SPATIO-TEMPORAL VARIABILITY IN WATER QUALITY AND MACROINVERTEBRATES ASSEMBLAGE OF LUNYANGWA RIVER, NORTHERN MALAWI

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SPATIO-TEMPORAL VARIABILITY IN WATER QUALITY AND MACROINVERTEBRATES ASSEMBLAGE OF LUNYANGWA RIVER, NORTHERN MALAWI

A THESIS SUBMITTED TO DEPARTMENT OF FISHERIES AND AQUATIC SCIENCES, FACULTY OF ENVIRONMENTAL SCIENCES IN PARTIAL FULFILMENT FOR THE AWARD OF MASTER OF SCIENCE IN FISHERIES AND AQUATIC SCIENCES

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DECLARATION

I hereby declare that the thesis titled "Spatio-Temporal Variability in Water Quality and Macroinvertebrates Assemblage of Lunyangwa River, Northern Malawi" has been written by me and is a record of my work. All citations, references, and borrowed ideas have been duly acknowledged. It is being submitted in fulfilment of the requirements for the award of Master of Science in Fisheries and Aquatic Sciences, Mzuzu University. None of the present work has been submitted previously for any degree or examination in any other University.

______________________ 5th January 2026

Aisha Lali Date

CERTIFICATE OF COMPLETION

We, the undersigned, certify that this thesis is a result of the author's work and that, to the best of our knowledge, it has not been submitted for any academic qualification within Mzuzu University or elsewhere. The thesis is acceptable in form and content, and the candidate demonstrated satisfactory knowledge of the field covered herein through an oral examination held on ……………………………………………

This thesis has been presented with our approval as university supervisors.

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DEDICATION

I dedicate this work to my parents for their sacrifices and unwavering love. To the future scientists who will ask better questions, may this be a steppingstone.

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I thank God for the grace and strength that propelled me through this journey.

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ACRONYMS

BMWP	Biological Monitoring Working Party.
BOD	Biochemical Oxygen Demand.
CCA	Canonical Correspondence Analysis.
COD	Chemical Oxygen Demand.
CPOM	Coarse Particulate Organic Matter.
DO	Dissolved Oxygen.
EC	Electrical Conductivity.
EPT	Ephemeroptera, Plecoptera, Trichoptera.
FPOM	Fine Particulate Organic Matter.
HBI	Hilsenhoff Biotic Index.
ITCZ	Inter-Tropical Convergence Zone.
M-IBI	Multimetric Index of Biotic Integrity.
MMI	Multimetric Indices.
NMDS	Non-metric Multidimensional Scaling.
NSO	National Statistical Office.
OHDP	Oligochaeta, Hirudinea, Diptera, Pulmonates.
PCA	Principal Component Analysis.

PERMANOVA	Permutational Multivariate Analysis of Variance.
PHQI	Physical Habitat Quality Index.
RDA	Redundancy Analysis.
SASS	South Africa Scoring System.
SDG	Sustainable Development Goals.
SIMPER	Similarity Percentage Analysis.
TBI	Trent Biotic Index.
TDS	Total Dissolved Solids.
TP	Total Phosphorus.
TSS	Total Suspended Solids.
USEPA	United States Environmental Protection Agency.
WQI	Water Quality Index.

ABSTRACT

Freshwater ecosystems in Sub-Saharan Africa are experiencing severe degradation due to agricultural expansion, urbanization, and poor sanitation, threatening the ecosystem services that riparian communities depend on. This study examined how land use affects water quality and benthic macroinvertebrate assemblages in the Lunyangwa River, Mzuzu City, Northern Malawi. Macroinvertebrates and water quality parameters were sampled at nine stations across three land-use types (forested, agricultural, and urban; n=3 each) during dry (Oct-Nov 2024) and rainy (Feb-Apr 2025) seasons. Data were analyzed using diversity indices, water quality standards, and multivariate statistics to assess spatial and temporal variation. Urban stations showed the most severe degradation, with very poor water quality (WQI: 186.40–204.25), lowest dissolved oxygen (3.07 ± 2.74 mg/L), highest biochemical oxygen demand (4.73 ± 1.17 mg/L), and macroinvertebrate communities dominated by pollution-tolerant taxa like Bellamya capillata and Tanypodinae, resulting in low diversity (Shannon H = 0.96). Agricultural stations displayed intermediate conditions, while forested stations maintained the highest water quality and macroinvertebrate diversity. Land use was the primary driver of variation in both water quality and macroinvertebrate composition, with temperature, dissolved oxygen, BOD, pH, conductivity, and salinity differing significantly among land-use types, while seasonal effects were minimal. The findings demonstrate that macroinvertebrates are effective bioindicators of water quality and provide scientific evidence for urgent conservation interventions, including riparian zone protection, improved wastewater treatment, and regular biomonitoring programs for sustainable river basin management in the Lunyangwa River and similar African freshwater systems.

TABLE OF CONTENTS

DECLARATION iii

CERTIFICATE OF COMPLETION iv

DEDICATION v

ACKNOWLEDGEMENT vi

ACRONYMS viii

ABSTRACT x

CHAPTER ONE. INTRODUCTION 1

1.1 Background Information 1

1.2 Statement of the problem 3

1.3 Objectives of the study. 5

1.3.1 Broad objective 5

1.3.2 Specific objective 5

1.4 Hypothesis 5

1.5 Justification 6

CHAPTER TWO. LITERATURE REVIEW 8

2.1 Physico-chemical water quality parameters in rivers 8

2.2 Water Quality Indices (WQIs) 12

2.3 Benthic Macroinvertebrate composition, diversity and distribution in rivers 13

2.4 Biotic Indices 16

2.5 Relationship between water quality and macroinvertebrates in rivers 17

2.6 Conclusions from the literature review 20

CHAPTER THREE. MATERIALS AND METHODS 22

3.1 Description of Study Area 22

3.1.1. Climate 23

3.1.2 Geology and soils 24

3.1.3 Land use 25

3.2 Selection of Sampling Stations 25

3.2.1 Description of sampling stations 26

3.3 Sampling design 29

3.3.1 Physico-chemical water quality parameters 30

3.3.2 Benthic macroinvertebrates 30

3.4 Laboratory Analysis 33

3.4.1 Nutrient Analysis 33

3.4.2 Macroinvertebrates 33

3.5 Benthic macroinvertebrates community diversity and water quality indices 34

3.5.1 Diversity indices 34

3.5.2 Macroinvertebrate Community Composition 35

3.5.3 Calculation of Water Quality Index (WQI) of the river 35

3.6 Data Analysis 39

3.7 Ethical Considerations. 40
CHAPTER FOUR. RESULTS 41
4.1 Spatio-temporal variability in water quality physico-chemical parameters and nutrients.. 41
4.1.1 Water Quality Index 45
4.2 Spatio-temporal variability in composition, distribution and diversity of benthic macroinvertebrates 46
4.3 Relationship between water quality physico-chemical parameters and benthic macroinvertebrates 57
CHAPTER FIVE. DISCUSSION 61
5.1 Spatio-temporal variability in water quality physico-chemical parameters. 61
5.2 Spatio-temporal variability in composition, distribution and diversity of benthic macroinvertebrates 66
5.3 Relationship between water quality physico-chemical parameters and benthic macroinvertebrates. 74
CHAPTER SIX. CONCLUSION AND RECOMMENDATION 82
6.1 CONCLUSIONS 82
6.2 RECOMMENDATIONS 83
6.2.1 Management and Policy Recommendations 84
REFERENCES 85
APPENDICES 103
APPENDIX I: Mzunirec Approval 103

APPENDIX II: Forest Access Permission Letter.. 104

APPENDIX III: Images of Macroinvertebrate Specimens Sampled...................................... 105

APPENDIX IV: SASS Score Sheet 106

APPENDIX V: Macroinvertebrate Presence/Absence Data.. 107

LIST OF TABLES

Table 1. Interpretation of SASS results based on SASS score and ASPT (Chutter, 1995). 35

Table 2. Normalization factors (Ci) and relative weights (Wi) for water quality variables used in the calculation of the Water Quality Index (WQI). 37

Table 3. Water quality classification based on WQI values (Bhateria and Jain, 2016). 38

Table 4. Two-way ANOVA results testing the effects of land use types (sites), seasons, and their interaction on water quality parameters in Lunyangwa River. Values in bold indicate statistically significant effects ($p < 0.05$). 42

Table 5. Mean values (± standard deviations) of water quality parameters across three land use categories during dry and wet seasons. (means with different letters across sites are significantly different). 43

Table 6. Spatial and Temporal Changes in Water Quality Index (WQI) across the Lunyangwa River. 46

Table 7. Chi-square test of seasonal abundance difference 47

Table 8. The diversity indices of macroinvertebrate structural communities across land use types in dry and wet seasons of the Lunyangwa river. 48

Table 9. Statistical results (two-way ANOVA) for differences in SASS Score, Number of Taxa, and ASPT across land use types and seasons. Significant results *($p < 0.05$)* are indicated in bold. 49

Table 10. Site-specific biological data for the dry and wet seasons. Sites are categorized by land use type. Water quality class is based on SASS5 guidelines: A (unimpacted) to E (seriously impacted). SASS= South African Scoring System, ASPT= Average Score Per Taxon 50

Table 11. Analysis of Similarities (ANOSIM) for benthic macroinvertebrate assemblages across land use types and seasons. 53

Table 12. Ranked abundance-based SIMPER analysis for macroinvertebrate taxa collected in Lunyangwa river for both wet and dry seasons. 54

Table 13. Pearson correlation coefficients between water quality parameters macroinvertebrate community diversity indices with Bonferroni correction for multiple comparisons. 60

LIST OF FIGURES

Figure 1. Map of Lunyangwa River showing different sampling sites. 22

Figure 2. PCA plots for both habitat quality and water quality physico-chemical (A)landuse types, (B)seasons, (C)dry season and (D)wet season of the Lunyangwa river. TDS = Total Dissolved Solids, TSS = Total Suspended Solids, BOD = Biological Oxygen Demand, TP = Total Phosphorus, DO = Dissolved Oxygen, EC = Electric Conductivity, TEMP = Temperature. 45

Figure 3. Seasonal variation in water quality indices across land use types. Box plots show SASS scores and ASPT values for agriculture, forested, and urban sites during dry season (gray boxes) and wet season (white boxes). Boxes represent interquartile ranges with median values (horizontal lines), whiskers show data range, and dashed horizontal lines indicate reference thresholds for water quality assessment. 50

Figure 4. Relative abundance of macroinvertebrate orders across land use types in dry and wet season of the Lunyangwa river, Northern Malawi 51

Figure 5. Relative abundance of EPT orders across land use types in dry and wet season of the Lunyangwa river, Northern Malawi 52

Figure 6. Non -metric Multidimensional Scaling (NMDS) plots illustrating macroinvertebrate community composition of (A) seasonal variations, (B) landuse types, (C) dry season and (D) wet season. 56

Figure 7. Redundancy Analysis (RDA) biplots showing the relationships between water quality parameters and macroinvertebrates where (A)landuse types, (B)seasonal variations, (C)wet season and (D)dry season. 59

CHAPTER ONE. INTRODUCTION

1.1 Background Information

Freshwater ecosystems, particularly rivers, are vital for life-support services and hold significant intrinsic value (Omary et al. 2023). They are crucial resources for drinking water, industry, commerce, and recreation. Globally, it is estimated that about 65% of freshwater habitats are considered moderately to severely threatened (Dudgeon et al. 2005; Schowe and Harding, 2014). In most cases, the quality of water in catchments is subject to both temporal and spatial changes, and depending on the specificity of the catchment or region, these changes are the result of various combinations of natural and/or anthropogenic factors (Ouattara et al. 2011; Huang et al. 2014; Lenart-Boroń et al. 2016, 2017). These threats include deforestation, habitat fragmentation, sedimentation, dam construction, urbanization, excessive water withdrawals, water pollution, and biological invasions (Masese et al. 2023). Climate variability and changing precipitation patterns significantly compound existing pressures on freshwater biodiversity by altering hydrological regimes and intensifying stressors (Masese et al. 2025).

Various water quality parameters like nutrients, dissolved oxygen and temperature are significantly impacted by different threats, primarily stemming from anthropogenic activities, land-use changes, and natural processes (Balaka and Chagoma, 2022). Human and industrial activities discharge contaminated water into streams and rivers, leading to deterioration. The cumulative effects of these multiple stressors create complex pollution gradients along river systems, where upstream activities cascade downstream to affect entire watersheds. Among these threats, one can mention land use, which in turn affects the type, number, and location of point, and non-point sources of pollution (USEPA, 2015). These pollution sources operate through distinct pathways, point sources such as industrial discharge pipes and sewage outfalls create localized contamination hotspots, while non-point sources like agricultural runoff and urban stormwater contribute diffuse pollution that can be more challenging to identify and regulate. Surface runoff carries sediments, nutrients, and contaminants from altered landscapes, while groundwater-surface water interactions can mobilize pollutants across broader temporal and spatial scales. This is especially true for (sub) tropical developing countries where intensification of land use for agriculture, urbanization and poor disposal of untreated waste has markedly degraded rivers and streams (Nyenje et al. 2010; Paisley et al. 2011; Bere and Nyamupingidza, 2014).

Rivers in sub-Saharan Africa, including Malawi, face complex and intensifying challenges due to rapid urbanization, agricultural expansion, and climate change (Fouchy et al. 2019; Mpopetsi et al. 2025). In Malawi specifically, river catchments, despite their importance, are deteriorating in terms of channel integrity, ecosystem health, and water quality due to human activities. The Likangala River catchment in southern Malawi, for instance, is affected by deforestation, agriculture, waste disposal, river bank cultivation, and river sand mining, leading to pollution from human activities (Pullanikkatil et al. 2015). Consequently, understanding and monitoring the health or "ecological integrity" of these systems is of paramount importance (Enabulele et al. 2024). Assessing ecological integrity requires integrated approaches that combine physical, chemical, and biological indicators to provide comprehensive understanding of ecosystem health and inform management decisions (Lukhabi et al. 2024).

To effectively assess this integrity, researchers increasingly rely on biological indicators, among which macroinvertebrates are particularly valuable. Macroinvertebrates are very good bioindicators in tropical African habitats (Enabulele et al. 2024). Their great taxonomic richness, communities that reflect environmental conditions over time and presence in nearly all freshwater habitats make them ideal for assessment (Dabessa et al. 2021). Their long larval life cycles enable researchers to assess long-term declines in environmental quality. They can reflect both short-term and long-term impacts on ecosystems by integrating the effects of various stressors over time (Enabulele et al. 2024). This sedentary behavior, combined with their variable sensitivity to multiple disturbances, including toxic pollutants, sedimentation, and habitat degradation, allows them to be used to detect diverse environmental responses (Abeke Ayoade and Adewumi Adeyemi, 2022). Efficient, adaptable biomonitoring tools like the South African Scoring System (SASS) and the Tanzania River Scoring System (TARISS) are already applied on the continent (Masese and Raburu, 2017). These biological tools are a critical addition to physico-chemical tools; they provide an overall picture of ecosystem well-being, track periodic pollution that cannot be captured by chemical snapshots, and offer a low-cost solution for long-term monitoring where resources are scarce.

