03	0.07	−0.03	0.06	−0.20	−0.09	−0.01	−0.10	−0.05	−0.03
F	0.16	**0.38****	0.02	0.04	0.04	−0.15	0.11	**−0.30***	−0.06	−0.10	−0.11	−0.03	0.08
K	0.10	0.15	0.01	0.02	0.02	−0.04	0.01	−0.13	0.02	−0.03	0.01	−0.02	0.01
Mg	−0.07	0.08	−0.01	−0.01	0.03	0.06	−0.02	−0.13	−0.12	0.05	−0.11	−0.05	−0.16
Na	−0.07	0.21	−0.11	−0.12	−0.08	−0.10	−0.10	**−0.26***	−0.19	−0.11	−0.20	0.05	−0.14
NO3	**0.27***	**0.29***	−0.16	−0.15	−0.17	**−0.39****	−0.09	−0.16	0.18	**−0.32***	0.08	0.14	**0.24***
SO4	0.01	0.20	−0.16	−0.13	−0.17	−0.16	−0.13	−0.17	−0.08	−0.19	−0.08	0.18	0.01
Al	0.14	**0.43***	**−0.45***	**−0.43***	**−0.44***	**−0.47***	**−0.34***	**−0.43***	−0.10	**−0.44***	−0.17	**0.49****	0.06
DOC	**0.41****	**0.43****	−0.16	−0.17	−0.17	**−0.40***	−0.09	**−0.23***	**0.23***	**−0.36***	0.15	0.16	**0.41***
DSi	0.16	0.13	−0.12	−0.08	−0.13	**−0.26***	−0.15	−0.10	0.08	−0.20	0.06	0.12	0.18
TP	−0.06	0.01	0.03	−0.03	0.05	−0.01	−0.07	−0.05	−0.03	−0.01	−0.04	−0.03	−0.09
TN	**0.26***	**0.72****	**−0.70****	**−0.65****	**−0.72****	**−0.72****	**−0.50****	**−0.72****	−0.11	**−0.74****	**−0.23***	**0.70****	0.12
TDN	−0.14	**0.29***	**−0.48***	**−0.48***	**−0.49***	**−0.32***	**−0.43***	**−0.41***	**−0.35***	**−0.36***	**−0.33***	**0.53****	−0.19

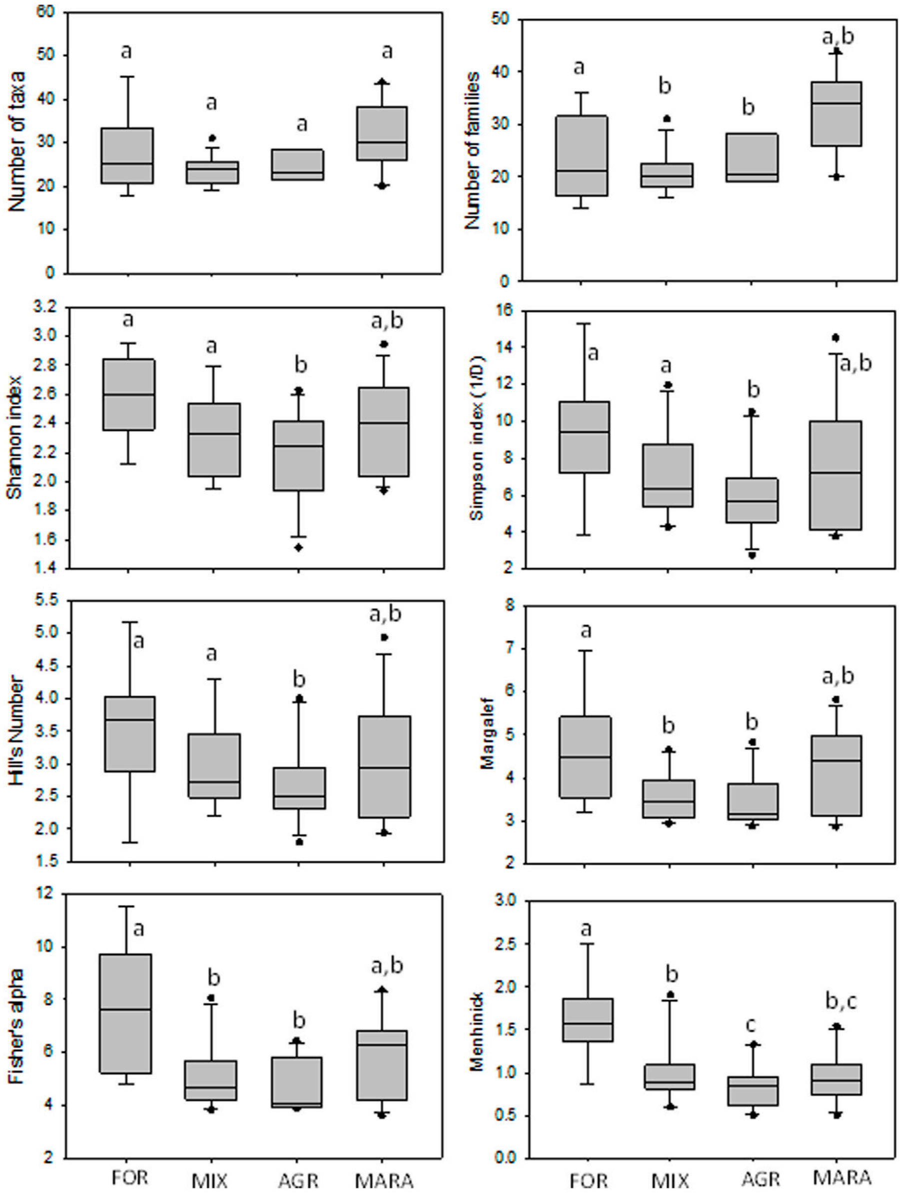

FIGURE 2
Performance of taxa richness and diversity indices across site categories in the Mara River Basin during the dry season. Similar letters on boxes indicate no significant difference in metric values among the four condition categories after One-Way ANOVA.

other metrics by having low correlation relationships ($r < 0.70$). Some metrics that are sensitive to the cessation of flows and river sedimentation were included in the M-IBI to capture the effects of land use change and excessive water withdrawals that have modified the flow regime of the river. These metrics include the percentage of rheophilic individuals, the percentage of non-insect individuals and the percentage of burrowing individuals (Table 4). In addition, the percentage of vegetation-associated taxa was included as a metric to capture the loss of riparian and instream vegetation caused by flash-flooding, disturbance by livestock and wildlife and human over-use of streams and rivers.

Metrics were also assessed for responsiveness to changes in water quality among the different sites. All macroinvertebrate metrics displayed significant Spearman correlations with various physico-chemical parameters (Table 5). SRP, F, K, and NO3-N returned only one significant relationship with M-IBI metrics. Significant correlations were among most water quality physico-chemical variables and M-IBI metrics (Table 5). The number of EPT taxa, number of tolerant taxa and percent Diptera individuals were the most responsive metrics, showing correlations with more than half of the 19 variables. The number of taxa and the number of predator individuals metrics were the least responsive metrics by having significant correlations with only three of the 19 physico-chemical variables.