Therefore, this study aims to contribute to river basin management and conservation efforts by assessing the spatial and temporal variation in macroinvertebrate assemblages and water quality along a disturbance gradient in the Lunyangwa River, located in Malawi. The Lunyangwa River flows through urban areas, particularly Mzuzu City, which is experiencing rapid urbanization. With annual and intercensal growth rates of 4.4% and 54% respectively (National Statistical Office, NSO, 2009), Mzuzu City's expansion poses potential risks to water quality and to the composition, distribution, and diversity of biological organisms in the river.

1.2 Statement of the problem

The escalating pollution of freshwater bodies due to human activities poses a significant threat to environmental sustainability and socio-economic development (Nyenje et al. 2010; Mpopetsi et al. 2025). In Malawi, one of the major drivers of river degradation is the widespread conversion of riparian zones into agricultural land, a practice that goes against national environmental policies and legislation. This pressure, combined with everyday uses such as laundry, domestic water supply, and activities across the catchment, has accelerated both biological and physical deterioration of many rivers (PACN, 2010). These pressures are already evident in the Lunyangwa River, which receives effluent from industrial areas, runoff from farms, and waste from surrounding settlements. Such inputs threaten the river's ability to supply water for domestic use, livestock, and aquatic life. These concerns make it necessary to assess the current state of the Lunyangwa River and generate information that can guide future management and protection of its ecosystem.

Physico-chemical water quality measurement techniques have been used to assess the ecological health of streams and rivers in Malawi including studies on the Bua River (Balaka and Chagoma, 2022). However, this traditional river health monitoring method is expensive and unreliable as it offers only instantaneous water quality status (Resende et al. 2010), and few studies integrate multiple water quality parameters comprehensively. Moreover, these techniques overlook the long-term impacts of disturbances on stream ecological health (Masese et al. 2013). Consequently, there is an urgent need to develop more comprehensive and cost-effective monitoring approaches that account for both short-term and long-term effects of pollution on river ecosystems.

While bioassessment studies have been conducted in streams and rivers globally and in other countries in Africa and proved to be superior in detecting the ecological health status of most lotic ecosystems (Kaaya et al. 2015; Musonge et al. 2019; Dallas, 2021; Feio et al. 2023; Kabore et al. 2024), there are limited studies in Malawi that have adopted macroinvertebrate-based tools. A few examples include works done on the Bua, Linthipe, Dzalanyama, and Shire rivers, which have provided useful baseline information. However, these studies have not examined ecological responses across disturbance gradients in smaller, urban-affected rivers, nor have they linked physico-chemical patterns with macroinvertebrate community changes across seasons. Lunyangwa River, which is exposed to multiple pressures from agriculture, settlements, aquaculture, and industry, remains one of the systems that have not been evaluated using an integrated bioassessment approach.

1.3 Objectives of the study.

1.3.1 Broad objective

The main objective of the study is to assess the spatial and temporal variability in macroinvertebrate assemblages and water quality in the Lunyangwa River, Malawi.

1.3.2 Specific objective

The specific objectives of the study were:

i. To determine the spatio-temporal variability in water quality physico-chemical parameters in response to land uses changes along the Lunyangwa River.
ii. To determine the spatio-temporal variability in the composition, distribution and diversity of benthic macroinvertebrates in response to land uses changes along the Lunyangwa River.
iii. To examine the relationship between water quality physico-chemical parameters and benthic macroinvertebrate communities in response to land uses changes along the Lunyangwa River.

1.4 Hypothesis.

H_{01}: There is no significant variability in selected physical and chemical parameters in response to land uses changes along the Lunyangwa River.

H_{02}: The composition and diversity of benthic macroinvertebrates do not show significant spatial and temporal variability in response to land uses changes along the Lunyangwa River.

H_{03}: There are no significant relationships between measured physico-chemical water quality parameters and benthic macroinvertebrates in response to land uses changes along the Lunyangwa River.

1.5 Justification

Assessing water quality exclusively through physicochemical parameters is considered a costly approach (Sitati et al. 2021). Furthermore, this method lacks the integrative capacity required to report effectively on the influence of pollution on biodiversity and the overall ecological integrity of aquatic resources (Sitati et al. 2021). On the other hand, biological indicators provide complete spatial-temporal integration of knowledge of freshwater ecosystems and their landscapes, making this approach an excellent method for effective evaluation of the ecological health of rivers and their landscapes (Couceiro et al. 2012; Uherek et al. 2014).

The Lunyangwa River, flowing through the rapidly urbanizing Mzuzu City, represents a critical system for assessing human impact on aquatic ecosystems. Previous studies on this river have primarily relied on physico-chemical water quality parameters, which have successfully identified point sources of pollution and established a basic baseline. However, these traditional approaches offer a limited, instantaneous snapshot and cannot fully capture the integrated, long-term ecological health of the river. What remains unknown is how these documented physico-chemical pressures translate into biological consequences for the river's ecosystem. This study leverages the river's clear gradient of human impact, from its pristine source in the Kaning'ina Forest Reserve through agricultural zones and into the urban core of Mzuzu, to address this critical gap.

Employing bioindicators enables the timely detection and application of appropriate conservation strategies (Mzungu, Yakub and Anyimba, 2022) compared to chemical and physical monitoring, which provides a "snapshot" of conditions. Biological monitoring provides a "moving picture" of past and present conditions, offering a more spatially and temporally integrated measure of ecosystem health (Carter, Resh and Hannaford, 2017). When properly carried out, biological surveillance can reveal the occurrence of ecologically-significant environmental changes and call attention to the need for further investigation.

CHAPTER TWO. LITERATURE REVIEW

2.1 Physico-chemical water quality parameters in rivers

Water quality is a general term describing the characteristics of water resources, including their physical and chemical properties (Lemessa et al. 2023). Investigating the concentration of water quality parameters is crucial for future river water quality monitoring (Lemessa et al. 2023). These parameters play important roles in assessing and monitoring river water and maintaining the ecological integrity of the river ecosystem (Lemessa et al. 2023). Knowledge about water quality parameters is a vital part of environmental monitoring and determining the condition of aquatic habitats (Tampo et al. 2021). The usability of surface water is based on the water quality which is a prerequisite in determining the health of any society (Ezemonye et al. 2016). Unfortunately, many literatures have reported of high levels of deterioration of river water quality occasioned by arrays of anthropogenic activities (Edori and Kpee, 2016). Therefore, the evaluation of water quality has been considered as an important aspect of water management strategy, but since it involves the measurement of large number of parameters, it has been considered as a complex task.

When water quality is poor, it negatively affects aquatic life and ecosystem health (Tampo et al. 2021). Several physico-chemical parameters are consistently examined to understand river health. These include core measurements like pH, temperature, and dissolved oxygen (DO), alongside indicators of mineralization, turbidity, and nutrient levels.

Electrical conductivity (EC) and total dissolved solids (TDS) are crucial indicators of water mineralization and salinity (Raphahlelo et al. 2022). EC reflects the ability of water to conduct current, which is influenced by the concentration of inorganic dissolved solids such as chlorides, nitrate, sulfate, phosphate, sodium, magnesium, calcium, iron, and aluminum ions (Lemessa et al.

2023). A sudden change in EC can indicate pollution. High EC values can negatively affect freshwater organisms, including macroinvertebrate communities (Lemessa et al. 2023) and Sitati et al. (2021) argued that disturbances can lead to increased conductivity in rivers.

The pH is a fundamental water quality parameter that indicates the strength of the acidic or alkalinity character of a solution (Omary et al. 2023). It is one of the most important parameters that can affect the suitability of water for various uses, including drinking, irrigation, recreation, habitat for aquatic life, and industrial operations (Omary et al. 2023), it is vital for the ecology of aquatic macroinvertebrates, as they are sensitive to its variations. Values outside the range of 5–9 can be harmful. One source found pH ranging from 6.4 to 7.8, generally neutral to slightly alkaline. Low pH can be associated with lower diversity of benthic macroinvertebrates. Some effluents can result in very high pH values (Omary et al. 2023).

Omary et al., (2023) said DO is the quantity of gaseous oxygen dissolved in water. He continued to argue that DO levels regulate the metabolic activities of organisms and manage the metabolism of the entire biological community, serving as an indicator of the water's trophic status. DO levels can be higher in the wet season compared to the dry season (Chen et al. 2022). Unpolluted freshwater ecosystems are expected to have DO levels close to, or somewhat above, saturation, typically ranging from 80% to 120% saturation (Ochieng et al. 2021). The lowest acceptable DO concentration for aquatic life is cited as ranging from 6 mg/L in warm water to 9.5 mg/L in cold water (Omary et al. 2023). In one study, a mean DO value around 6–7 mg/L with the 75th percentile around 9.75 mg/L suggested that water samples could be classified from good to excellent quality according to international standards (Tampo et al. 2021). However, another study (Chen et al. 2022a) noted that observed DO values around 7 mg/L were found even at sites with otherwise poor water quality, highlighting the need for localized standards and suitable DO limits.

According to Fekadu et al., (2022), water temperature is influenced by solar radiation intensity, evaporation, and river water influx he further continued that factors like presence and absence of vegetation cover/shading effect, anthropogenic activities in the watershed, and water depth can cause spatial variations in temperature.

Turbidity affects sunlight penetration and aquatic life by scattering and obstructing light with suspended particulate matter like mud, algae, detritus, and fecal material clay (Lemessa et al. 2023). High turbidity can be linked to land surface activities and environmental conditions (Gbedzi et al. 2022). Factors like land cover changes, mining impact, deforestation, farming, and the nature of the water body and human disturbance activities contribute to turbidity variation (Gbedzi et al. 2022). For instance, in the Lunyangwa River catchment in Malawi, Wanda et al. (2014) recorded turbidity levels in raw water ranging from 130–225 NTU, far exceeding the WHO guideline of 5 NTU. This was attributed to deforestation and soil erosion in the upper catchment, exacerbated by population-poverty-environment pressures. It is often higher in wet seasons due to increased sediment input with floodwaters (Chen et al. 2022). Less disturbed streams tend to have lower turbidity (Wanderi et al. 2022). Higher turbidity was noted at downstream sites compared to upstream ones in one study (Raphahlelo et al. 2022).

Total suspended solids (TSS) is related to turbidity and represents the mass of solid material suspended in the water column (Masese et al. 2023). High TSS values can be linked to increased anthropogenic activities (Sitati et al. 2021) and tend to be higher at more impacted sites (Masese et al. 2023). According to Tampo et al. (2021) high concentrations of suspended solids can be considered a form of pollution that reduces the quality of habitat for aquatic organisms.

Salinity is an indicator of the total amount of dissolved ions in water and it's a wide word than total dissolved solids (Allan et al. 2021). According to Hanrahan (Hanrahan et al. 2012) and (Montagna et al. 2013), salinity is usually less than 0.5 ppt. Salinity levels are referred to as oligohaline (0.5-5.0 ppt), mesohaline (5.0-18.0 ppt), polyhaline (18.0 to 30.0 ppt) and the average salinity of the ocean is 35 ppt. River's salinity has been indirectly determined by means of electrical conductivity (Alley et al. 2007). Typically expressed in ppt (part per thousand).

Nutrients like total phosphorus (TP), total nitrogen (TN), nitrate (NO3-), nitrite (NO2-), ammonium (NH4+), and orthophosphate (PO43-) are critical indicators of pollution, often originating from sources like agriculture (fertilizers), livestock/wildlife, septic systems, and domestic or industrial wastewater (Kengne Fotsing et al. 2022). High nutrient concentrations, such as nitrates and phosphates, have been observed in polluted sites (Makumbe et al. 2022). Nutrient levels can vary spatially (Ochieng et al. 2021) and seasonally (Masese et al. 2023). Ochieng et al. (2021) observed significant seasonal differences in mean values for TN (higher in wet season) and nitrate (higher in dry season) However, TP did not show significant seasonal differences in his study. Phosphates showed significantly higher mean values during the wet season as reported by Munyai et al. (2025). Wet season often brings influxes of runoffs, potentially impacting nutrient levels.

Biochemical oxygen demand (BOD) measures the quantity of oxygen required by bacteria for breaking down decomposable organic matter present in water (Omary et al. 2023). A higher BOD indicates a higher intake of oxygen by microorganisms to degrade organic materials. The greater the BOD, the more rapidly oxygen is depleted in the water body, as microorganisms use up DO (Lemessa et al. 2023). According to Omary et al. (2023), high BOD can be attributed to factors like agricultural activities, surface runoff, bathing, washing, livestock keeping, and underground water movement containing leachates from solid waste landfills. Adesakin et al. (2023) said landfilling materials rich in organic content can also lead to high BOD by increasing anaerobic microbial activities during decomposition. High water influx during the rainy season might also contribute to high BOD values.

2.2 Water Quality Indices (WQIs)

While individual water quality parameters like BOD and salinity are crucial for understanding specific aspects of water health, a Water Quality Index (WQI) serves to provide a more holistic picture1.A WQI is a tool that describes the overall water quality by combining complex and technical information from multiple parameters into a single, unitless numerical value (Tyagi et al. 2020). It simplifies communication about water quality to non-experts, policymakers, and the public. WQIs predict water quality status and allow for spatial and temporal comparisons (Wu et al. 2021). Chemical parameters are the most frequently used characteristics in WQIs, followed by physical and microbiological parameters (Lukhabi et al. 2023). They further explain that WQIs reflect the impact of multiple water quality parameters and allow for spatial-temporal comparisons. Parameter selection for WQIs depends on the intended use of the water (e.g., drinking, irrigation, aquatic life protection, recreation). The resulting WQI value can categorize water quality into levels such as "extremely poor," "poor," "medium," "good," and "excellent" (Chen et al. 2022). Anthropogenic activities like industrial discharge and urbanization can lead to the deterioration of water quality, which can be reflected in WQI values (Lemessa et al. 2023). While useful, WQI development can have limitations, particularly in parameter selection and classification schemes and incorporating biological parameters is suggested for a more comprehensive evaluation (Lukhabi et al. 2023).

2.3 Benthic Macroinvertebrate composition, diversity and distribution in rivers

Benthic macroinvertebrates are organisms that live on the bottom substrates of aquatic ecosystems and are visible to the naked eye. They are widely used for assessing the ecological integrity and water quality of river ecosystems through biomonitoring (Ollis et al. 2006). Their community structure, encompassing composition, diversity, and distribution, provides valuable insights into the health of freshwater systems, as their life cycles are typically long enough to reflect changes induced by anthropogenic activities (Munyai et al. 2025).