Metric scoring for the M-IBI was based on distributions of reference FOR sites and the most impacted (AGR and MARA) sites (Table 6). The scoring criteria were based on dry season samples because metric values are more stable and representative of environmental conditions

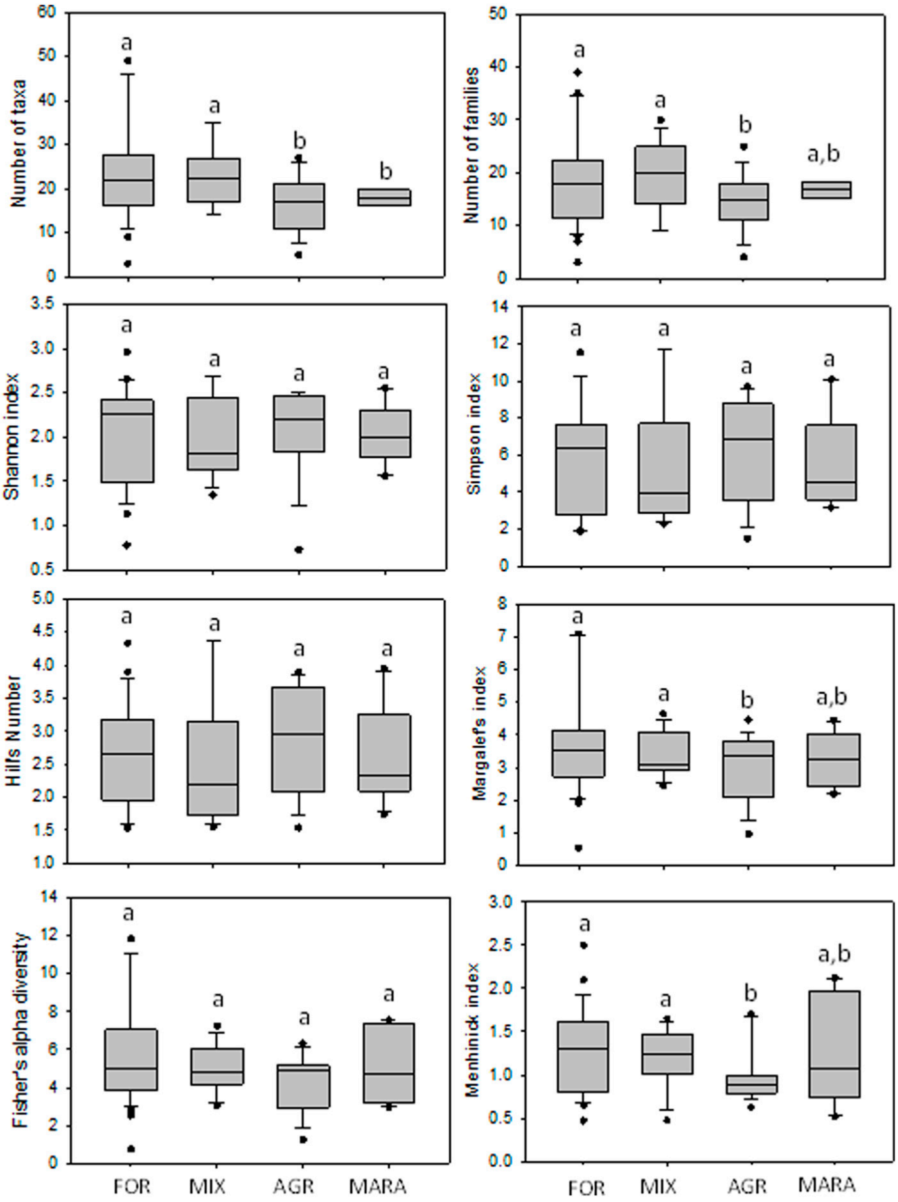

FIGURE 3
Performance of taxa richness and diversity indices across site categories in the Mara River Basin during the wet season. Similar letters on boxes indicate no significant difference in metric values among the four condition categories after One-Way ANOVA.

as compared with wet season conditions. Consequently, metrics were scored separately for the dry and wet seasons.

3.4.2 Performance of the M-IBI

The ability of the M-IBI to discriminate between the four levels of disturbance or site categories was evaluated using whisker-and-box plots (Figure 5). During the dry (Figure 5A) season, the performance of the M-IBI was able to discriminate between FOR and MIX sites, which were least disturbed, and AGR and MARA, which were the most disturbed human activities. However, during the wet season, the discriminatory ability of the M-IBI was much reduced as the four categories of sites were essentially inseparable (Figure 5B). The FOR and MIX sites scored higher values than the AGR and MARA sites, where water quality was much reduced. Overall, the M-IBI effectively assessed perturbations, including water quality and habitat disturbance, throughout the Mara River basin.

3.5 Relationships between biotic indices and M-IBI

Significant correlations existed among the total number of taxa, number of families, regional biotic indices, and the Mara River M-IBI (Table 7). During the dry season, the number of taxa had a significant positive correlation with the number of families, SASS5 score, TARISS score, and ETHbios score. The number of

TABLE 3 List of taxa collected in the study area and their sensitivity weightings for rapid bioassessment protocols (RBPs) used in Africa, including the South African Scoring System (SASS, Dickens, and Graham, 2002), Tanzania River Scoring System (TARISS, Kaaya et al., 2015) and ETHbios (Aschalew and Moog, 2015). (–) indicate that sensitivity weights don't exist in the three RBPs for the taxon.

Taxon	SASS	TARISS	ETHBIOS
PORIFERA	5	5	
COELENTERATA (Cnidaria)	1	1	
TURBELLARIA (Flatworms)	3	3	
ANNELIDA			
Oligochaeta (Earthworms)	1	1	3
Hirudinea (Leeches)	3	3	
ARACHNIDA			
COLEMBOLA (Springtails)			
CRUSTACEA			
Atyidae (shrimps)	8	8	
Potamonautidae (Crabs)	3	3	7
HYDRACARINA (Water mites)	8	8	
PLECOPTERA (Stoneflies)			
Perlidae	12	12	10
EPHEMEROPTERA (Mayflies)			
Baetidae 1sp	4	4	4
Baetidae 2 sp	6	6	6
Baetidae >2sp	12	12	9
Caenidae	6	6	6
Heptageniidae	13	13	9
Leptophlebiidae	9	9	9
Tricorythidae	9	9	8
Dicercomyzidae		10	
Oligoneuridae	15	15	
Polymitarcyidae	10	10	
Propistomatidae	15	15	
ODONATA (Damselflies and Dragonflies)			
Aeshnidae	8	8	7
Coenagrionidae	4	4	
Corduliidae	8	8	
Gomphidae	6	6	6
Lestidae	8	8	7
Libellulidae	4	4	5
Protoneuridae	8	8	
Synlestidae	8	8	
LEPIDOPTERA (Aquatic Caterpillars/ Moths)			
Crambidae (= Pyralidae)	12	12	
HEMIPTERA (Bugs)			
Belostomatidae	3	3	3
Cicadellidae	–	–	–
Corixidae	3	3	4
Galastocoridae	–	–	–
Gerridae	5	5	5
Hebridae	–	–	–
Hydrometridae	6	6	
Nepidae	3	3	3
Notonectidae	3	3	3
Pleidae	4	4	4
Saldidae	–	–	–
Veliidae/Mesoveliidae	5	5	
MEGALOPTERA (Fishflies, Dobsonflies, and Alderflies)			
Sialidae	6	6	
Corydalidae	8	8	
NEUROPTERA			
Sisyridae			
TRICHOPTERA (Caddisflies)			
Hydropsychidae 1 sp	4	4	
Hydropsychidae 2 sp	6	6	
Hydropsychidae >2 sp	12	12	
Calamoceratidae	11	11	
Ecnomidae	8	8	8
Glossiphoniidae	3	1	3
Hydroptilidae	6	6	
Lepidostomatidae	10	10	10
Leptoceridae	6	6	8
Philopotamidae	10	10	10
Pisuliidae	10	10	
Platycnemididae	–	–	–
Polycentropodidae	12	12	
Psychomyiidae	8	8	

(Continued in next column)

TABLE 3 (*Continued*) List of taxa collected in the study area and their sensitivity weightings for rapid bioassessment protocols (RBPs) used in Africa, including the South African Scoring System (SASS, Dickens, and Graham, 2002), Tanzania River Scoring System (TARISS, Kaaya et al., 2015) and ETHbios (Aschalew and Moog, 2015). (–) indicate that sensitivity weights don't exist in the three RBPs for the taxon.