By convention, “macro” generally refers to invertebrate fauna retained by a 500-µm mesh net, although smaller early life stages important for ecological understanding are often included. Benthic macroinvertebrates are animals without backbones that are visible to the naked eye and spend at least part of their life cycle inhabiting the bottom substrate of freshwater ecosystems like streams and rivers (Yazdian et al. 2014). They occupy a critical and central position within aquatic food webs and are vital to energy transfer and nutrient cycling in freshwater ecosystems (Ochieng et al. 2021). Many macroinvertebrates function as primary consumers of plant products, including algae, diatoms, mosses, and decaying leaves (Yazdian et al. 2014). They play a significant role in processing organic matter, which is crucial for ecosystem functioning (Addo-Bediako, 2021). This processing includes; shredders, these organisms consume coarse particulate organic matter (CPOM), such as leaf litter, often found within riparian corridors. Collector-Gatherers; they utilize fine particulate organic matter (FPOM) found on the river bottom. Collector-Filterers; these species filter and consume FPOM from both the stream column and bottom. The presence and composition of different functional feeding groups can reflect the availability of specific food resources in a stream (Carter et al. 2017).

The life cycles of benthic macroinvertebrates make them particularly sensitive to environmental changes in several key ways, making them valuable as bio-indicators (Mzungu et al. 2022). They have life cycles that are long enough for temporal changes caused by perturbations to be detected (Carter et al. 2017). Despite their long enough life cycles for temporal detection, their life spans are also short enough to enable the observation of recolonization patterns following perturbation (Carter et al. 2017). This relatively short life cycle allows for a quick reflection of environmental changes (Fekadu et al. 2022), meaning they respond to changes well before the manifestation of a problem (Makumbe et al. 2022).

Macroinvertebrates are particularly effective bioindicators for several reasons: their communities respond interactively to changes from multiple spatial or time scales, providing a "moving picture" of past and present conditions; they exhibit differing sensitivities to environmental stressors(Abdel Gawad, 2019), including varying degrees of tolerance to pollution; and they represent an integral part of lotic systems, playing crucial roles like processing organic matter and transferring energy to higher trophic levels (Carter et al. 2017). For example, some species like stoneflies, mayflies, and water pennies require high levels of dissolved oxygen and indicate good water quality, while others, such as certain Oligochaeta and Gastropoda, are indicators of organic pollution (Abdel Gawad, 2019). The consistent presence of pollution-sensitive species often indicates clean water conditions, whereas the dominance of resistant species can signal water quality deterioration.

Benthic macroinvertebrate community composition changes are often more informative than simple abundance measures because they provide a more nuanced and integrated understanding of environmental health (Carter et al. 2017). The composition of macroinvertebrate communities is strongly influenced by various environmental factors. Mzungu et al. (2022) say that physico-chemical parameters such as water temperature, pH, dissolved oxygen (DO), electrical conductivity (EC), total dissolved solids (TDS), nitrates, and turbidity play a significant role in macroinvertebrate composition. Different taxa have varying survival rates in different pollution conditions. For example, high EC values can affect certain taxa, and pH values outside a range like 6.5–8.5 have been associated with lower diversity. High concentrations of various parameters like salinity, chloride, sodium, pH, COD, BOD, Mn, Ni, Cd, nitrate, and Fe can affect composition (Adesakin et al. 2023). Habitat characteristics are also crucial, including the availability of microhabitats and food resources (Raphahlelo et al. 2022), sediment deposition, channel flow status, bank stability, and vegetative protection. Anthropogenic activities and land use changes are significant drivers of changes in macroinvertebrate composition (Raphahlelo et al. 2022). Human impacts can lead to habitat degradation and changes in aquatic. Disturbance can create degraded habitat conditions that favor tolerant macroinvertebrate taxa.

Macroinvertebrate diversity in rivers is a fundamental aspect studied to evaluate the ecological health and water quality of these systems (Ochieng et al. 2021). Diversity refers to the variety and abundance of different macroinvertebrate taxa present in a given area (Munyai et al. 2025). It is commonly measured using metrics such as species richness (the number of taxa), and various diversity indices like the Shannon-Wiener diversity index (H'), Simpson index, Pielou evenness, and others like Menhinick's index and Margalef's species richness index (Ochieng et al. 2021).

This study explains that, generally, higher macroinvertebrate diversity and richness are associated with better water quality and less disturbed or pristine environmental conditions. Conversely, low diversity and richness are typically observed in areas with poor water quality or high anthropogenic impact. Further says, in polluted waters, the macroinvertebrate community structure shifts, leading to a decrease in diversity and a dominance by a few pollution-tolerant taxa. For example, Chironomidae and Oligochaeta are often prevalent in urban or disturbed sites with poor water quality, while pollution-sensitive groups like Ephemeroptera, Plecoptera, and Trichoptera (EPT) are associated with higher diversity and minimally disturbed conditions (Makumbe et al. 2022).

2.4 Biotic Indices

Biotic indices are numerical expressions that integrate quantitative values of species diversity with qualitative information on the ecological sensitivity of each taxon (Yazdian et al. 2014). The fundamental assumption of biomonitoring and bioassessment using biotic indices is that measurements of the responses, condition, or community integrity of biota can be used to assess an ecosystem's ecological integrity (Ollis et al. 2006). Biomonitoring using macroinvertebrates is considered a particularly effective and internationally recognized approach for determining riverine ecological conditions and assessing water quality (Kaaya et al. 2015). Historically, the Saprobien or Saprobic System, developed in German rivers in the early 1900s, is generally considered the first biological scoring system for water quality assessment (Ollis et al. 2006). Numerous biotic indices have been developed and are successfully applied globally for river bioassessment, often based on the pollution tolerances of benthic macroinvertebrates (Mehrjo et al. 2024). These indices are such as Trent Biotic Index (TBI), Hilsenhoff's Biotic Index (HBI), South African Scoring System (SASS), Tanzania River Scoring System (TARISS), Zambian Invertebrate Scoring System (ZISS) and Diversity indices. Diversity Indices are indices quantify species diversity in a community, considering both species richness (number of species) and evenness (distribution of individuals among species) (Yazdian et al. 2014). The examples of these indices are Shannon-Wiener Index (H′), Margalef Index Simpson Index. Biotic indices are often used in conjunction with physico-chemical parameters and habitat assessments to provide a comprehensive picture of river health (Yazdian et al. 2014).

Although widely used for biomonitoring, biotic indices suffer from various limitations including geographic specificity (impeding cross-regional use) (Masese et al. 2023), reduced sensitivity to intermediate degradation, and taxonomic resolution hindrance, particularly in tropical systems where local pollution sensitivity is poorly elaborated. Seasonal hydrological variability also makes their reliability difficult, with the majority of indices showing irregular functioning during rainy and dry seasons. A primary limitation occurs when tolerant taxa replace sensitive species without reducing overall richness, so that diversity measures cannot identify ecological decline. Such issues must be properly calibrated locally by iterative validation, even though this process is normally thwarted by a lack of long-term data. In an attempt to compensate for these limitations, integrative methods such as Multimetric Indices (MMIs) have become popular, integrating structural (e.g., EPT richness) and functional (e.g., feeding groups) metrics into composite indicators (e.g., Benthic-IBI) that more strongly discriminate multiple stressor effects along ecological gradients (Carter et al. 2017).

2.5 Relationship between water quality and macroinvertebrates in rivers

Numerous studies have indeed established strong links between water quality variables and the structure of macroinvertebrate communities. These relationships are fundamental to understanding how environmental stressors impact aquatic biodiversity and are frequently utilized to detect ecological degradation across various land use gradients (Orozco-González and Ocasio-Torres, 2023). For example, in the Mutshundudi River in South Africa, both water quality and sediment chemistry were identified as driving factors for macroinvertebrate communities, with degradation observed due to high nutrient content from sewage, waste disposal, and poor farming practices (Munyai et al. 2024). Benthic macroinvertebrates serve as valuable biological indicators for the health of river ecosystems (Ndichu et al. 2023). Their presence, abundance, and community structure reflect the environmental conditions of the water body over time (Ochieng et al. 2021). The sources reinforce this, with several studies utilizing macroinvertebrate indices and community composition to assess water quality and the impact of disturbances (Ndichu et al. 2023).

These organisms represent the abiotic and biotic status of an environment and demonstrate how environmental changes affect the habitat, community, or ecosystem (Munyai et al. 2025). Because they are relatively sedentary, present, easily collected, and visible to the naked eye, they are practical for water quality assessment. Invertebrate assemblages integrate environmental changes over several months, and examining their structure and composition can effectively inform on the ecological status of water bodies and show the effects of past and present environmental degradation (Kengne Fotsing et al. 2022). Different macroinvertebrate taxa respond differently to environmental pressures such as habitat loss, degradation, pollution, and siltation, allowing their abundance and diversity to be quantified to predict the environmental conditions of a river (Sitati et al. 2021).

The abundance of macroinvertebrates in an aquatic environment mirrors the water quality because different taxa have varying requirements to live (Omary et al. 2023). Further explains that, some macroinvertebrates need good water quality to survive, often requiring clear or non-turbid water and/or high dissolved oxygen levels. Examples include stoneflies, water pennies, mayflies, and caddisflies, collectively known as the Ephemeroptera, Plecoptera, and Trichoptera (EPT). EPT taxa are sensitive to pollutants. Lower numbers of EPT taxa often coincide with water quality degradation (Sitati et al. 2021). Omary et al. (2023) explain more than others invertebrates can survive in fair water quality with less strict habitat requirements. Examples include crane flies, crayfish, dragonflies, damselflies, sow bugs, clams, and scuds. Other macroinvertebrates can survive in poor water quality due to adaptations allowing them to tolerate turbid, nutrient-enriched, or low dissolved oxygen conditions (Omary et al. 2023). Examples include leeches, pouch snails, aquatic worms, midges (Diptera), water striders, back swimmers, true bugs, Chironomidae, and Oligochaeta. Chironomids are noted to dominate degraded areas (Tela and Masayi, 2023).

Various physico-chemical parameters significantly influence the distribution and abundance of macroinvertebrates (Raphahlelo et al. 2022). DO is of the highest importance for the survival of aquatic biota. When waters are polluted, the decomposition of organic matter increases, causing DO to decrease, which in turn causes variation in macroinvertebrate assemblages (Ochieng et al. 2021). Pollution-tolerant organisms can survive in low DO, while sensitive taxa like EPTO are affected by DO levels. Elevated DO can be associated with certain taxa like Pseudagrion sp. (Odonata) (Ochieng et al. 2021). Increased nutrient loads can negatively affect macroinvertebrate abundances and diversities (Munyai et al. 2025). Makumbe et al. (2022) argue that, a significant negative relationship has been found between macroinvertebrate diversity and nitrate and phosphate levels; diversity was higher where these levels were lower. Nitrates may be a better indicator of sewage or manure pollution than phosphates because they dissolve more readily (Ndichu et al. 2023). Water temperature is a key physico-chemical driver (Kengne Fotsing et al. 2022). Different macroinvertebrates require varied optimal temperatures to survive. Temperature can be negatively correlated with the presence of certain sensitive taxa (Munyai et al. 2025). Seasonality influences temperature and other variables, impacting community structure (Fekadu et al. 2022). pH is considered an important parameter in the ecology of aquatic macroinvertebrates (Tampo et al. 2021). Benthic macroinvertebrates are sensitive to pH variation; values below 5 or above 9 are considered harmful. Low pH values are associated with lower diversity. EPTO taxa are sensitive to pH (Tampo et al. 2021). Pollution-tolerant organisms can survive in turbid water (Omary et al. 2023). A turbidity threshold might exist, after which macroinvertebrate abundance decreases. Turbidity, along with suspended sediments, can be higher in areas impacted by agriculture and grazing (Wanderi et al. 2022).

Beyond water chemistry, habitat characteristics significantly influence macroinvertebrate distribution (Raphahlelo et al. 2022). Further says, this includes the availability of microhabitats and food resources, interactions among habitat characteristics, physico-chemical variables, and structural/hydrological features, streambed substrate, riparian vegetation cover, habitat heterogeneity, and physical disturbances. Human activities are major drivers of changes in both water quality and habitat, thereby impacting macroinvertebrate communities (Fekadu et al. 2022). Activities like municipal discharge, agricultural runoff, deforestation, sand mining, waste disposal, and water abstraction alter physico-chemical conditions and physical habitats, leading to shifts in macroinvertebrate diversity, abundance, and composition (Munyai et al. 2025). Areas with intense anthropogenic activities often show degraded water quality and are dominated by tolerant macroinvertebrate taxa (Tela and Masayi, 2023).

The strong and direct links between these water quality variables and macroinvertebrate community structure reinforce their utility as early warning signals of pollution and ecological distress, emphasizing the critical need for their conservation and consistent biomonitoring in freshwater ecosystems globally (Arimoro et al. 2021).

2.6 Conclusions from the literature review

This literature review shows that there is a clear and positive relationship between river water quality and benthic macroinvertebrate assemblages as effective bioindicators of ecological health. Macroinvertebrates are widely recognized and utilized as effective bioindicators of a river's ecological health, as their composition, diversity, and distribution are directly influenced by and thus reflect the surrounding environmental conditions, including water quality. However, there has been minimal study on Malawian rivers, and findings from other locations are not directly transferable due to local faunal differences, pollution sensitivities, and environmental conditions. Furthermore, studies have often examined either water chemistry or biological indicators in isolation. This research will bridge that gap by performing a diligent, correlative examination of both physico-chemical parameters (e.g., BOD, nutrients) and biological measures (e.g., EPT richness, diversity measures) to identify key ecological drivers. It will also close the gap in spatial analysis by measuring the effects of human pressures along an upstream-downstream gradient and will provide valuable baseline information on seasonal variation (wet vs. dry seasons) to inform effective long-term monitoring programs that account for temporal variation. Lastly, the literature reviewed is largely focused on ecological interactions and provides little evidence of how water quality-macroinvertebrate relationships translate into consequences for ecosystem services that local communities depend on, e.g., water supply, fisheries support, and cultural values.

CHAPTER THREE. MATERIALS AND METHODS

3.1 Description of Study Area

The study was conducted in Lunyangwa River (Figure 1), located 33°48’0” E and 11°24’0” S in Mzuzu city. The Lunyangwa River watershed comprises the area lying between Kaning’ina ridges on the east and the lower undulating plains on the north east and south-west. The watershed is largely covered by the Lunyangwa forest, which is located at about 10 km north- east of Mzuzu City Center. The catchment area for the Gulliver dam is the protected Kaning’ina forest reserve and is approximately 25 km^2.