(Continued on following page)

TABLE 3 (*Continued*) List of taxa collected in the study area and their sensitivity weightings for rapid bioassessment protocols (RBPs) used in Africa, including the South African Scoring System (SASS, Dickens, and Graham, 2002), Tanzania River Scoring System (TARISS, Kaaya et al., 2015) and ETHbios (Aschalew and Moog, 2015). (–) indicate that sensitivity weights don't exist in the three RBPs for the taxon.

Taxon	SASS	TARISS	ETHBIOS
COLEOPTERA (Beetles)			
Carabidae	–	–	–
Chrysomelidae	–	–	–
Curculionidae	–	–	–
Dytiscidae/Noteridae	5	5	5
Elmidae/Dryopidae	8	8	7
Gyrinidae	5	5	5
Haliplidae	5	5	
Helophoridae	–	–	–
Hydraenidae	8	8	
Hydrophilidae	5	5	5
Lamphyridae	–	–	–
Naucoridae	7	7	6
Psephenidae	10		
Scirtidae/Helodidae	12	12	10
DIPTERA (Flies)			
Athericidae	10	10	
Ceratopogonidae	5	5	5
Chaoboridae	–	–	–
Chironomidae	2	2	1
Culicidae	1	1	1
Dixidae	10	10	
Emphididae	6	6	
Ephydridae	3	3	
Tipulidae	5	5	7
Muscidae	1	1	2
Psychodidae	1	1	1
Sciomyzidae	–	–	–
Simulidae	5	5	
Stratiomyidae	–	–	–
Tabanidae	5	5	6
Tipulidae	5	5	7
GASTROPODA (Snails)			
Bithyniidae	–	–	–
Bulinae	3	3	
Planorbidae	3	3	3
Thiaridae	3	3	
Lymnaeidae	3	3	
Viviparidae	5	5	
PELECYPODA (Bivalves)			
Corbuculidae	5	5	
Sphaeriidae	3	3	
Unionidae	6	6	

EPT taxa displayed a similar response as the total number of taxa. The SASS5 score had a highly significant positive relationship with the TARISS score. Both the SASS5 score and TARISS score had highly significant relationships with the number of taxa, the number of families and ETHbios score. The M-IBI index showed significant positive relationships with the SASS5 score and ASPT, TARISS score and ASPT, and ETHbios Score. However, the M-IBI didn't display any significant relationship with the number of taxa and families, nor ETHbios ASPT.

Notably, all Spearman correlations among the total number of taxa, number of families, regional biotic indices, and the Mara River M-IBI were positive and significant (Table 7). This implies that the discriminatory ability of all the indices was reduced and could not display variability among sites.

3.5.1 Assessment of ecological condition

Four condition categories of excellent, good, fair, and poor were established to distinguish the different environmental conditions of the sampling sites (Table 8). The ecological condition and integrity classes were interpreted relative to the levels of human disturbance at the different groups of sampling sites. Narrative descriptions were established for different categories or classes of sites using physico-chemical data, individual metrics, final M-IBI scores, habitat conditions and flow conditions for each site (Table 8). A notable response was the association of stable substrate and clear water quality with high diversity and abundances of the EPT and rheophilic taxa in the river. However, habitat modifications caused by trampling and input of organic matter and nutrients by large mammalian herbivores (livestock and hippos), and increased erosion causing turbidity from agricultural areas, were associated with low numbers of intolerant EPT taxa and high numbers and dominance of tolerant taxa such some species among Diptera Hemiptera, Coleoptera, and Oligochaeta.

4 Discussion

We sought to compare the performance of macroinvertebrate-based diversity (including richness) indices, regional biotic indices, and an index of biotic integrity (M-IBI) to assess the ecological

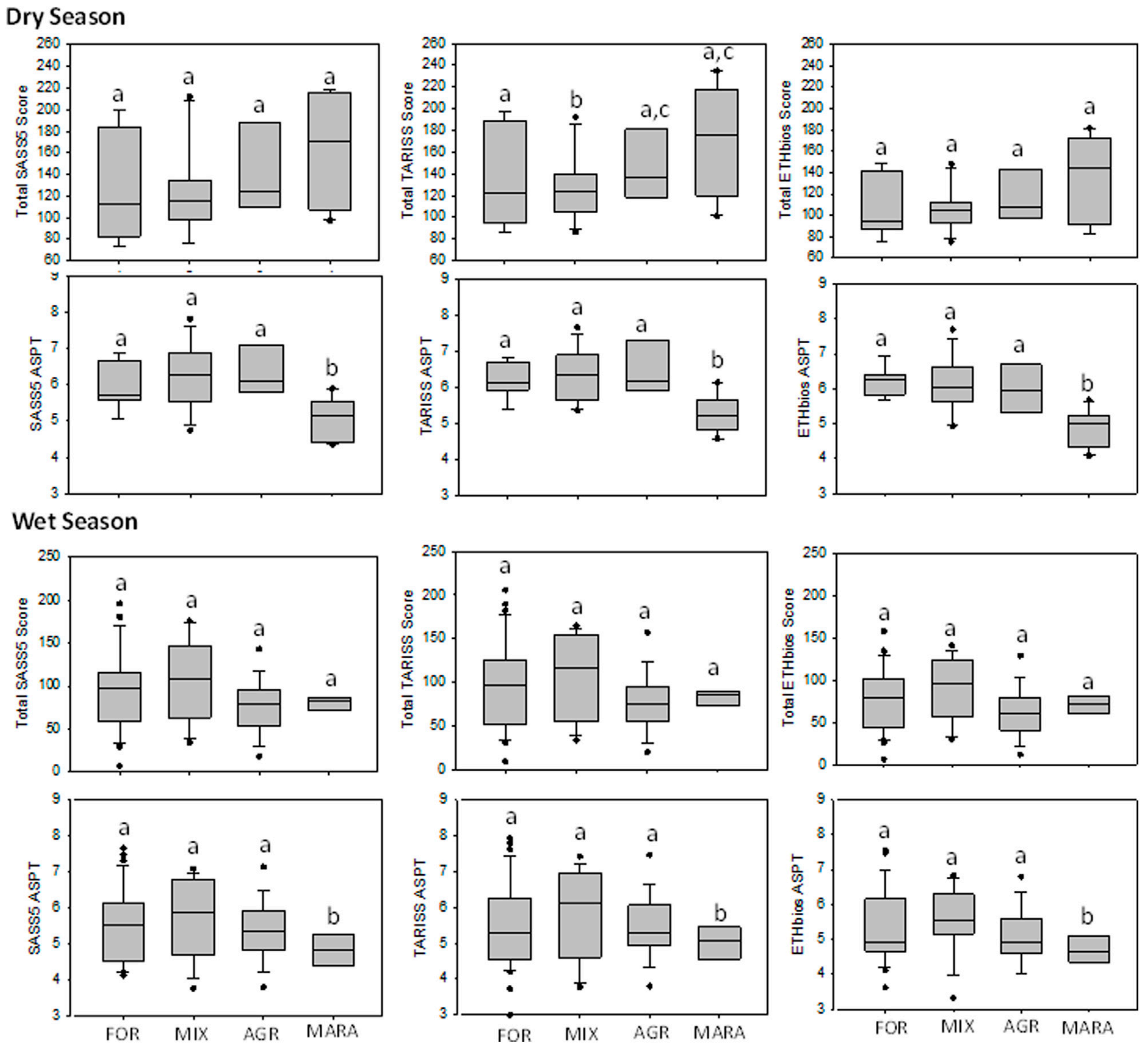

FIGURE 4
Box-and-whisker plots comparing the ability of SASS5, TARISS, and ETHbios total index scores and average score per taxon (ASPT) to discriminate levels of disturbance among the four site categories (FOR, MIX, AGR, and MARA) in the Mara River Basin during the dry and wet seasons. Boxes show interquartile ranges (25th and 75th percentiles, and middle lines are medians. FOR, forested; MIX, mixed; AGR, Agricultural, and MARA, Mara River mainstem and seasonal tributaries. Similar letters on boxes indicate no significant difference in metric values among the four condition categories after One-Way ANOVA.

condition of the Mara River, Kenya/Tanzania. The river is influenced by multiple stressors arising from agriculture and animal grazing, organic matter and nutrient loading, and excessive water withdrawals. Significant differences in water quality variables among the site categories influenced the structure of macroinvertebrates communities. Overall, the M-IBI performed better than biotic and diversity indices by having a higher discriminatory ability of site categories according to different levels and types of disturbance. Diversity indices performed poorly and failed to discriminate between stressor gradients in the river. All the indices were very sensitive to seasonality. During the dry season, the indices were more stable and could distinguish between different levels of disturbances in the river. However, during the wet season, the indices performed poorly and largely failed to differentiate between the different disturbance levels among the site categories.

4.1 Water quality physico-chemical variables

Most physico-chemical variables differed among the site categories (Table 1), with turbidity, conductivity, TSS, nutrients and some major ions recording the lowest values in FOR and MIX

TABLE 4 Final metrics selected for inclusion in the Mara River M-IBI for assessment of the present ecological condition of the river. Details on definition and rationale or significance for inclusion, their historical use in Lake Victoria basin and predicted responses to perturbations and reduced flows are provided.

Metric	Metric definition and significance	Predicted response to increased perturbation (including flow reduction)
1. Number of taxa (genera or families)[b,e]	Total number of all taxa at a site	Decrease
2. Number of EPT taxa[a,b,c,d]	Total number of taxa from mayfly, stonefly, and caddisfly orders	Decrease
3. Number of tolerant taxa	Total number of taxa that are tolerant to pollution or disturbance	Increase
4. Percent EPT individuals[a,b,c,d,e]	Percent individuals from mayfly, stonefly, and caddisfly orders	Decrease
5. Percent Diptera individuals[d]	Percent midge individuals	Increase
6. Percent individuals in 3 dominant taxa[a,b,d]	Relative abundance of the three most dominant taxa (number of individuals)	Increase
7. Percent rheophilic individuals[g]	Percent of individuals of taxa requiring moderate to fast flows. Rheophilic individuals considered include families Hydropsychidae, Tricorythidae, Simuliidae, Elmidae, some Baetidae (*Centroptiloides* sp. and *Pseudocloeon* sp. and *Afroptilum* sp., etc)	Decrease
8. Percent vegetation-associated individuals[g]	Percent of individuals of taxa occurring in the vegetated zone. Vegetation-associated taxa include Naucoridae, Belostomatidae, Lestidae, Hydrometridae, Nepidae, Curculionidae, Leptoceridae (plant cased), Pisuliidae, etc.	Variable
9. Percent predator individuals[a,b,c,d]	Carnivores- scavengers, engulf or pierce prey	Variable
10. Percent non-insect individuals[b]	Percent of individuals of taxa that do not belong to class Insecta	Increase
11. Percent scraper + filterers: gatherer individuals[f]	Ratio of individuals that feed on algae and periphyton and those that filter food from water currents to those that feed on organic matter on the benthos	Variable
12. Percent burrowing individuals[g]	Percent of individuals of taxa with a habit of burrowing in soft substrate (GSM). Burrowing taxa considered include Chironomidae, Oligochaeta, Polymitarcyiidae, Gomphidae and Libellulidae, etc	Increase

NB: EPT, ephemeroptera, Plecoptera, and Trichoptera. Superscript numbers of metrics denote historical use of the metric in the Lake Victoria Basin.

[a] Masese et al. (2009a).

[b] Raburu et al. (2009a).

[c] Raburu et al. (2009b).

[d] Aura et al. (2010).

[e] Kobingi et al. (2009).

[f] Masese et al. (2014a).

[g] Denote metrics used for the first time in the Lake Victoria basin.

sites and highest values in AGR and MARA sites. Turbidity, TSS and nutrients are good indicators of disturbances at the local scale, such as human activity and the loading of organic matter and nutrients by livestock and large wildlife through defecation and excretion (Masese et al., 2017; Dutton et al., 2018; Iteba et al., 2021). In contrast, electrical conductivity and major ions are surrogates for land use impacts or changes in geology on water physico-chemistry at the catchment scale (Gaillardet et al., 1999; Ibarra et al., 2016).

The negative impact of agriculture on water quality and ecological condition of Afrotropical streams and rivers through sediments and nutrient inputs from farmlands have been well-documented (Minaya et al., 2013; Masese et al., 2014a; Masese et al., 2014b; Masese et al., 2017; Fugère et al., 2018; Dalu et al., 2019; Kroese et al., 2020). Other stressors that negatively affect water quality and ecological integrity include the loading of organic matter from human settlements and livestock grazing areas (Dutton et al., 2018; Iteba et al., 2021). Excessive water withdrawal and changes in the natural flow regimes of streams and rivers are also emerging as significant stressors of river ecosystems. Extreme cases of flow alteration that lead to cessation of flows or supra-reduced flow levels during the dry season have been reported in the Mara River and other rivers in eastern Africa (Dutton et al., 2018; Stears et al., 2018). Reduced flows lead to the accumulation of organic matter and ammonia, increased concentration of solutes and an overall decline in DO concentration (Dutton and Subalusky, 2021; Wanderi et al., 2022).

Seasonality played a significant role in influencing water quality variables in the river through its influence on runoff, erosion and leaching of solutes, as also shown in a previous study (Wanderi et al., 2022). Solute concentrations and electrical conductivity were much higher during the dry season, probably because of increased evaporation and water temperatures. Studies show that inter-site differences can increase during the dry season as reach-scale influences (e.g., daily animal and human disturbance) increase during this period (Mathooko, 2001; Yillia et al., 2008; Minaya et al., 2013). In contrast, runoff and leaching during the wet season can increase the delivery of sediments, nutrients, and DOC into streams and rivers (Elsenbeer, 2001; Saunders et al., 2006). However, during the wet season, dilution and increased longitudinal connectivity can homogenize conditions in rivers, leading to reduced inter-site differences in water quality and ecological conditions (Leung et al., 2012; Masese et al., 2014b).

4.2 Performance of diversity indices

Since there are many diversity indices, it is difficult to decide *a priori* which one is best for a given region or study objective. By comparing the performance of eleven indices that are rarely or never used, we found that most of the indices performed poorly as discriminators of human disturbance in the river. This poor performance is probably due to taxa replacements with increasing levels of disturbance and not necessarily a loss or decline in taxa richness or diversity. For instance, the Mara River mainstem recorded the highest taxa richness, but most often, these were either tolerant or moderately tolerant to poor water quality. This is a weakness of most diversity indices as they don't consider the tolerance of individual taxa or species to different levels of disturbance but the composition of the community in terms of the number of species and their abundances.