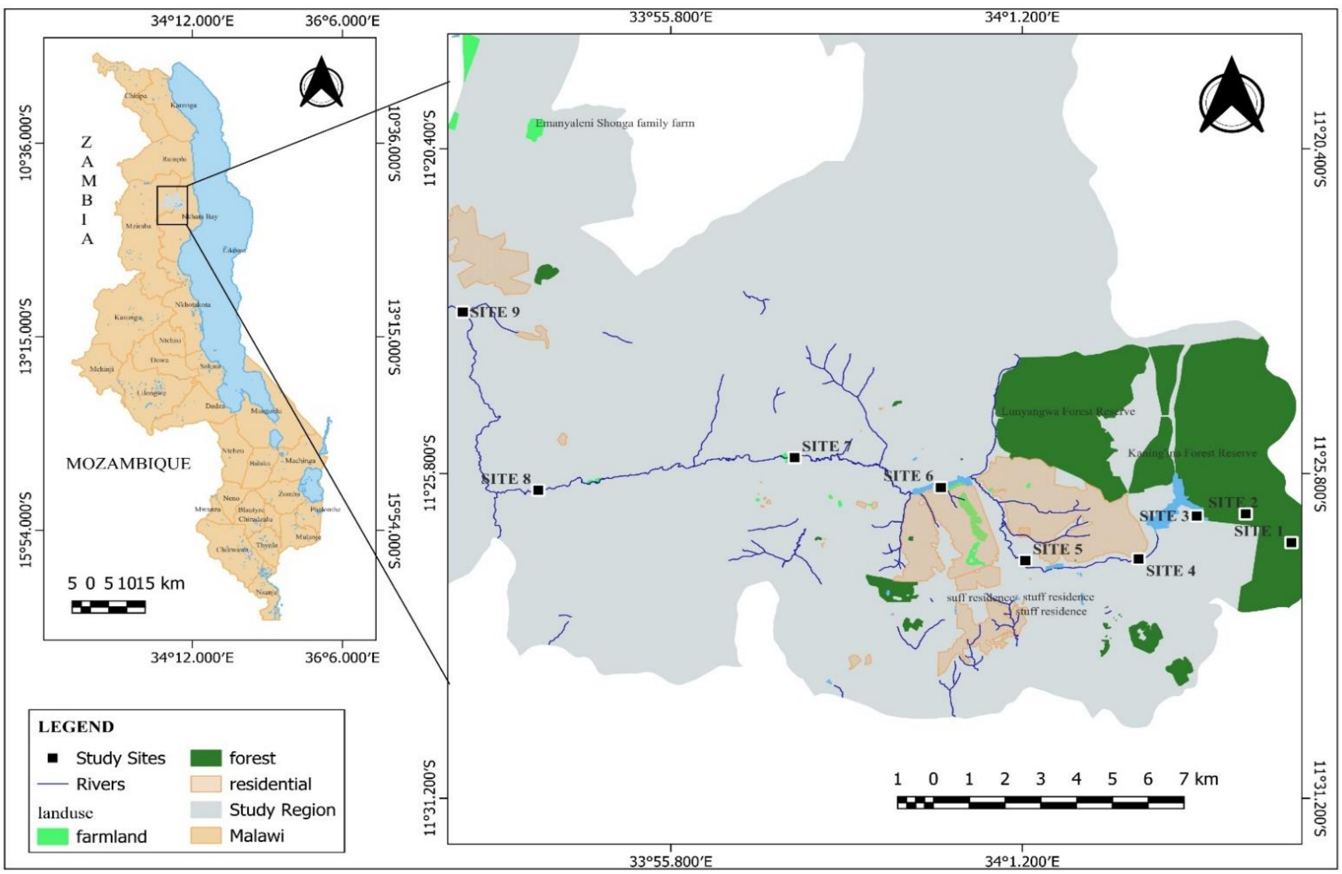


Figure 1. Map of Lunyangwa River showing different sampling sites.

3.1.1. Climate

The Lunyangwa River originates from the Kaning'ina Forest Reserve, located to the east of Mzuzu City in northern Malawi. This forested highland area sits at elevations of over 1,500 meters above sea level and forms part of the Viphya Plateau (Bisani, 2016). The region, including Mzuzu and the Lunyangwa River catchment, experiences a subtropical highland climate, characterized by cool temperatures and distinct wet and dry seasons (MetMalawi, 2023). The average annual temperature in Malawi's highland zones typically falls between 15 °C and 20 °C, with areas like the uplands of the northern and central regions such as Kaning'ina tending toward the cooler end of that range (Earth Site). In nearby Mzuzu, which shares similar highland characteristics, average annual temperatures hover around 20 °C, with July being the coolest month at about 16 °C. Meanwhile, Malawi's cool, dry season runs from June to August. Daytime temperatures during this period are generally moderate (around 17–27 °C), but nighttime temperatures in higher elevations often dip, sometimes falling between 4 °C and 10 °C (Department of Climate Change and Meteorological Services, 2024).

The region receives 1,200 to 1,500 mm of rainfall annually. The rainy season occurs from November to April, peaking between January and March. The dry season, from May to October, is marked by minimal or no rainfall, especially from July to September ((Ministry of Water Development (MoWD), 2005). Rainfall is strongly influenced by the Inter-Tropical Convergence Zone (ITCZ) and is enhanced by orographic effects due to the plateau and forested hills. Humidity is highest during the rainy season, with levels often exceeding 80% (MDPI, 2023; Climate Centre, 2023; MetMalawi, 2023). The dry season sees lower humidity and occasional dry winds. Winds are generally mild but may intensify around the transition into the rainy season.

The Kaning'ina Forest plays a crucial role in regulating the microclimate of the Lunyangwa River's headwaters. Its dense vegetation helps maintain moisture, reduce surface runoff, and protect against erosion. However, deforestation and land use change around the forest pose a threat to the river's seasonal flow and water quality.

3.1.2 Geology and soils

The region's geology is predominantly composed of Precambrian to Lower Palaeozoic low-grade metamorphic gneisses, part of the Basement Complex. These ancient rocks influence the river's catchment characteristics, such as mineral composition and erosion susceptibility. Overlying these metamorphic rocks are widespread alluvial and colluvial deposits, consisting of sand, silt, gravel, and clays, which have accumulated over time due to erosion and sedimentation processes (Wanda et al. 2014). These deposits contribute to the diverse soil types found in the region.

The soils in the Lunyangwa River catchment are primarily ferruginous, especially in the lower lands, derived from the weathering of the underlying metamorphic bedrock. These soils are typically, Sandy loam to clay loam in texture, with moderate to low natural fertility, Acidic in reaction, particularly in upland and forested zones and prone to erosion in sloped areas, especially due to land use changes such as agriculture, deforestation, and urban development in Mzuzu (FAO, 2020). Closer to the riverbanks, the soil transitions into alluvial deposits, which are more fertile and consisted of a mix of silt, sand, and organic material. These support riparian vegetation but are also susceptible to degradation from human activities, including farming, waste disposal, and sand mining.

3.1.3 Land use

The land use types in the Lunyangwa River catchment include agricultural land use, urban land use, and conservation land use, which can be found adjacent to the river, as seen in the Kaning'ina Forest Reserve, which is located at the source of the river (catchment) (Luka and Manda, 2024) The Kaning'ina Forest Reserve is important for the management of water flow and reducing soil erosion, but it is under threat from increased deforestation and unsustainable harvesting of wood (Ministry of Natural Resources, Energy and Mining, 2017). The Lunyangwa Dam impounds the river and provides water for the city, but it is experiencing increased anthropogenic impacts from the growing number of residential neighborhoods and industrial activity from the Mzuzu Industrial Area. Agricultural land use is the main land use type throughout the river basin and consists mainly of small-scale subsistence cultivation of maize, cassava, sunflower, and tobacco etc (UN-Habitat, 2011). In the lower and middle parts of the basin this has resulted in soil loss, nutrient loss, and siltation of the river from erosion. The floodplains also have rice farming as a result of fertile alluvial soils, and development of Mzuzu City has led to drastic land use changes in the river basin, such as conversion of agricultural land and forests to residential, commercial, and industrial land. Increased impervious surfaces as a result of urban development has increased stormwater runoff and contributed to water quality degradation in the river. Net, land use changes in the Lunyangwa River catchment represent significant water quality degradation, ecosystem health issues, and sustainability needs for the river.

3.2 Selection of Sampling Stations

To facilitate a comprehensive longitudinal comparison of anthropogenic effects on the Lunyangwa River, nine sampling stations were strategically selected following a targeted approach. Selection criteria prioritized habitat diversity and riparian land use patterns along the river's course (Plate 1). The selected nine stations are Kaning'ina Forest, Mzuzu water treatment plant, Royal bridge, Chinese, Zyambo, Kampingo and Ekwendeni water board. Stations within the forest reserve (site 1–3) are relatively natural conditions prior to major human interference. Site 4, where the water treatment plant station is located, was chosen in order to show effects related to dams as well as impacts of early settlement. Site 5 and 6 at Royal Bridge and Chinese Station reflect increasing urban pressures as aquaculture effluent runoff, domestic use, and waste disposal. Zyambo and Kampingo (site 7 and 8) are farming and mixed land use, respectively, with additional effects of mining at Kampingo. The Ekwendeni station (site 9) shows downstream pressures from agriculture, grazing, and water abstraction.

3.2.1 Description of sampling stations

Selected sampling stations that were sampled for testing the study's hypotheses are described below (Plate 1).

3.2.1.1 Kaning'ina Forest stations

Kaning'ina Forest Reserve, which is located in the Nkhata-Bay District with an estimated terrain elevation above sea level comprises three sampling sites (sites 1, 2 and 3) and is 1264 meters lying at latitude 11° 26' 48" S and longitude 34° 7' 9" E. There are three control sites along the Lunyangwa River within the Kaning'ina Forest. The river flows downhill from the first site upstream through the second and third sites before entering the dam. The water is clear at the first two sites, with sandy bottoms and natural vegetation along the slopes. However, at the third site, the water becomes less clear as the river transitions into the stagnant conditions of the dam. Fishing activity takes place here, as evidenced by the presence of fish traps. The surrounding forest shows signs of natural regeneration, with microhabitats forming along moisture gradients from the top to the bottom of the slopes. These sites represent the river's natural state before it reaches the dam and areas influenced by human activity downstream. Monitoring stations were spaced approximately 1.5-2 km apart along the river corridor,

3.2.1.2 Mzuzu Water Treatment plant station

The station comprises only one site (site 4) which lies within a transitional zone where natural riverine vegetation meets human settlement located at latitudes 11° 27' 13.46" S and longitudes 34° 2' 59.17" E. The area features tall grasses, shrubs, and scattered trees. The riverbed is composed of pebbles and sand, with some bedrock. Surrounding the river are moist soils supporting diverse plant life, gradually shifting to drier ground with signs of land use change. There are a few small farms nearby alongside. The site is located just after the forest, immediately downstream of the dam, with a water treatment plant facility nearby. It was selected to study the effects of the dam on the river ecosystem and how human activities, including agriculture and water management, impact this riparian zone.

3.2.1.3 Royal Secondary School Bridge station

This station also comprising one site (site 5) which is a shallow and muddy area located in an urban area. It lies just downstream of an aquaculture farm at 11°27.3′S, 34°01.2′E, likely receiving nutrient-rich runoff. The water is slightly turbid, with a muddy substrate and signs of sedimentation. People were observed washing clothes and drawing water directly from the river, indicating regular domestic use. This site was chosen to evaluate how combined pressures of urban runoff, aquaculture discharge, and direct domestic use that affect river conditions.

3.2.1.4 Chinese Station

The Chenese station is the sixth site (site 6) that is located at an urban river of Mzuzu city with latitude of approximately 11°26.0′S and longitude approximately 33°59.9′E. The river is a narrow and slow-moving river with a substrate composed primarily of fine sediments, with clay and silt. The river had some reddish deposits, possibly because of river erosion and runoff. The site is impacted by urban activities including agriculture and waste disposal, as household waste can easily enter the river. The site appears to have moderate to high impacts based on urban anthropogenic activities.

3.2.1.5 Zyambo station

This study site 7 located at 11°25’32 S and 33°57’42 E along the Lunyangwa River is a narrow, fast-flowing turbid water surrounded by vegetated section which is characterized by tall grasses, reeds, and nearby agricultural crops, indicating a riparian or wetland area influenced by human activity. The river features shallow, flowing water with aquatic vegetation and a natural substrate of mud and sand, supporting diverse macroinvertebrate communities. Fish traps were observed in the water, showing that the site is also used for local fishing. The surrounding farmland and visible dirt paths suggest potential stressors such as fertilizer runoff or sedimentation. This site, with its mix of natural and anthropogenic features, offers a suitable setting for examining how seasonal and spatial factors influence water quality and macroinvertebrate assemblages, aligning well with the goals of the thesis.

3.2.1.6 Kampingo station

This site 8 is located at 11°26′04.5″S, 33°53′45.8″E, lies within an agricultural area with a mine upstream. The river water is muddy and turbid, reflecting sediment runoff from both farming and mining activities. Dense reeds and tall grasses line the riverbanks, forming natural channels that guide the flow. The substrate is reddish-brown, indicating iron-rich sediments common in the region. There are signs of human modification, such as embankments or constructed edges for water management or access. The site sits in a valley surrounded by rocky hills, blending natural landscape features with human land use.

3.2.1.7 Ekwendeni Water Board station

This station is named site 9 and is located in Ekwendeni, at approximately 11°23.1′S and 33°52.6′E. The river at this point is relatively fast-flowing, with a sandy substrate and muddy banks. The surrounding area is primarily agricultural, with extensive cultivation close to the river. Livestock grazing is common, and animals regularly access the river to drink, contributing to bank erosion and disturbance of riparian vegetation. Vegetation along the banks includes grasses, shrubs, and occasional trees, though much of it is impacted by human activities. The site was chosen to reflect a mix of managed and natural features, with pressures from water abstraction, agriculture, and grazing likely influencing both water quality and ecological conditions.

3.3 Sampling design

Data on physico-chemical water quality variables, habitat quality and macroinvertebrate assemblages were collected across two distinct seasonal periods. The first collection phase took place during the dry season in October and November 2024, while the second phase was carried out during the wet season in February and April 2025. This approach allowed for comparison of seasonal variations in the measured parameters.

3.3.1 Physico-chemical water quality parameters

At each site, duplicate measurements of pH, dissolved oxygen (DO), temperature, electrical conductivity 23 (EC), total dissolved solids (TDS), and salinity were carried out in-situ using a YSI multi-probe water quality meter (YSI 550) calibrated with standard solutions (SMEWW, 1998). Additionally, triplicate water samples for the analysis of total phosphorous (TP), ammonia (NH_4^+), nitrate (NO_3^-), nitrite (NO_2^-) and total suspended solids (TSS) were collected and kept in 500 mL plastic bottles, previously rinsed with distilled water. After collection, the water samples were labelled indicating the time, date, and name of the sampling station and stored in a cooler box in the field before being transported to Mzuzu University for laboratory analysis.

3.3.2 Benthic macroinvertebrates

At each sampling site, a multi-habitat sampling approach was employed to ensure representative coverage of the different microhabitats present. This involved collecting benthic macroinvertebrates from a composite of habitat types, such as riffles, runs, pools, and vegetated margins, in proportion to their representation at each site.