Although the diversity indices performed poorly, most were essentially in agreement regarding differences in macroinvertebrate diversity among the four site categories (Figures 2, 3). Low values of the Shannon index (<2.5) in MIX, AGR and MARA sites indicate widespread degradation affecting macroinvertebrate communities in the river. The same trend of declining richness and diversity of macroinvertebrates in MIX and AGR sites was captured by Simpson's, Hill's, Margalef's, Fisher's, and Menhinick's indices (Figure 2). Agricultural streams had the lowest diversity indices, while MARA sites had slightly higher values, although there was a lot of variability within this site category. The lack of variation in most diversity indices across the site categories indicates the similarity of macroinvertebrate communities (Jost, 2007), especially regarding the abundance of common taxa. Menhinick's diversity index had better differentiation among the site categories, suggesting that it was less sensitive to the numerical dominance of macroinvertebrate communities by a few common taxa. Hence, the index (Menhinick) is better suited for assessing anthropogenic influences on the diversity of macroinvertebrates in Afromontane-savanna rivers where the relative abundance of a few taxa (3–5) can be very high. In the Mara River, the relative abundance of the three most common taxa ranged from 30% to 93%. Shannon's, Simpson's (the reciprocal form), Hill's, and Fisher's indices could not capture declining water quality conditions when the diversity of macroinvertebrates was high. Hence, they are better suited to assessing disturbance that causes taxa richness and composition variation.

Like diversity indices, the number of taxa and families did not perform any better in discriminating different river disturbance forms. For instance, MARA sites recorded the highest number of taxa and families, but water quality was the most degraded. The MARA sites also experienced extreme flow variation, with seasonal tributaries ceasing flowing during the dry season. Given that this is the nature of savanna rivers—seasonal flows, high levels of organic matter, turbidity, water temperatures, and electrical conductivity (dissolved solutes) and low dissolved oxygen concentration (Wanderi et al., 2022), these taxa may be adapted to live in these conditions while maintaining high diversity and abundances. Indeed, some of the most sensitive taxa to poor water quality, such as Ephemeroptera and Trichoptera, recorded high taxa richness and abundance in organically polluted sites downstream of hippo pools and livestock watering points (Masese and Raburu, 2017). Similarly, Coleoptera, Hemiptera and Odonata had high taxa richness and abundance in MARA sites, suggesting that these orders are essential for monitoring extreme cases of flow reduction (including flow cessation), organic matter and nutrient loading in streams and rivers (Masese et al., 2018; Masese et al., 2021).

4.3 Performance of biotic indices

The three biotic indices (SASS5, TARISS and ETHbios) performed similarly in assessing the ecological status of different site categories in the Mara River (Figure 4). However, the indices' total score and ASPT derivatives provided contrasting results. While the total scores indicated improved ecological conditions from the FOR to MARA sites, the ASPT derivative showed that the MARA sites had the poorest ecological condition. Thus, the ASPT derivatives captured the true conditions of the sites according to water quality variables. Higher ASPT scores indicate a high number of taxa that are sensitive to pollution or disturbance (Chutter, 1972; Chutter, 1998; Dickens and Graham, 2002). Overall, the results of the biotic indices indicate a general deterioration in water quality and habitat diversity in the Mara River mainstem and the seasonal tributaries. On the contrary, sites in the upper reaches of the Amala and Nyangores tributaries had natural water quality and high habitat diversity.

Despite having the lowest number of taxa represented in the Mara River—only 40 out of 96- ETHbios performed better than expected in comparison to both SASS5 and TARISS, which had 78 and 77 taxa, respectively, represented in the river (Table 3). The good performance of ETHbios shows that even with a subset of the taxa, sites can be assessed to determine their ecological status. However, this can be difficult when the sensitivities of native taxa are unknown, and values from elsewhere are assigned to native taxa. TARISS, which borrows heavily from SASS5, was expected to perform better than the other two indices (SASS5 and ETHbios) because it is the only biotic index empirically developed in East Africa, and macroinvertebrates in Tanzania and Kenya share similar characteristics. For instance, Dicercomyzidae in TARISS, which is missing in both SASS5 and ETHbios, is represented in the Mara River.

Similar to diversity indices, the biotic indices also showed dependence on seasonality and flow conditions for performance. The three biotic indices performed better during the dry season by distinguishing between the extremes of ecological conditions (FOR and MARA) in the river. In contrast, there were declines in the discriminatory ability of the indices from the dry to the wet season. Poor performance of the biotic indices is likely due to improvements in environmental conditions or water quality caused by the dilution and flushing of the streams and rivers during the rainy season. Improvement of water quality in the MARA sites during the wet season was captured by the presence of sensitive taxa such as Perlidae, Heptageniidae and Philopotamidae, which did not occur

TABLE 5 Spearman correlations among water quality variables and component metrics of the Mara River M-IBI. Values in bold are significant at $p < 0.05$.

Physico-chemical variables	MIBI metrics											
	Total number of taxa	Number of EPT taxa	Number of tolerant taxa	Percent EPT individuals	Percent Diptera individuals	Percent 3 dominant taxa (abundance)	Percent rheophilic individuals	Percent VEG associated individuals	Percent predator individuals	Percent non-insect individuals	Ratio of scrapers + filterers to gatherers	Percent burrowing individuals
Conductivity	−0.06	**−0.39**	**0.47**	−0.17	0.06	**0.27**	−0.02	−0.15	−0.09	0.02	**0.24**	−0.06
DO	0.22	**0.25**	−0.10	0.17	−0.01	**−0.24**	0.22	0.11	0.18	**−0.36**	0.11	**−0.28**
Temperature	0.12	0.07	**0.46**	0.13	−0.23	0.08	**0.38**	−0.18	−0.13	**−0.29**	**0.36**	**−0.35**
Turbidity	0.02	**−0.56**	**0.56**	0.07	**0.27**	0.20	**0.34**	**−0.31**	−0.18	−0.20	**0.55**	**−0.53**
NH4	**−0.39**	**−0.52**	−0.04	−0.08	−0.01	**0.50**	−0.24	−0.21	0.05	**0.33**	−0.18	−0.08
SRP	−0.12	−0.18	0.23	0.16	**−0.33**	0.04	0.19	0.08	0.21	−0.13	0.15	−0.26
TSS	−0.09	0.02	**0.36**	0.08	**−0.24**	**0.27**	0.21	**−0.27**	−0.14	−0.09	**0.36**	**−0.43**
Ca	**−0.25**	**−0.52**	**0.38**	**−0.24**	0.12	0.02	−0.19	0.18	0.15	−0.01	0.07	0.15
Cl	−0.14	**−0.41**	**0.38**	**−0.36**	**0.45**	0.07	−0.09	−0.08	−0.04	−0.19	0.23	0.17
F	0.23	−0.08	**0.41**	0.13	−0.21	0.06	0.17	−0.18	0.08	−0.18	0.14	−0.16
K	−0.12	**−0.32**	0.20	−0.18	**0.30**	0.00	−0.07	0.14	0.03	−0.06	0.16	0.11
Mg	−0.22	**−0.47**	**0.29**	**−0.29**	**0.30**	0.00	−0.11	**0.24**	0.08	−0.10	0.11	0.22
Na	**−0.28**	**−0.54**	**0.31**	**−0.29**	**0.32**	0.16	−0.01	−0.02	−0.06	−0.15	0.20	0.03
NO_3-N	−0.09	0.07	0.08	0.07	0.14	0.12	0.10	−0.07	**−0.36**	0.07	−0.13	−0.18
SO_4	−0.16	**−0.35**	**0.33**	−0.21	0.20	0.18	0.01	−0.15	−0.05	−0.25	**0.36**	−0.03
DOC	0.01	−0.06	**0.25**	−0.12	**0.30**	0.08	**0.40**	−0.02	−0.26	−0.02	0.19	−0.17
DSi	−0.16	−0.15	0.05	**−0.41**	**0.43**	0.08	−0.01	0.07	**−0.36**	0.15	**0.25**	0.23
TP	0.12	0.03	**0.38**	0.01	−0.09	0.00	−0.09	0.01	0.00	**0.34**	0.05	0.17
TN	0.20	0.18	**0.38**	**0.43**	**−0.24**	**0.27**	**0.60**	**−0.38**	**−0.29**	**−0.44**	0.22	**−0.30**

TABLE 6 Metric percentiles (25th, 50th, and 75th) for reference (FOR) sites and impacted sites (AGR and MARA) used for metric calibration.

	Reference sites			Impacted sites		
Metric variable	25th	50th	75th	25th	50th	75th
Total number of taxa	23	25	28	17	21	24
Number of EPT taxa	8	11	14	3	5	7
Number of tolerant taxa	2	3	5	5	7	8
Percent EPT individuals	37.8	54.5	64.5	28.6	37.4	53.3
Percent Diptera individuals	15.3	18.3	21.3	22.5	34.7	45.7
Percent 3 dominant taxa (abundance)	25.4	37.2	43.1	42.9	47.2	68.3
Percent rheophilic individuals	33.1	44.4	53.6	20.5	29.2	35.0
Percent VEG associated individuals	8.4	9.3	12.2	3.7	5.6	9.1
Percent predator individuals	14.3	28.5	30.8	11.5	21.0	39.1
Percent non-insect individuals	1.7	2.9	3.3	4.7	9.1	17.3
Ratio of Scrapers + Filterers to Gatherers	1.7	2.8	3.7	0.8	1.2	1.6
Percent burrowing individuals	17.7	23.9	30.7	25.9	39.8	61.0

during the dry season. Similar findings have been reported in other rivers, whereby replenishing of water and removal of fine sediments in the benthos by river flows leads to the recolonization of streams and rivers with sensitive taxa that cannot withstand sedimentation and poor water quality during the dry season (Shivoga, 2001; Masese et al., 2009b).

The performance of the SASS5 and TARISS biotic indices was very similar. This can be explained by the fact that TARISS was derived from SASS5, with all the sensitivity weightings in the two indices being similar except for three taxa (Dicercomyzidae, Ephemerythidae and Neridae) missing in SASS5 but included in TARISS. Dicercomydidae occur in the Mara River, implying that the distribution of taxa in regions sampled for the development of TARISS, including northern Tanzania, is like that in the Mara River basin. This isn't surprising because the Mara River is transboundary between Kenya and Tanzania. In contrast, ETHbio's poor performance was largely due to many taxa (40) in the Mara River that are missing in the index, as opposed to only 16 taxa in the Mara that aren't in either SASS5 or TARISS (Table A1).

4.4 Performance of M-IBI

The M-IBI developed for the Mara River performed better than diversity and biotic indices in discriminating the different levels and types of stressors among the four site categories. IBIs are more rigorous in their assessment of ecological conditions because they incorporate metrics whose utility or sensitivity to different forms of disturbance is already tested and assured. Although most of the 12 metrics included in the M-IBI have previously been used to develop macroinvertebrate IBIs in the Lake Victoria basin (Masese et al., 2009a; Raburu et al., 2009a; Raburu et al., 2009b; Kobingi et al., 2009; Aura et al., 2010; Masese et al., 2014a), and other river systems in Africa (Thorne and Williams, 1997; Lakew and Moog, 2015; Alemneh et al., 2019; Tampo et al., 2020; Kaboré et al., 2022), three new metrics were incorporated to capture specific stressors that are unique to Afrotropical savanna rivers as indicated above. The first new metric included in the M-IBI was the percentage of individuals of rheophilic taxa. Rheophilic taxa require a stable substrate and moderate to fast flows to meet their ecological requirements, mainly attachment for feeding, dissolved oxygen uptake and osmoregulation. Rheophilic taxa in the Mara River include Hydropsychidae, Tricorythidae, Simuliidae, Elmidae, and some Baetidae (*Centroptiloides* sp. and *Pseudocloeon* sp. and *Afroptilum* sp.), among others (Masese et al., 2021). Simuliidae, Hydropsychidae, Tricorythidae, and some Baetidae have been identified to be sensitive to flow alteration in African rivers (O'Keeffe and de Moor, 1988; Dallas, 2007; Rivers-Moore et al., 2007; Thirion, 2016; Masese et al., 2021). When water levels and flow velocities drop, they cannot feed and obtain enough dissolved oxygen, and consequently, they are eliminated. Incorporating rheophilic species in multimetric indices is crucial for monitoring changes in natural flow regimes of rivers caused by land use change and excessive water withdrawals. These taxa are also important for setting environmental flow requirements for streams and rivers.

The second new metric included in the M-IBI was the percent of individuals in vegetation-associated taxa, which is the number of individuals in taxa occurring in or preferring vegetated zones. Vegetation-associated taxa included Naucoridae, Belostomatidae, Lestidae, Hydrometridae, Nepidae, Notonectidae, Naucoridae, Lepidostomatidae, Leptoceridae (plant cased), Pisuliidae, among others. Most of these taxa belong mainly to Coleoptera, Hemiptera and Odonata, and some Trichoptera, especially detritivores. The sensitivity of Trichoptera to different forms of disturbance in streams and rivers is undisputed (Barbour et al., 1999). Taxa among Odonata, Coleoptera, and Hemiptera are fast colonizers tolerant to water flow reduction or cessation and poor water quality (Velasco and Millan, 1998; Boulton and Lake, 2008;

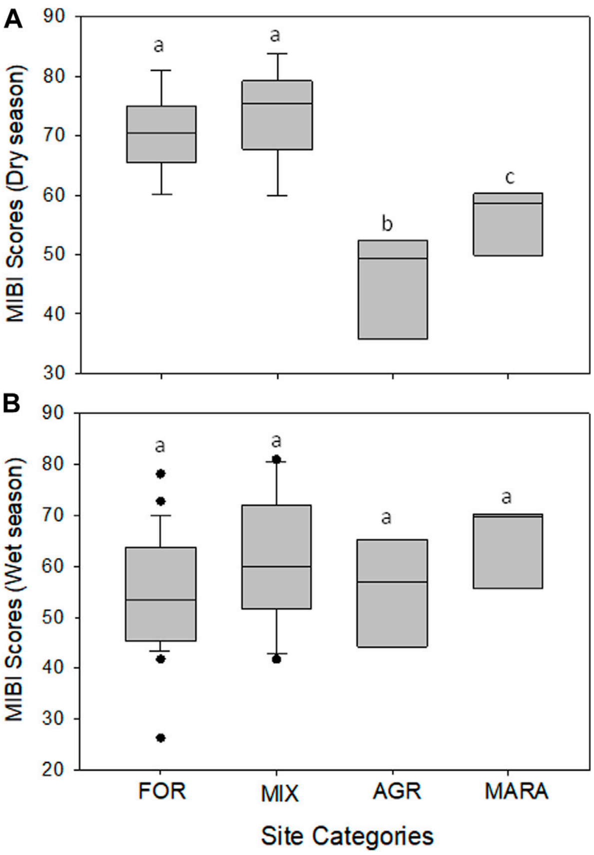

FIGURE 5
Box-and-whisker plots comparing the ability of the M-IBI to discriminate between the four levels of disturbance or site categories (FOR, MIX, AGR and MARA) in the Mara River Basin during the dry **(A)** and wet **(B)** seasons. Boxes show interquartile ranges (25th and 75th percentiles), and the middle lines are medians. FOR, forested; MIX, mixed; AGR, Agricultural, and MARA, Mara River mainstem and seasonal tributaries. Similar letters on boxes indicate no significant difference in metric values among the four condition categories after One-Way ANOVA.