Sampling was conducted using a D-frame kick-net with a mesh size of 500 µm and a surface area of 300 mm by 300 mm. The kick-net sampling method involved disturbing the substrate upstream of the net and allowing the dislodged organisms to be carried into the net by the current. At each habitat type, a standardized sampling effort was applied, with a minimum of 1 m^2 of substrate area sampled. Each habitat type was sampled in replicate, and the collected samples were preserved in the field using 10% formalin. Sampling was conducted during the wet and dry season(s) to capture the diversity and abundance of benthic communities, taking into account factors such as life cycles, emergence patterns, and hydrological conditions.

(a)

(b)

(c)

(d)

(e)

(f)

Plate 1. Field sampling stations in the Lunyangwa River (a,b,c) Forest areas and (d,e,f) urban-influenced downstream locations which are water treatment plant, royal bridge and Chinese.

(g)

(h)

(i)

Plate 2. Field sampling stations in the Lunyangwa River, (g,h,i) agricultural influenced locations which are zyambo, kampingo and ekwendeni water board.

3.4 Laboratory Analysis

3.4.1 Nutrient Analysis

In the laboratory, nutrient analyses were determined using standard colorimetric methods (APHA, 2005). Unfiltered water samples were used for TP. For TP analysis, after persulfate digestion, samples were analyzed using the ascorbic acid method with absorbance read at a wavelength of 885 nm (APHA, 2005), while Nitrate (NO_3^-) and Nitrite (NO_2^-) soluble nutrients were analyzed using the Salicylate method with the spectrophotometric absorbance read at a wavelength of 543 nm (APHA, 2005). Ammonium (NH_4^+) was analyzed through the reaction between sodium salicylate and hypochlorite solutions with the spectrophotometric absorbance of the treated sample being read at a wavelength of 665 nm (APHA, 2005). Beer-Lambert law ($A = \varepsilon l C$) (Bartram and Ballance, 1996) and APHA (2005) (A = absorbance, l = cuvette width, ε = extinction coefficient) was used to convert the obtained absorbance of each nutrient to its corresponding concentration. The total suspended solids (TSS) concentrations were determined by filtering a known volume of lake water through GF/C filters that were first dried and pre-weighed and then oven-dried after filtration. The final weights were taken to determine the difference (eq. 1) as the TSS weight (g) per unit volume of the sample (Rodier et al. 2009).

$$TSS\ (mg/L) = \frac{[(Final\ weight - Initial\ weight) \times 1000]}{0.5}$$ **Equation 1**

3.4.2 Macroinvertebrates

In the laboratory, benthic macroinvertebrate samples were rinsed through a series of sieves of 250 µm mesh sizes to remove fine sediments, debris and formalin. Organisms were sorted, enumerated, and identified to the lowest practicable taxonomic level, typically genus or species, using appropriate taxonomic keys and reference materials (Merritt et al. 2019; Thorp and Rogers, 2019; Vieira et al. 2020). Then stored in 70% ethanol.

3.5 Benthic macroinvertebrates community diversity and water quality indices

3.5.1 Diversity indices

Total numerical abundance and Shannon Weaver Diversity Index (H') were all computed for each sampling location. Each sampling site's diversity was determined using the Shannon Weaver diversity index (H'), following the formula

$$H' = -\sum p_i \ln(p_i)$$ **Equation 2**

Where H' is the Diversity Index and pi is the percentage of a certain taxon/species in a sample that belongs to the ith species (Shannon & Weaver, 1949; 1963). Furthermore, considered in the index was richness and evenness.

Total numerical abundance was estimated as the overall number of individuals per taxon per site. Because sampling was semi-quantitative (kick net), these values cannot be expressed as density per unit area. However, because the sampling effort was standardized across sites, comparisons of absolute abundances between sites are valid.

SASS (South African Scoring System) is a bioassessment method that uses aquatic macroinvertebrates to determine river health. Each taxon is assigned a tolerance/sensitivity score, which are summed to provide a Total Score. The Average Score per Taxon (ASPT) is calculated to give a complete picture of ecosystem health (Dallas, 1995). At each site ASPT was determined by,

$$ASPT = \frac{SASS\ Sore}{Number\ of\ Taxa}$$ **Equation 3**

The SASS score indicates the richness of sensitive taxa present, while the ASPT provides insight into the average sensitivity of the community. When combined, they allow for a more reliable assessment of river health. The guideline values developed by Chutter (1995) are presented in Table 1, showing how different score ranges relate to water quality and habitat condition.

Table 1. Interpretation of SASS results based on SASS score and ASPT (Chutter, 1995).

SASS score	Average score per taxon (ASPT)	Interpretation
> 100	> 6	Water quality natural, habitat diversity high
< 100	> 6	Water quality natural, habitat diversity reduced
> 100	< 6	Borderline between water quality natural and some deterioration. Interpretation should be based on the extent by which the SASS exceeds 100 and the ASPT < 6
50 – 100	< 6	Some deterioration in water quality
< 50	Variable	Major deterioration in water quality

3.5.2 Macroinvertebrate Community Composition

Community composition was determined by identifying all macroinvertebrates collected at each sampling site to the lowest possible taxonomic level, mainly family or genus, using standard identification keys. For each site, all individuals were sorted and counted to generate a list of taxa present and their absolute numbers.

3.5.3 Calculation of Water Quality Index (WQI) of the river

The water quality index (WQI) is a rating that reflects the composite influence of different water quality parameters (Sahu and Sikdar, 2008; Ramakrishnaiah et al. 2009). Firstly, each of the chemical parameters (e.g. pH, TDS, turbidity, total alkalinity, hardness, fluoride, nitrate, nitrite, silica, phosphate) is assigned different weights (wi) on a scale of 1 (least effect on water quality) to 5 (highest effect on water quality) based on its perceived effects on human health and according to its relative importance in the drinking water or groundwater quality (Brown et al., 1972; Sener et al. 2017). The highest weight of 5 is assigned to parameters that have critical health effects and whose presence above the critical concentration limits could hinder the usability of water for domestic and drinking purposes (Bhateria and Jain, 2016; Sener et al. 2017). In this study, nutrients were assigned the highest weight (5) because of their health influence (WHO, 2008) and importance in water quality assessment while, a minimum weight of 1 was assigned to total alkalinity and electrical conductivity parameters due to their least importance in water quality assessment (Brown et al., 1972; Katyal, 2011).

The relative weight (RW_i) which is the contribution of each parameter to the WQI was then computed from the following equation (Sener et al. *2*017):

$$RW_i = \frac{w_i}{\sum_i^n x w_i}$$ **Equation 4**

Where, w_i is the assigned weight of each parameter (Table 2) and n is the number of parameters. Then, a quality rating (Q_i) for each parameter except pH and DO was assigned by dividing its concentration (C_i) in each water sample by its limits values/standards given by the WHO (2008, 2011) and the result multiplied by 100:

$$Q_i = \frac{C_i}{S_i} \times 100$$ **Equation 5**

Where:

Q_i = the quality rating,

C_i = the concentration of the chemical parameter in each water sample in mg/L, and

S_i = is the drinking water standard for the chemical parameter in mg/L according to the guidelines of WHO (2008).

The quality rating for pH or DO (QpH, DO) was calculated following Alobaidy et al. (2010) as:

$$Q_{p^h}, DO = \frac{[C_i - V_i]}{[S_i - V_i]} \times 100 \qquad \textbf{Equation 6}$$

Where, V_i = the ideal value which is considered as 7.0 for pH and 14.6 for DO (WHO, 2011).

Table 2. Normalization factors (Ci) and relative weights (Wi) for water quality variables used in the calculation of the Water Quality Index (WQI) (Brown et al. (1972).

Variable	Units	Relative weight (*Wi*)	Normalization factor (*Ci*) 100	90	80	70	60	50	40	30	20	10	0
Temp.	°C	3	21/16	22/15	24/14	26/12	28/10	30/5	32/0	36/−2	40/−4	45/−6	> 45/< -6
pH	Unit pH	1	7	7–8	7–8.5	7–9	6.5–7	6–9.5	5–10	4–11	3–12	2–13	1–14
EC	μS/cm	1	< 750	< 1000	< 1250	< 1500	< 2000	< 2500	< 3000	< 5000	< 8000	≤ 12000	> 12000
DO	mg/L	4	≥ 7.5	> 7	> 6.5	> 6	> 5	> 4	> 3.5	> 3	> 2	≥ 1	< 1
TDS	mg/L	2	< 100	< 500	< 750	< 1000	< 1500	< 2000	< 3000	< 5000	< 10000	≤ 20000	> 20000
Turb.	mg/L	4	< 5	< 10	< 15	< 20	< 25	< 30	< 40	< 60	< 80	≤ 100	> 100
SiO_4^{4-}	mg/L	1	< 25	< 50	< 75	< 100	< 150	< 250	< 400	< 600	< 1000	≤ 1500	> 1500
PO_4^{3-}	mg/L	1	< 0.025	< 0.05	< 0.1	< 0.2	< 0.3	< 0.5	< 0.75	< 1	< 1.5	≤ 2	> 2
NH_4^+	mg/L	3	< 0.01	< 0.05	< 0.1	< 0.2	< 0.3	< 0.4	< 0.5	< 0.75	< 1	≤ 1.25	> 1.25
NO_2^-	mg/L	2	< 0.005	< 0.01	< 0.03	< 0.05	< 0.1	< 0.15	< 0.2	< 0.25	< 0.5	≤ 1	> 1

Equations (4) and (5) ensure that Qi = 0 when a pollutant is totally absent in the water and Qi = 100 when the value of this parameter is just equal to its permissible value (Bhateria and Jain, 2016). Hence, the higher the value of Qi, the more polluted is the water.

Consequently, to calculate WQI, firstly, sub-index (SIi) value is determined for each water quality parameter and then used to derive WQI with the following equations (Bhateria and Jain, 2016; Kumar et al. 2018):

$$SI_i = RW_i \; x \; Qi$$ **Equation 7**

$$WQI = \sum_1^n x \; Sl_i$$ **Equation 8**

Where, SI_i is the sub-index of ith parameter; Qi is the quality rating based on the concentration of ith parameter. The computed WQI values were classified into five categories ranging from 1 (excellent) to 5 (unsuitable for drinking) as in **Table 3** following the equation proposed by Bhateria and Jain (2016). Thus, the highest WQI reflects the poorest water quality of the lake in space and time.

Table 3. Water quality classification based on WQI values (Bhateria and Jain, 2016).

N°	WQI Values	Water quality	Colors allocated to water quality status
1	< 50	Excellent water	Blue
2	50 – 100	Good water	Green
3	100 – 200	Poor water	Yellow
4	200 – 300	Very poor water	Orange
5	> 300	Unsuitable for drinking	Red

3.6 Data Analysis

A combination of univariate and multivariate statistical techniques was employed. All statistical analyses were performed using R software version 4.5.0. Descriptive statistics, including measures of central tendency (mean) and measures of dispersion (standard deviation), were calculated for each water quality parameter at each site and disturbance level. Two-way analysis of variance (ANOVA) was used to test the influence of multiple factors (sampling sites and seasons) on the physico-chemical variables simultaneously. For factors showing significant effects ($p < 0.05$), one-way ANOVA and the Tukey–Kramer multiple comparison post hoc test were applied to compare mean values of individual factors (either site or season) and identify which specific sites or seasons differed significantly. Principal Component Analysis (PCA) was performed to identify clustering patterns of water quality parameters across land-use types and seasons.

One-way Analysis of Variance (ANOVA) was also applied to test for significant differences on log-transformed macroinvertebrate abundance and diversity indices across land-use types, with Tukey's post hoc test used to identify specific differences between. The macroinvertebrate data were log-transformed before ANOVA to meet assumptions for parametric tests (Zar, 2010). Multivariate techniques such as Non-metric Multidimensional Scaling (NMDS), was used to explore patterns in macroinvertebrate community composition across the disturbance gradient.

Two-way nested analysis of similarities (ANOSIM) was used to compare average rank similarities in macroinvertebrate community structure composition among land-use types and between seasons, with land-use types nested within seasons. This analysis was performed to check if macroinvertebrate community structure and composition varied spatially “land-use types” and seasonally “season”.

Similarity Percentage Analysis (SIMPER) was conducted to determine the contribution of taxa to community dissimilarities between land-use types and seasons, based on abundance data.

Redundancy Analysis (RDA) was performed to examine relationships between physico-chemical parameters and macroinvertebrate community composition. Pearson's correlation coefficient (r) was used to assess the strength and direction of the relationships between individual water quality parameters (e.g., dissolved oxygen, pH, nutrient's concentrations) and macroinvertebrate community metrics (e.g., species richness, diversity and biotic indices). These analyses provided insights into the potential influence of specific physico-chemical variables on the macroinvertebrate communities.

3.7 Ethical Considerations.

This study sought ethical clearance approval from the Mzuzu University Ethical Clearance Committee (MZUNIREC). The fieldwork has been approved with Protocol Reference number MZUNIREC/DOR/24/110: SPATIO-TEMPORAL VARIABILITY IN WATER QUALITY AND MACROINVERTEBRATES ASSEMBLAGE OF LUNYANGWA RIVER, NORTHERN MALAWI. Permission from the Department of Forestry Mzuzu under the Ministry of Natural Resources and Climate Change was obtained to access the forest for data collection.

CHAPTER FOUR. RESULTS

4.1 Spatio-temporal variability in water quality physico-chemical parameters and nutrients

Most of the physico-chemical variables measured during this study showed significant differences among land use types (Table 4). Specifically, seven variables, temperature, dissolved oxygen (DO), DO saturation (DO%), biochemical oxygen demand (BOD), conductivity, pH, and salinity varied significantly between sampling sites ($p < 0.05$). In contrast, seasonal variation (sampling months) did not significantly influence these water quality parameters ($p > 0.05$).

Forested sites consistently recorded higher DO and DO% mean values (6.44 ± 0.26 mg/L and 70.90 ± 3.40%) compared to agricultural (3.58 ± 2.66 mg/L and 44.88 ± 31.42%) and urban areas (3.07 ± 2.74 mg/L and 33.60 ± 29.40%) (Table 5). Similarly, BOD was significantly lower in forested plots (1.37 ± 0.47 mg/L) than in agricultural (4.53 ± 1.30 mg/L) and urban sites (4.73 ± 1.17 mg/L). Conductivity and salinity reached their highest mean values in agricultural sites (145.38 ± 43.86 µS/cm and 65.00 ± 11.46 ppm, respectively), indicating higher ionic concentration likely due to fertilizer and runoff inputs. According to Table 4, only hydrological parameters velocity and discharge exhibited significant differences between both sites ($F = 13.17$, $p < 0.001$; $F = 42.50$, $p < 0.001$) and across seasons ($F = 5.43$, $p = 0.030$; $F = 30.30$, $p < 0.001$). Discharge values were higher during the wet season across all land use types (e.g., agriculture: 8.51 ± 1.93 m^3/s in the wet season vs. 3.37 ± 1.10 m^3/s in the dry season; Table 5).

The interaction between season and land use was significant only for pH ($F = 9.27$, $p = 0.001$) and discharge ($F = 6.28$, $p = 0.008$) (Table 4). This interaction reflected pronounced seasonal variation in pH at forested and urban sites, with values rising from 7.07 ± 0.07 in the dry season to 9.34 ±

0.34 in the wet season at forested sites, and from 6.72 ± 0.12 to 7.35 ± 1.14 at urban sites (Table 5).

Table 4. Two-way ANOVA results testing the effects of land use types (sites), seasons, and their interaction on water quality parameters in Lunyangwa River. Values in bold indicate statistically significant effects (p < 0.05).

Parameter	**F**	**p**	**F**	**p**	**F (Interaction)**	**p (Interaction)**
	Land use		Season			
Temperature (°C)	8.34	**0.002**	0.17	0.686	2.06	0.153
DO (mg/L)	4.66	**0.022**	0.27	0.612	0.04	0.961
DO%	4.08	**0.033**	0.39	0.540	0.03	0.975
BOD (mg/L)	27.44	<**0.001**	2.20	0.153	0.04	0.959
TDS (g/L)	0.78	0.474	1.01	0.328	0.57	0.574
TSS (g/L)	0.74	0.489	1.76	0.199	0.23	0.797
Conductivity	14.45	<**0.001**	0.01	0.942	0.16	0.855
pH	5.72	**0.011**	2.47	0.132	9.27	**0.001**
Salinity (ppm)	13.48	<**0.001**	0.06	0.810	0.11	0.898
Total Phosphorus	1.10	0.352	2.61	0.122	1.08	0.357
Ammonium	1.22	0.315	0.32	0.578	0.17	0.845
Nitrites	0.56	0.580	3.15	0.091	0.63	0.544
Nitrate	0.61	0.554	3.29	0.085	0.24	0.790
Width (m)	2.81	0.086	0.98	0.335	0.16	0.849
Velocity (m/s)	13.17	**<0.001**	5.43	**0.030**	0.86	0.439
Discharge (m^3/s)	42.50	**<0.001**	30.30	**<0.001**	6.28	**0.008**

Table 5. Mean values (± standard deviations) of water quality parameters across three land use categories during dry and wet seasons. (means with different letters across sites are significantly different).

Parameter	Season	Agriculture	Forested	Urban
Temperature (°C)	Dry	24.93 ± 1.85^{a}	20.93 ± 3.44^{ab}	21.47 ± 1.39^{ab}
	Wet	23.97 ± 1.41^{ab}	19.68 ± 0.14^{b}	23.39 ± 2.08^{ab}
DO (mg/L)	Dry	3.58 ± 2.66^{a}	6.44 ± 0.26^{a}	3.07 ± 2.74^{a}
	Wet	3.47 ± 2.00^{a}	5.97 ± 1.77^{a}	2.38 ± 2.38^{a}
DO%	Dry	44.88 ± 31.42^{a}	70.9 ± 3.4^{a}	33.6 ± 29.4^{a}
	Wet	36.65 ± 20.81^{a}	63.0 ± 2.07^{a}	29.92 ± 26.9^{a}
BOD (mg/L)	Dry	4.53 ± 1.30^{a}	1.37 ± 0.47^{b}	4.73 ± 1.17^{a}
	Wet	5.25 ± 0.76^{a}	1.87 ± 0.45^{b}	5.22 ± 0.9^{a}
TDS (g/L)	Dry	0.08±0.02	0.02±0.0	0.14±0.24
	Wet	0.08±0.02	0.01±0.0	0.04±0.03
TSS (g/L)	Dry	35.0±29.14	12.0±2.0	21.33±8.82
	Wet	61.5±39.91	16.67±23.69	62.67±100.3
Conductivity (µS/cm)	Dry	145.38 ± 43.86^{a}	28.37 ± 5.14^{b}	68.25 ± 38.22^{ab}
	Wet	134.2 ± 33.38^{a}	30.33 ± 0.58^{b}	77.15 ± 53.33^{ab}
pH	Dry	8.38 ± 0.54^{ab}	7.07 ± 0.07^{bc}	6.72 ± 0.12^{c}
	Wet	7.22 ± 1.05^{bc}	9.34 ± 0.34^{a}	7.35 ± 1.14^{bc}
Salinity (ppm)	Dry	65.0 ± 11.46^{a}	16.03 ± 4.29^{b}	37.87 ± 19.64^{ab}
	Wet	62.08 ± 13.1^{a}	19.8 ± 4.16^{b}	41.33 ± 22.26^{ab}
Total Phosphorus	Dry	0.03±0.01	0.02±0.02	0.28±0.43
	Wet	0.01±0.01	0.01±0.02	0.01±0.02
Ammonium (mg/L)	Dry	0.01 ± 0.01	0.02 ± 0.02	0.07 ± 0.11
	Wet	0.05 ± 0.01	0.03 ± 0.02	0.08 ± 0.09
Nitrites (mg/L)	Dry	0.02 ± 0.02	0.02 ± 0.04	0.00 ± 0.01
	Wet	0.41 ± 0.81	0.00 ± 0.00	0.65 ± 1.00
Nitrate	Dry	2.7±3.02	2.55±2.24	1.61±1.31
	Wet	0.86±0.25	1.54±1.94	0.8±0.39
Depth (m)	Dry	0.45 ± 0.07^{ab}	0.18 ± 0.09^{b}	0.4 ± 0.22^{ab}
	Wet	0.61 ± 0.06^{ab}	0.25 ± 0.12^{ab}	0.69 ± 0.36^{a}
Velocity (m/s)	Dry	1.29 ± 0.38^{ab}	0.6 ± 0.42^{bc}	0.38 ± 0.26^{c}
	Wet	1.45 ± 0.34^{a}	0.72 ± 0.35^{abc}	0.87 ± 0.34^{abc}
Discharge (m^3/s).	Dry	3.37 ± 1.10^{b}	0.38 ± 0.11^{c}	0.36 ± 0.34^{c}
	Wet	8.51 ± 1.93^{a}	1.21 ± 1.31^{bc}	2.27 ± 1.51^{bc}

The Principal Component Analysis (PCA) biplots allowed for the unambiguous clustering of water quality parameters across land use type and season, with the first two principal components explaining 53.5% to 65.2% of variance (Figure 2). Agricultural and urban sites cluster together, separated from forest sites along pollution indicator gradients such as BOD, conductivity, salinity, and temperature. Forest sites show consistent clustering regardless of season, indicating homogeneous water quality, whereas agricultural and urban sites exhibit greater variability.

The PERMANOVA results showed that the land use type significantly affected the variation in community composition ($F = 8.80$, $df = 2$, $p = 0.001$). This result indicate that taxa present differed depending on the land use type whether the site was agricultural, urban, or forest. In contrast, season had no effect ($F = 0.53$, $df = 1$, $p = 0.59$), indicating that community structure as a whole did not vary greatly between wet and dry seasons. Likewise, no significant interaction was detected between land use type and season ($F = 0.24$, $df = 2$, $p = 0.94$), this suggests that land use affected community structure the same way regardless of season.

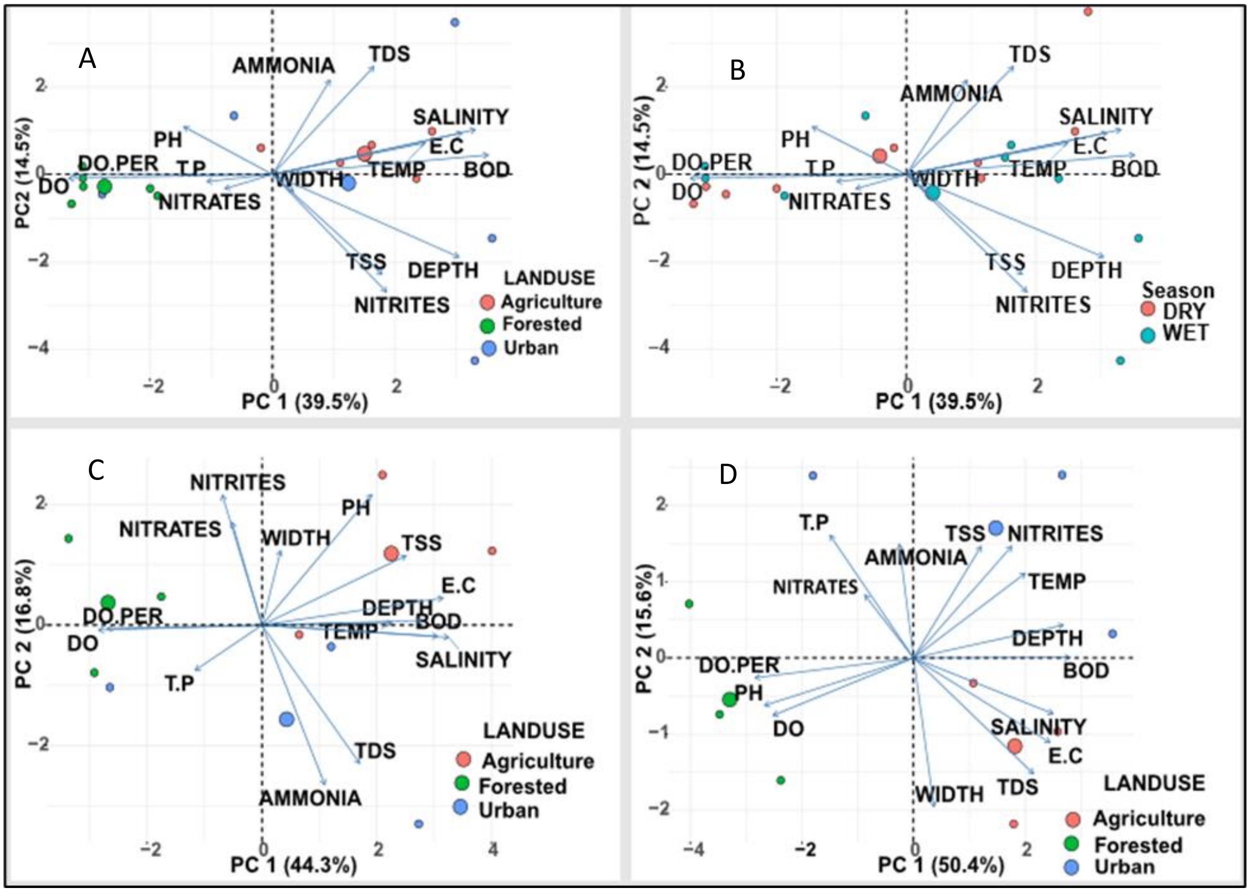


Figure 2. PCA plots for both habitat quality and water quality physico-chemical (A)landuse types, (B)seasons, (C)dry season and (D)wet season of the Lunyangwa river. TDS = Total Dissolved Solids, TSS = Total Suspended Solids, BOD = Biological Oxygen Demand, TP = Total Phosphorus, DO = Dissolved Oxygen, EC = Electric Conductivity, TEMP = Temperature.

4.1.1 Water Quality Index

The WQI values demonstrated a considerable range, from an excellent quality status with low water quality index value of 28.52 at the forested Station (Site 2) during the dry season to a very poor water quality status characterized by high water quality index value of 204.25 at the urban Station (Site 6) named Chinese during the wet season (Table 6). A two-factor analysis of variance (ANOVA) revealed that the differences in WQI values between the sampling stations were statistically significant ($F = 82.36$, $p < 0.001$). Furthermore, a significant effect of season was observed (F = 18.37, p = 0.003), with mean WQI values generally higher in the wet season. However, the interaction between site and season was not statistically significant ($F_{(8, 9)} = 0.98$, p = 0.480).

Table 6. Spatial and Temporal Changes in Water Quality Index (WQI) across the Lunyangwa River.

		DRY SEASON		WET SEASON	
Sites	Stations	WQI Values	Water Quality	WQI Values	Water Quality
Site 1	Forest	30.05	Excellent	36.84	Excellent
Site 2	Forest	28.52	Excellent	35.21	Excellent
Site 3	Forest	42.14	Excellent	58.32	Good
Site 4	Water Treatment	60.13	Good	75.44	Good
Site 5	Royal	155.60	Poor	170.05	Poor
Site 6	Chinese	186.40	Poor	204.25	Very Poor
Site 7	Zyambo	167.64	Poor	189.53	Poor
Site 8	Kampingo	182.64	Poor	176.43	Poor
Site 9	Ekwendeni	131.53	Poor	152.14	Poor

4.2 Spatio-temporal variability in composition, distribution and diversity of benthic macroinvertebrates

During the study, we recorded 5,469 macroinvertebrate individuals representing 72 taxa across 13 orders and 36 families. Abundance was significantly higher during the dry season (3,953 individuals) compared to the wet season (1,516 individuals). A chi-square test (Table 7) confirmed this seasonal difference was highly significant ($\chi^2(1) = 1085.93$, $p < 0.001$) with a medium-to-large effect size (Cohen's w = 0.45), indicating both statistical significance and ecological importance.

Table 7. Chi-square test of seasonal abundance difference

Statistical Test	χ^2	df	p	Cohen's w
Season comparison	1085.93	1	< 0.001	0.45

The macroinvertebrate diversity analysis revealed distinct patterns across land-use categories and seasonal variations. During the wet season, forested locations demonstrated the highest taxa richness (Taxa S = 8.7) and lowest dominance (Dominance D = 0.29), indicating greater diversity in forested areas compared to agriculture (Taxa S = 7, Dominance D = 0.35) and urban (Taxa S = 4.3, Dominance D = 0.65) stations. Urban localities consistently showed the lowest diversity metrics during this study . However, abundance of macroinvertebrates did not vary significantly between land-use categories in the wet season ($F_{(2, 6)} = 1.996$, $p = 0.217$).

In the dry season, forested localities maintained the highest taxa richness (Taxa S = 9) and relatively low dominance (Dominance D = 0.33), confirming their greater diversity. Urban localities had markedly lower taxa richness (Taxa S = 7.7) and highest dominance (Dominance D = 0.61), while agricultural sites showed intermediate values (Taxa S = 6.7, Dominance D = 0.36). Significant differences were detected in macroinvertebrate abundance between land-use categories during the dry season ($F_{(2, 6)} = 18.65$, $p = 0.003$).

Urban land-use types consistently held the lowest mean abundance of macroinvertebrates per location across both seasons (wet season: 101 individuals; dry season: 585 individuals). This was markedly lower compared to forested sites (wet: 171; dry: 138) and agricultural locations (wet: 93; dry: 89). The comparison between agricultural and forest sites showed variable patterns depending on the season.