Raburu et al., 2017) but very sensitive to the loss of instream or riparian vegetation that they use for attachment. Odonata was particularly abundant in seasonal streams and river sections with emergent and submerged macrophytes. In other savanna rivers, marginal vegetation supports abundant odonate larvae and adults (Samways and Steytler, 1996), and, as a result, Odonata and other taxa that prefer marginal vegetations are good indicators of riparian deforestation or clearance of instream vegetation (macrophytes) by floods or other disturbances (da Silva Monteiro et al., 2013).

The third new metric included in the M-IBI was the percentage of individuals in burrowing taxa. Taxa that burrow in a soft substrate (gravel, sand, or mud) prefer slow-flowing sections of rivers (backwaters and pools) and are soft-bodied. Burrowing taxa include some Chironomidae, Oligochaeta, Polymitarcyiidae, Gomphidae, Libellulidae, Leptophlebiidae, Tubificidae, Gyrinidae, and Corixidae, among others. The abundance of burrowing taxa is negatively related to discharge and flow velocities in streams and rivers (Masese et al., 2021). In the Njoro River, drying of the river resulted in a tremendous increase in oligochaetes while other sensitive taxa among the EPT were eliminated (Shivoga, 2001; Mathooko et al., 2005). In the Moiben River, the peak of the dry season coincided with a significant decline in sensitive taxa and a proliferation of burrowing and pool-associated taxa, such as Belostomatidae, Corixidae, Gomphidae, and oligochaetes (Masese et al., 2009a; Masese et al., 2009b). Similar findings have been reported in other river systems suggesting that burrowing or pool-associated taxa are good indicators of flow reduction, sedimentation or organic pollution in streams and rivers.

4.5 Comparison of diversity, biotic and multimetric (M-IBI) indices

The discriminatory ability of the different indices was in the order M-IBI > biotic indices > diversity indices. Incorporating various metrics that responded differently to various forms and levels of disturbance gave the M-IBI an edge over diversity and biotic indices in the Mara River. Despite the notable better performance of the M-IBI, the three classes of indices were insensitive to low and moderate levels of disturbance in the river, specifically among FOR and MIX sites, MIX and AGR sites and AGR and MARA sites. Only the extreme ecological conditions in FOR and MARA sites were distinguishable, and even so, most diversity and biotic indices couldn't differentiate FOR and MARA site categories. Failure of diversity and biotic indices to identify MARA sites as disturbed shows that the indices are biased in assessing disturbed sites with high taxa richness. It has been noted that diversity measures describe community structure but not the pollution level of water bodies (Washington, 1984). Thus, when community structure remains unchanged, for instance, by replacing sensitive taxa with tolerant taxa when water quality degrades or the level of disturbance increases, most diversity indices cannot capture this change. Similarly, biotic indices are insensitive to the number of taxa but to the sensitivity ratings or scores of individual taxa. However, most derivatives of the biotic indices were positively correlated with the number of taxa, not the number of families.

4.6 Considerations for using diversity, biotic and multimetric indices

Although the diversity and biotic indices have a long history of use as discriminators of different levels of pollution and other forms of disturbance in streams and rivers, the findings of this study show that they can be limited when distinguishing low to moderate levels of disturbance. Diversity indices were particularly ineffective in identifying changes in macroinvertebrate community composition caused by the replacement of taxa, and not loss of taxa. On the other hand, biotic indices were insensitive to the number of taxa and instead relied more on individual taxa's sensitivity ratings or scores. This made it challenging to detect moderate forms of disturbance that maintain or increase taxa richness. These shortcomings of diversity and biotic indices are addressed in multimetric indices

TABLE 7 Pair-wise Spearman correlations among regional biotic indices and the macroinvertebrate Index of Biotic Integrity (M-IBI) for the Mara River. The top-right half represents the wet season, while the bottom-left half is for the dry season.

	Number of taxa	Number of families	SASS5-score	TARISS-score	ETHbios-score	SASS5-ASPT	TARISS-ASPT	ETHbios-ASPT	M-IBI
Number of Taxa	–	0.91***	0.87***	0.87***	0.85***	0.64**	0.61**	0.64**	0.38*
Number of Families	0.85***	–	0.91***	0.92***	0.94***	0.69**	0.69**	0.73**	0.43*
SASS5-Score	0.78**	0.82***	–	0.97**	0.95***	0.83***	0.80***	0.74**	0.52*
TARISS-Score	0.79**	0.81***	0.97***	–	0.97***	0.82***	0.84***	0.78**	0.58*
ETHbios-Score	0.82***	0.79**	0.94***	0.94***	–	0.75**	0.77**	0.82***	0.51*
SASS5-ASPT	0.25	0.07	0.50*	0.47*	0.49*	–	0.94***	0.79**	0.54*
TARISS-ASPT	0.01	−0.23	0.15	0.19	0.19	0.89***	–	0.83***	0.59*
ETHbios-ASPT	−0.09	−0.38*	−0.08	−0.03	0.02	0.71**	0.88***	–	0.38*
M-IBI	0.20	0.17	0.58*	0.53*	0.57*	0.66**	0.68**	0.31	–

Correlation is significant at the alpha level: ***<0.001, **0.01, *0.05

TABLE 8 Classification categories of total Mara River M-IBI scores, their integrity classes and narrative description based on the riverine habitats and flow conditions in the study area.

Total M-IBI score and integrity classes	Narrative description
Excellent >81 (A class)	No human activity within 100 m of the riparian zone, stable banks and natural or re-grown vegetation stabilizing the banks. Instream habitat dominated by a stable substrate. Water is clear (you can see the bottom). Percent EPT individuals >50% and number intolerant EPT taxa >14
Good, 66–81 (B class)	Minimal human activity within 50 m of the riparian zone, %EPT, >40%, and number intolerant EPT taxa >8. The bottom substrate is dominated by stones and vegetal materials. Water clear as the bottom can be seen
Fair, 52–65 (C class)	Minimal human activity in the river or riparian zone. Natural vegetation maintained along the reach, %EPT >30%, %Diptera <30%, number of EPT taxa >5, substrate mainly stable in riffles and runs
Poor, <52 (D class)	Collapsed and eroded riverbanks, human activity includes agriculture, animal watering points, number of intolerant EPT taxa <5, % EPT <30%, % tolerant taxa >30% and dominated by chironomids and oligochaetes. Substrate dominated by soft sediments and organic material, water turbid (cannot see the bottom)

that incorporate various metrics of macroinvertebrates whose utility is individually evaluated before inclusion in the final index.