During the wet season, forested sites exhibited higher diversity as revealed by indices such as Shannon-H (1.5), Evenness (0.54), and Margalef (1.55) (Table 8), indicating more diverse and even communities. Urban sites consistently displayed lower values for these diversity measures (Shannon-H = 0.74, Evenness = 0.67, Margalef = 0.68), demonstrating reduced diversity despite higher evenness values. Agricultural sites showed intermediate diversity patterns (Shannon-H = 1.37, Evenness = 0.62, Margalef = 1.54) (Table 8).

Similarly, during the dry season, forested sites demonstrated the highest diversity as indicated by various indices, including Shannon-H (1.55), Evenness (0.58), and Margalef (1.62), showing more affluent and even assemblages. Urban sites again showed the lowest values for most diversity parameters (Shannon-H = 0.96, Evenness = 0.41, Margalef = 1.24), supporting reduced diversity patterns. Agricultural sites often displayed diversity measures intermediate between urban and forest sites (Shannon-H = 1.29, Evenness = 0.57, Margalef = 1.27) (Table 8).

Table 8. The diversity indices of macroinvertebrate structural communities across land use types in dry and wet seasons of the Lunyangwa river.

	Wet Season			Dry Season		
Index	Forested	Urban	Agriculture	Forested	Urban	Agriculture
Taxa_S	8.7	4.3	7	9	7.7	6.7
Individuals	171	101	93	138	585	89
Dominance_D	0.29	0.65	0.35	0.33	0.61	0.36
Simpson_1-D	0.71	0.35	0.65	0.67	0.39	0.64
Shannon_H	1.5	0.74	1.37	1.55	0.96	1.29
Evenness_e^H/S	0.54	0.67	0.62	0.58	0.41	0.57
Brillouin	1.4	0.69	1.17	1.44	0.9	1.18
Menhinick	0.73	0.45	1.04	0.78	0.53	0.72
Margalef	1.55	0.68	1.54	1.62	1.24	1.27
Equitability_J	0.7	0.41	0.73	0.72	0.44	0.7
Fisher_alpha	2.06	0.88	2.39	2.23	1.62	1.69
Berger-Parker	0.39	0.77	0.54	0.48	0.69	0.52
Chao-1	8.8	4.5	9	9.8	9.3	7

During the dry season, forested sites maintained the highest ecological integrity (*p* = 0.051, Table 9), with a mean SASS Score of 87.3 ±54.5 and an ASPT of 6.13 ±2.27. Agricultural sites displayed intermediate quality (SASS: 63.7 ± 22.9; ASPT: 6.84 ± 0.91), while urban sites exhibited the most degraded conditions, with the lowest scores (SASS: 46.7 ± 28.9; ASPT: 3.98 ± 1.12) (Table 10). In the wet season, forested sites again demonstrated the highest scores (*p = 0.009*, Table 9) (SASS: 88.7 ±14.8; ASPT: 7.87 ±1.62), with ASPT showing a notable seasonal increase. The performance of agricultural sites was variable but intermediate (SASS: 68.3 ±34.3; ASPT: 6.69 ±1.85) (Table 10). Urban sites consistently yielded the poorest biological indicators, with SASS Scores declining further to 28.7 ±15.0, while ASPT remained low and was estimated at 4.13 ±0.70.

Two-way ANOVA revealed significant differences among land use types for ASPT scores ($F_{2,12}$ = 7.043, *p* = 0.009), with a marginally significant effect on SASS scores ($F_{2,12}$ = 3.852, *p* = 0.051). Season alone didn't significantly influence any of the water quality parameters, and neither did the combined effect of land use and season together (all *p* > 0.05). The number of taxa also remained consistent across all treatments without significant variation.

Table 9. Statistical results (two-way ANOVA) for differences in SASS Score, Number of Taxa, and ASPT across land use types and seasons. Significant results *(p* < 0.05*)* are indicated in bold.

	df	SASS Score		No. of Taxa		ASPT	
		F	*p*	F	*p*	F	*p*
Landuse	2	3.85	0.051	1.22	0.32	7.04	**0.009**
Season	1	0.07	0.792	0.87	0.370	0.66	0.431
Landuse * Season	2	0.23	0.801	0.74	0.497	0.68	0.527

** *p* < 0.01*,* * *p* < 0.05

Table 10. Site-specific biological data for the dry and wet seasons. Sites are categorized by land use type. Water quality class is based on SASS5 guidelines: A (unimpacted) to E (seriously impacted). SASS= South African Scoring System, ASPT= Average Score Per Taxon

		Dry Season				Wet season			
SITES	LANDUSE	Sass Score	Taxa	ASPT	Class	Sass Score	Taxa	ASPT	Class
1	FORESTED	126	16	7.88	A	85	11	7.73	B
2	FORESTED	111	16	6.94	A	105	11	9.55	A
3	FORESTED	25	7	3.57	E	76	12	6.33	B
4	URBAN	78	15	5.20	D	28	7	4.00	E
5	URBAN	41	11	3.73	E	14	4	3.50	E
6	URBAN	21	7	3.00	E	44	9	4.89	D
7	AGRICULTURE	38	6	6.33	E	39	8	4.88	D
8	AGRICULTURE	71	9	7.89	B	60	7	8.57	B
9	AGRICULTURE	82	13	6.31	B	106	16	6.63	A

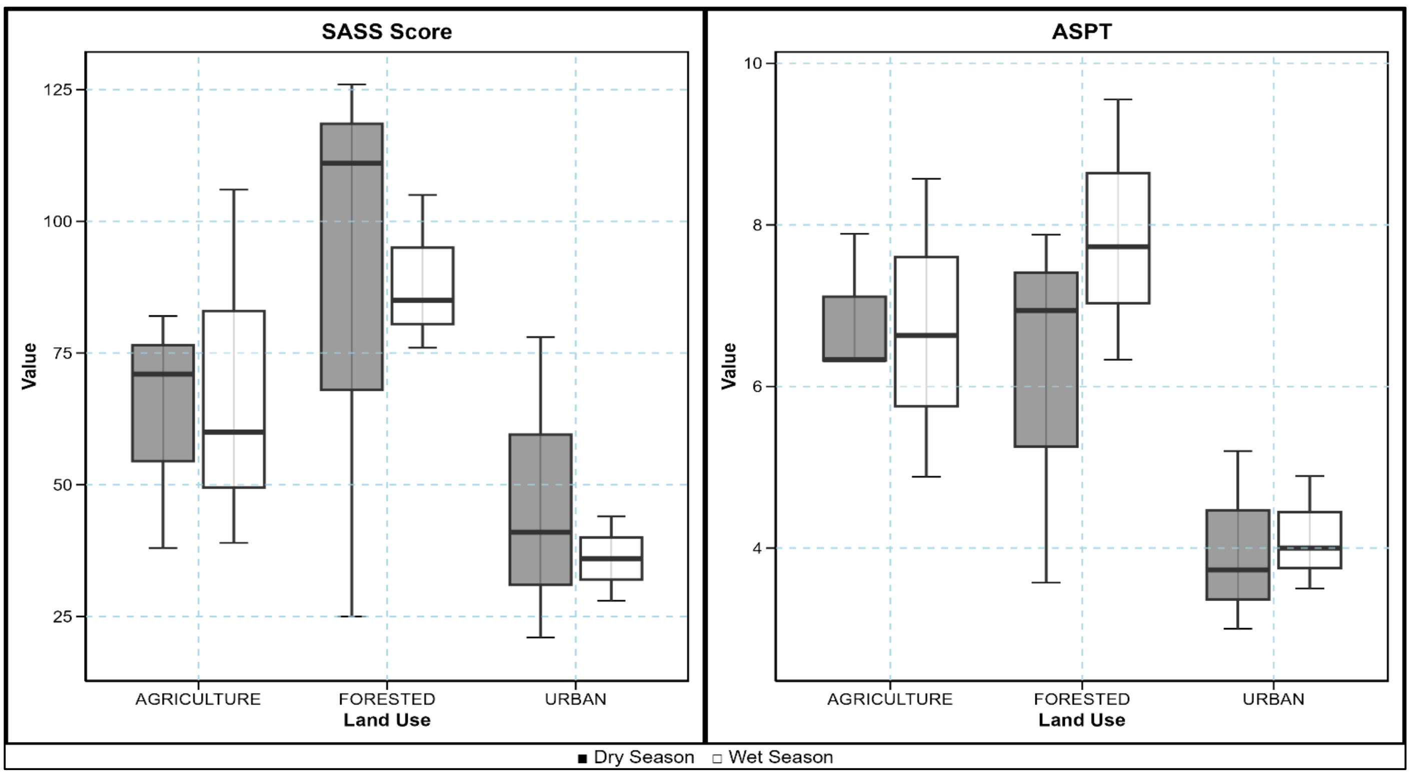
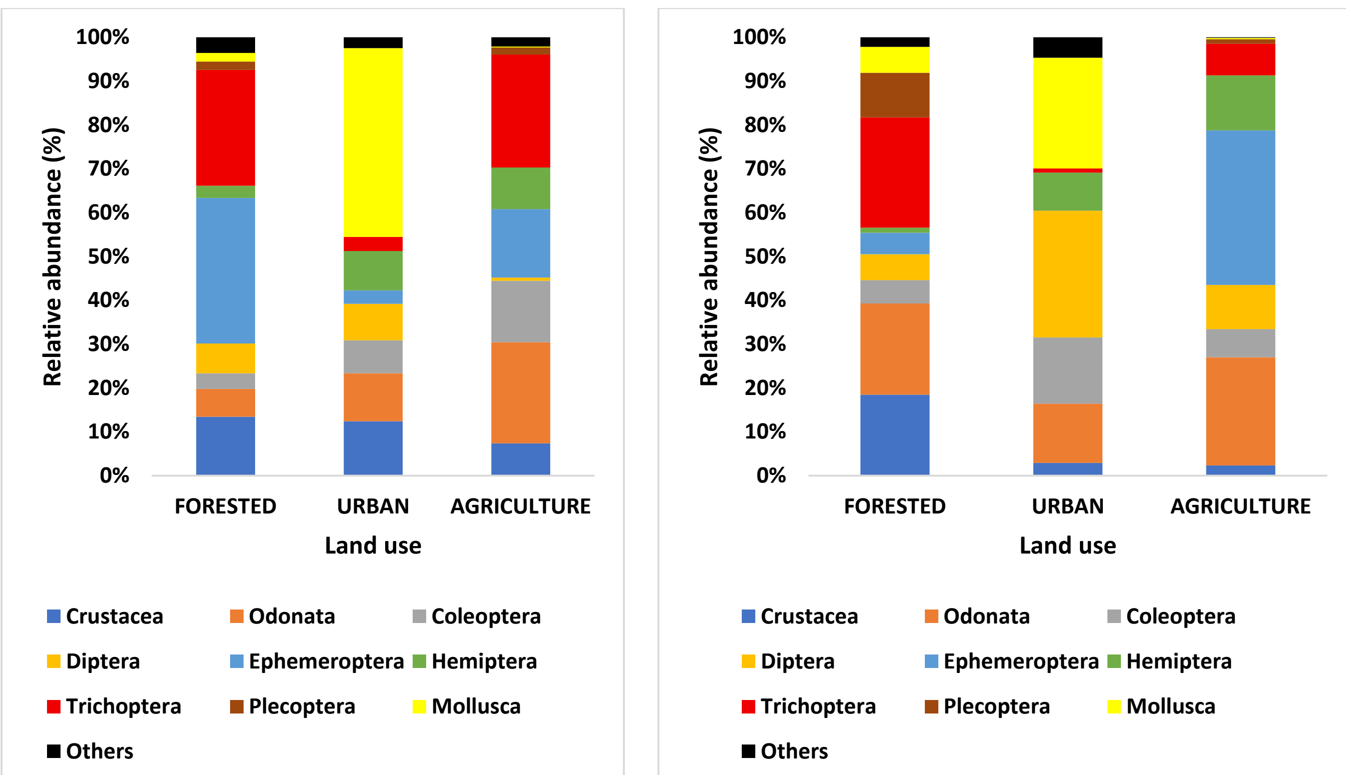


Figure 3. Seasonal variation in water quality indices across land use types. Box plots show SASS scores and ASPT values for agriculture, forested, and urban sites during dry season (gray boxes) and wet season (white boxes). Boxes represent interquartile ranges with median values (horizontal lines), whiskers show data range, and dashed horizontal lines indicate reference thresholds for water quality assessment.

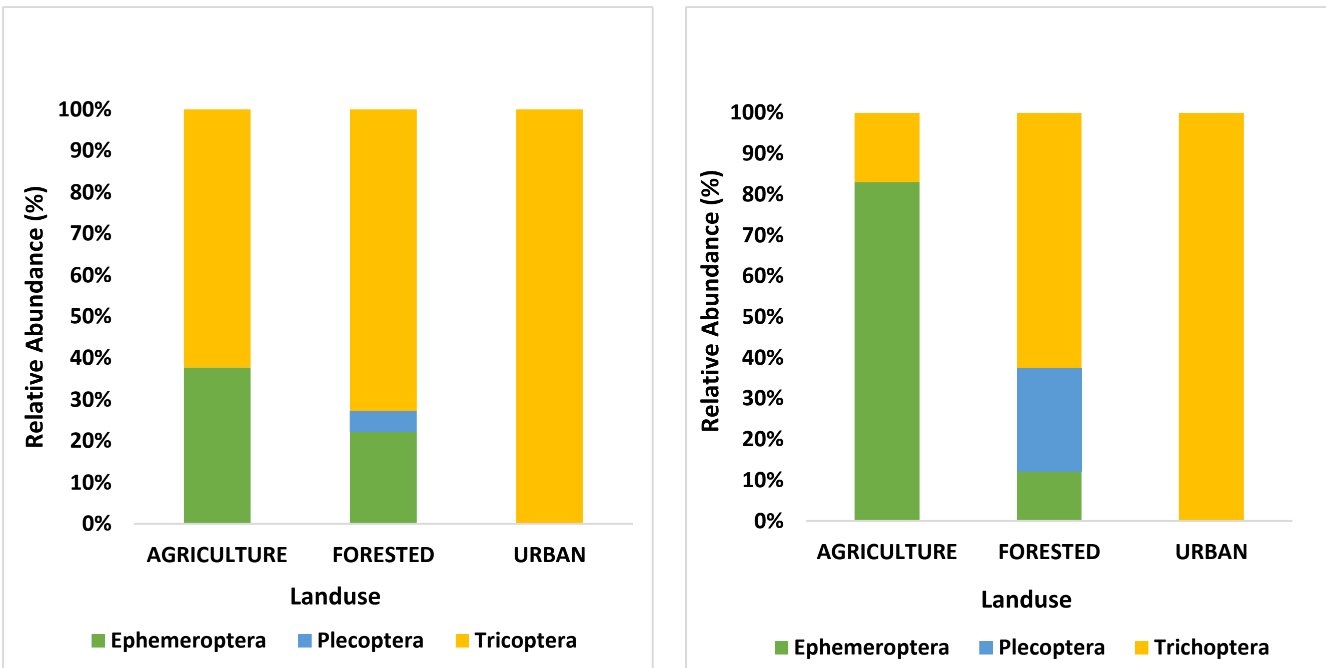
During the dry season, significant variation in log-transformed macroinvertebrate abundance was detected across land-use types ($F_{(2, 6)} = 18.65$, $p = 0.003$). Tukey's post hoc test indicated that the urban land use category had the lowest average number of macroinvertebrate individuals per site (mean = 42), which was significantly lower ($p = 0.002$) than both the forest (mean = 283) and agricultural (mean = 197) categories. However, the difference between the forest and agricultural sites was not statistically significant. In contrast, during the wet season, macroinvertebrate abundance did not vary significantly across land use categories ($F_{(2, 6)} = 1.996$, $p = 0.217$).