There is growing interest in developing biotic indices for the assessment of surface waters in Africa (Dallas, 2021; Masese et al., 2022). Thus, the performance of existing indices should be evaluated to inform the development of new ones. Most importantly, it is vital to determine the situations and conditions under which their use is most appropriate. There is also a need to improve the performance of biotic indices by empirically establishing the sensitivity ratings or scores of native taxa instead of relying on values in the literature. Although TARISS and SASS5 could assess the ecological condition of the different sites in the Mara River, their performance in terms of discriminating different levels of disturbance in the river was inadequate. Thus, if any one of the indices is to be used for regular biomonitoring of the river, several improvements must be made. First, all the taxa in the Mara River which could not be scored because of missing sensitivity weights or ratings in the two indices must be assigned sensitivity scores. This can be done using water quality and distribution data or by comparing the distribution with taxa already scored in other indices. Secondly, the sampling methods need to be tested for representativeness, and data on abundance incorporated as part of the bioassessment protocols. Although abundance data is estimated in SASS5 (Dickens and Graham, 2002), it isn't clear how these data are incorporated into the categorization of sites into different ecological classes. Thirdly, the sensitivity scores of the existing taxa should be re-assessed to ensure that they are congruent with the displayed responses in water quality and the overall condition of the sampled sites.

Concerns have been raised over the possibility of regional differences in the sensitivity of similar or related taxa to the same forms of disturbance (Kaaya et al., 2015; Masese et al., 2017; Dallas, 2021). Lastly, the issue of seasonality should be addressed to determine the optimum conditions for sampling. This is necessary because water resources are dwindling, and increasing demand has led to excessive withdrawals that convert once permeant streams and rivers into seasonal ones. In semi-arid and sub-humid savanna grasslands, streams and rivers experience hydrological extremes characterized by flooding during spates and cessation of flows during the dry season or droughts. This makes it difficult to develop an index suitable for all flow conditions. For instance, the SASS5 protocol is not recommended for high-flow (wet) conditions (Chutter, 1972; Dickens and Graham, 2002) because it is too variable and unreliable. However, it is very good for assessing the effects of low flow on ecological conditions and establishing minimum flow requirements (environmental flows) for sustaining the functioning of rivers (Dickens and Graham, 2002; O'Keeffe and Dickens, 2008).

Even with the development of regional indices such as TARISS and ETHbios in Eastern Africa, there is still a need to develop a biotic index for river systems in Kenya and other African countries. However, this doesn't need to start from scratch as existing regional indices such as SASS or TARISS can be used as foundations. Most importantly, these indices must be validated and tested using native taxa and environmental conditions. Differences in climate, geology, altitude, longitude, and latitude among regions and African countries may contribute to differences in physico-chemical characteristics of rivers, resulting in differences in macroinvertebrate assemblages and sensitivities to disturbance gradients and general impairment of ecosystem functioning.

5 Conclusion

The findings of this study show that while diversity and richness indices that measure the structure of macroinvertebrate communities are widely used in determining the level of anthropogenic disturbance in streams and rivers, their performance in the Afromontane-savanna rivers isn't adequate. Most diversity and richness indices tested performed poorly and failed to discriminate among different levels of human disturbance. Overall, the M-IBI performed better than biotic and diversity indices by having a higher discriminatory ability among site categories. The poor performance of regional biotic indices in assessing the river's ecological condition provides more evidence for the need to test and validate indices developed elsewhere before their use in bioassessment programs and decision-making.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

FM, EW, KF, and MM: Conceptualization, experimental design; FM, EW, KF, and MM: Data collection; FM, EW, AA, and KN: Data analysis; FM and EW: Drafting of the manuscript; All authors commented on the manuscript and approved it for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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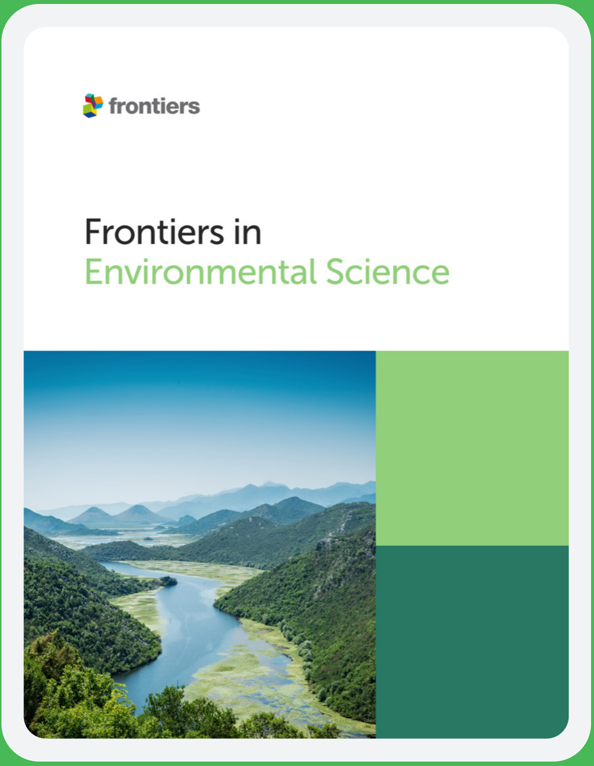
Appendix

TABLE A1 List of taxa (families) in the Mara River basin that are missing sensitivity scores in the South African Scoring System version 5 (SASS5), Tanzanian River Scoring System (TARISS) and the biotic index for the Ethiopian highlands (ETHbios) biotic indices.

#	Phylum/ Class/ Order	SASS5	TARISS	ETHbios
1	Coleoptera	Chrysomelidae	Chrysomelidae	Chrysomelidae
2	Arachnida	Arachnida	Arachnida	Arachnida
3	Collembola	Collembola	Collembolla	Collembolla
4	Diptera	Stratiomyidae	Stratiomyidae	Stratiomyidae
5	Hemiptera	Saldidae	Saldidae	Saldidae
6	Hemiptera	Cicadellidae	Cicadellidae	Cicadellidae
7	Coleoptera	Carabidae	Carabidae	Carabidae
8	Coleoptera	Curculionidae	Curculionidae	Curculionidae
9	Coleoptera	Lampyridae	Lampyridae	Lampyridae
10	Coleoptera	Helophoridae	Helophoridae	Helophoridae
11	Diptera	Chaoboridae	Chaoboridae	Chaoboridae
12	Mollusca	Bithyniidae	Bithyniidae	Bithyniidae
13	Hemiptera	Hebridae	Hebridae	Hebridae
14	Neuroptera	Sisyridae	Sisyridae	Hydroptilidae
15	Diptera	Sciomyzidae	Sciomyzidae	Sciomyzidae
16	Odonata	Synlestidae	Synlestidae	Synlestidae
17	Ephemeroptera			Oligoneuriidae
18	Ephemeroptera			Prosopistomatidae
19	Ephemeroptera			Polymitarciidae
20	Diptera			Dixidae
21	Diptera			Simuliidae
22	Diptera			Athericidae
23	Diptera			Empididae
24	Trichoptera			Calamoceratidae
25	Trichoptera			Pisuliidae
26	Trichoptera			Polycentropodidae
27	Trichoptera			Psychomyiidae
28	Lepidoptera			Pyralidae
29	Megaloptera			Sialidae
30	Neuroptera			Sisyridae
31	Crustacea			Atyidae
32	Turbellaria			Tricladida
33	Coleoptera			Corduliidae
34	Odonata			Platycnemididae
35	Odonata			Protoneuridae
36	Hemiptera			Hydrometridae
37	Lepidoptera			Crambidae/ Pyralidae
38	Pelecypoda			Unionidae
39	Gastropoda			Lymnaeidae



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