Wet season Dry season

Figure 4. Relative abundance of macroinvertebrate orders across land use types in dry and wet season of the Lunyangwa river, Northern Malawi

Land use significantly affected EPT macroinvertebrate communities and urbanization was identified as the main degrading agent (Figure 5). Urban streams had vastly reduced EPT abundance ($F_{2, 15} = 5.47$, $p = 0.016$), Ephemeroptera abundance ($F_{2, 15} = 10.09$, $p = 0.002$), and diversity ($F_{2, 15} = 4.90$, $p = 0.023$) relative to agricultural and forest streams. Results of post-hoc tests also showed that EPT abundance was lower particularly within urban streams relative to agricultural streams ($p = 0.016$). Seasonal difference (dry vs. wet) did not significantly impact any EPT measurements (all $p > 0.05$). Overall, results show that urbanization has a significant negative impact on sensitive stream communities despite seasonal changes.



Wet Season Dry Season

Figure 5. Relative abundance of EPT orders across land use types in dry and wet season of the Lunyangwa river, Northern Malawi

Analysis of Similarities (ANOSIM) revealed that land-use type had a significant overall effect on benthic macroinvertebrate community composition (Global R = 0.26, $p = 0.02$), while seasonality did not (Global R = 0.04, $p = 0.61$) (Table 11). Consequently, SIMPER analyses were conducted to discern the taxa responsible for these differences, stratified by season due to the strong interaction between land use and seasonal hydrology.

Table 11. Analysis of Similarities (ANOSIM) for benthic macroinvertebrate assemblages across land use types and seasons.

Factor	Global R	p
Land use	0.26	**0.02***
Season	0.04	0.61

Significant effects ($p < 0.05$) are indicated in bold, with asterisks

The SIMPER analysis revealed distinct indicator taxa driving the compositional differences between land use types, with several species showing consistent responses to urbanization and agricultural conversion across both seasons. During the dry season, a distinct difference in macroinvertebrate community composition was observed between urban and forested sites. *Bellamya capillata* (freshwater snail) contributed most to the dissimilarity (24.95%), being nearly absent in forested areas (mean = 3.33) but highly abundant in urban sites (mean = 445). Similarly, Tanypodinae (chironomid larvae) were absent in forests but present in urban areas (mean = 146), accounting for 21.72% of the dissimilarity. In contrast, *Hydropsyche* (net-spinning caddisfly) showed the opposite pattern, with substantially higher abundance in forested sites (mean = 42.7) compared to urban sites (mean = 3.67).

Comparison between forested and agricultural sites revealed Elassoneuria (stonefly nymph) as the main contributor to dissimilarity (11%), being absent in forested areas but abundant in agricultural sites (mean = 46.7). *Hydropsyche* again displayed higher abundance in forested areas (mean = 42.7) than in agricultural areas (mean = 22.7), indicating its sensitivity to habitat disturbance.

During the wet season, Potamonautes (freshwater crabs) contributed most to the dissimilarity between forested and urban sites (19.94%), with higher abundance in forested areas (mean = 70) than in urban streams (mean = 20.3). Hydropsyche maintained its trend of higher abundance in forested areas (mean = 57) than in urban areas (mean = 5.33), while *Bellamya capillata* exhibited the reverse pattern (absent in forest, mean = 45.7 in urban). In comparisons between forested and agricultural areas, Potamonautes again showed the greatest contribution (25.93%), being more abundant in forested areas (mean = 70) than in agricultural areas (mean = 10.7). Hydropsyche continued to display a preference for less disturbed habitats, though with a smaller difference in abundance (mean = 57 in forested areas and 37.3 in agricultural areas).

Table 12. Ranked abundance-based SIMPER analysis for macroinvertebrate taxa collected in Lunyangwa river for both wet and dry seasons.

Dry Season

Forest vs urban					
Taxon	Av. dissim	Contrib. %	Cumulative %	Mean FORESTED	Mean URBAN
Bellamya capillata	22.11	24.95	24.95	3.33	445
Tanypodinae	19.25	21.72	46.67	0	146
Potamodytes	6.65	7.50	54.17	7	44.3
Hydropsyche	5.54	6.25	60.42	42.7	3.67
Bulinus Africanus	3.92	4.43	64.85	0	79
Pseudagrion sp.	3.22	3.63	68.47	3	12.7
Potamonautes sp.	3.17	3.58	72.05	21.7	11.7
Trithemis	3.03	3.42	75.47	15	23.7
Ranatra	0.05	0.06	99.95	0	0.33
Trychorythus	0.04	0.05	100	0.33	0

Forest vs Agriculture					
Taxon	Av. dissim	Contrib. %	Cumulative %	Mean FORESTED	Mean AGRICULTURE
Elassoneuria	9.00	11.0	11.0	0.0	46.7
Hydropsyche	8.03	9.80	20.8	42.7	22.7
Trychorythus	7.53	9.19	29.99	0.33	38.3
Acanthiops	7.41	9.05	39.04	0.66	26
Crenigomphus	6.85	8.37	47.41	5.67	35.7
Rhagovelia	5.31	6.49	53.9	1.33	27.7
Trithemis	4.30	5.26	59.16	15	3.33
Potamonautes	4.29	5.24	64.4	21.7	7.33
Neoptela	3.39	4.15	68.55	17.3	0.0
Pseudancyronyx	3.065	3.743	72.3	0	11.7

Wet Season

Forest vs Urban					
Taxon	Av. dissim	Contrib. %	Cumulative %	Mean FORESTED	Mean URBAN
Potamonautes	16.46	19.94	19.94	70	20.3
Hydropsyche	13.93	16.88	36.81	57	5.33
Bellamya capillata	9.65	11.69	48.5	0	45.7
Bulinus Africanus	3.80	4.60	53.11	0	18
Tanypodinae	3.49	4.22	57.34	0	11.7
Tipula	3.09	3.75	61.09	12.7	2
Afronurus	3.08	3.74	64.83	12.3	0
Appasus	3.06	3.70	68.54	1	10.7
Gyrinus	2.97	3.60	72.15	0	8.33
Pseudagrion	2.45	2.97	75.12	0	7.67

Forest vs Agriculture					
Taxon	Av. dissim	Contrib. %	Cumulative %	Mean FORESTED	Mean AGRICULTURE
Potamonaute s	20.45	25.93	25.93	70	10.7
Hydropsyche	15.81	20.04	45.97	57	37.3
Acanthiops	4.45	5.64	51.61	5	20
Potamodytes	4.26	5.41	57.02	4.33	16.7
Tipula	3.52	4.47	61.49	12.7	0.66
Afronurus	3.49	4.43	65.93	12.3	1
Helisoma duryi	2.60	3.30	69.23	4.33	0
Angelena	2.56	3.25	72.48	5.33	0.66
Crenigomphu s	2.41	3.05	75.54	4.33	9.67
Trithemis	2.38	3.02	78.56	3.33	5.33

NMDS ordination analysis revealed strong patterns of community composition along environmental gradients (Figure 6). Seasonal analysis (upper left) revealed strong separation of dry and wet season communities with minimal overlap (stress = 0.156). Land-use effects (upper right) showed strong clustering of urban, forest, and agricultural sites, with agricultural communities midway between urban and forest sites (stress = 0.156). Separate seasonal analyses indicated tighter grouping during dry season (stress = 0.072) compared to wet season (stress = 0.132), with land-use differentiation more pronounced under dry conditions (Figure 6).

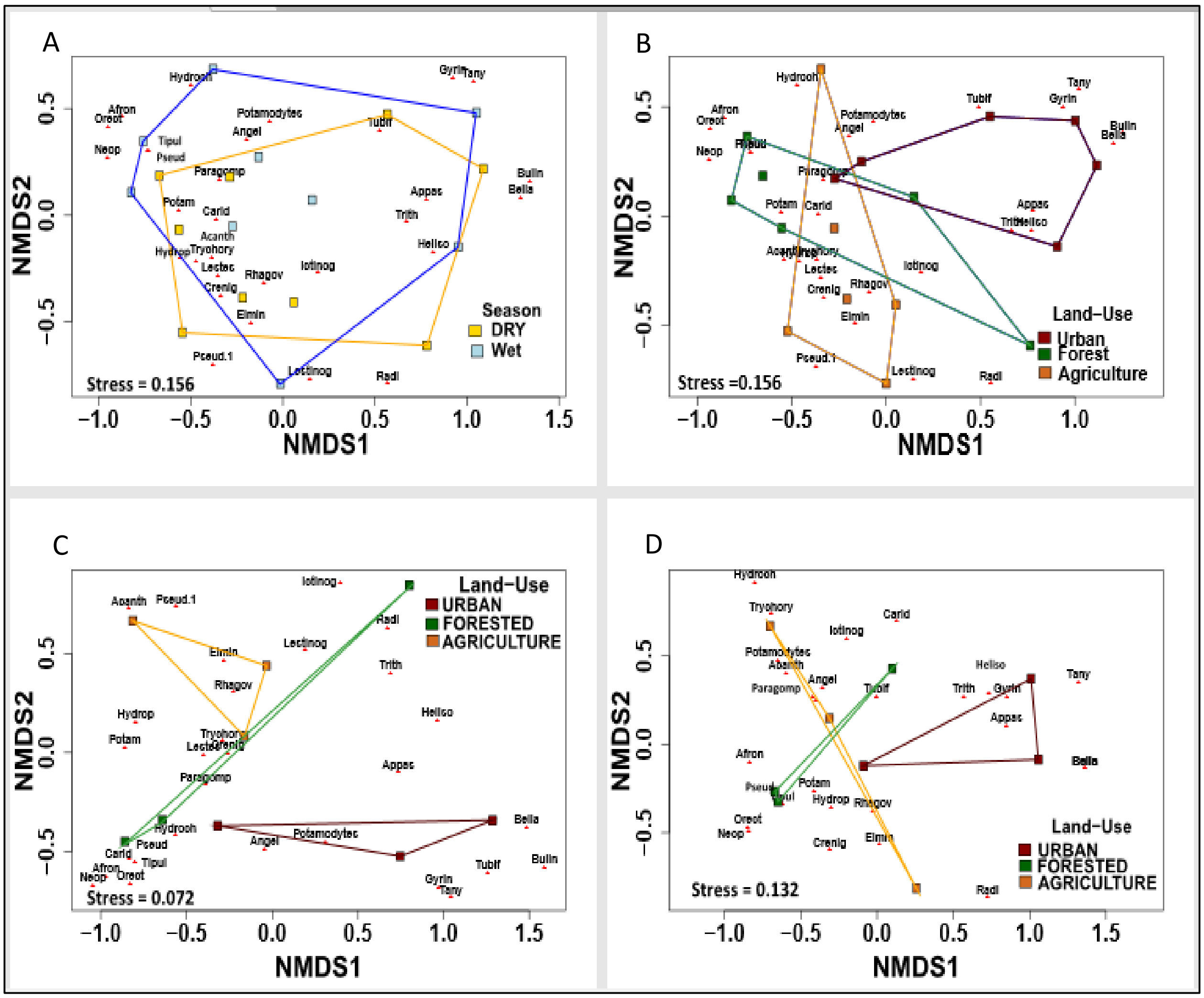


Figure 6. Non -metric Multidimensional Scaling (NMDS) plots illustrating macroinvertebrate community composition of (A) seasonal variations, (B) landuse types, (C) dry season and (D) wet season.

4.3 Relationship between water quality physico-chemical parameters and benthic macroinvertebrates

The RDA results indicated close associations among water quality parameters and benthic macroinvertebrate community composition (Figure 7). The first two axes explained 26.97% and 19.48% of the variability, suggesting that the patterns in the communities are strongly controlled by physico-chemical factors. Dissolved oxygen (DO) was the most explanatory variable, with taxa like Potamocytes, Hydropsych, and aragomp clustered in areas of higher DO concentration. The pH also exerted a pronounced influence on species distribution, as did nutrients especially nitrates and nitrites reflectinged species-specific response to nitrogen inputs. Temperature and salinity were moderate in influence, with some macroinvertebrate taxa, e.g., Lestinog, Cratil, and Tryonory, reflecting affinities for particular temperature-salinity regimes. Macroinvertebrate taxa, in general, showed well-defined environmental preferences that reflected differences in tolerances and ecological requirements under different water quality regimes.

Generation of the Pearson's linear correlation matrix used 13 water quality parameters and 13 biological variables to determine the functional relationships between the limnological parameters and the macroinvertebrate distributions along the river. A Bonferroni correction was applied to all tests to control for multiple comparisons and the results revealed that ammonium was the paramount environmental stressor, showing an extremely strong positive correlation with the Berger-Parker dominance index ($r = 0.92$) and Dominance_D ($r = 0.95$), while exhibiting equally strong negative correlations with the Brillouin ($r = -0.92$), Shannon_H ($r = -0.92$), and Equitability_J ($r = -0.93$) diversity indices (Table 13). This indicates that ammonium enrichment leads to a severe reduction in community diversity and a pronounced increase in dominance by a limited number of tolerant species. Furthermore, Total Phosphorus was strongly correlated with total community abundance (Individuals: r = 0.99), suggesting it acts as a key driver of biomass, though this may come at the cost of community balance, as shown by its negative correlation with evenness (Evenness_e_H_S: r = -0.88). Total Suspended Solids (TSS) emerged as another critical stressor, demonstrating a very strong negative correlation with species richness (Taxa_S: r = -0.95, Chao-1: r = -0.84), indicating that increased turbidity significantly reduces the number of species present. Conversely, dissolved oxygen (DO and DO %) parameters were positively correlated with diversity metrics (e.g., Brillouin: r = 0.79 and 0.73; Shannon_H: r = 0.72 and 0.67) and negatively correlated with dominance, confirming their role as fundamental proxies for a healthy, and stable ecosystem. BOD also showed a strong negative correlation with diversity (Brillouin: r = -0.77), providing further evidence of organic pollution stress. These correlations indicate that chemical pollutants, particularly bioavailable nutrients like ammonium and conditions leading to high TSS, are strongly associated with degraded ecological conditions, characterized by reduced species diversity and loss of community balance.

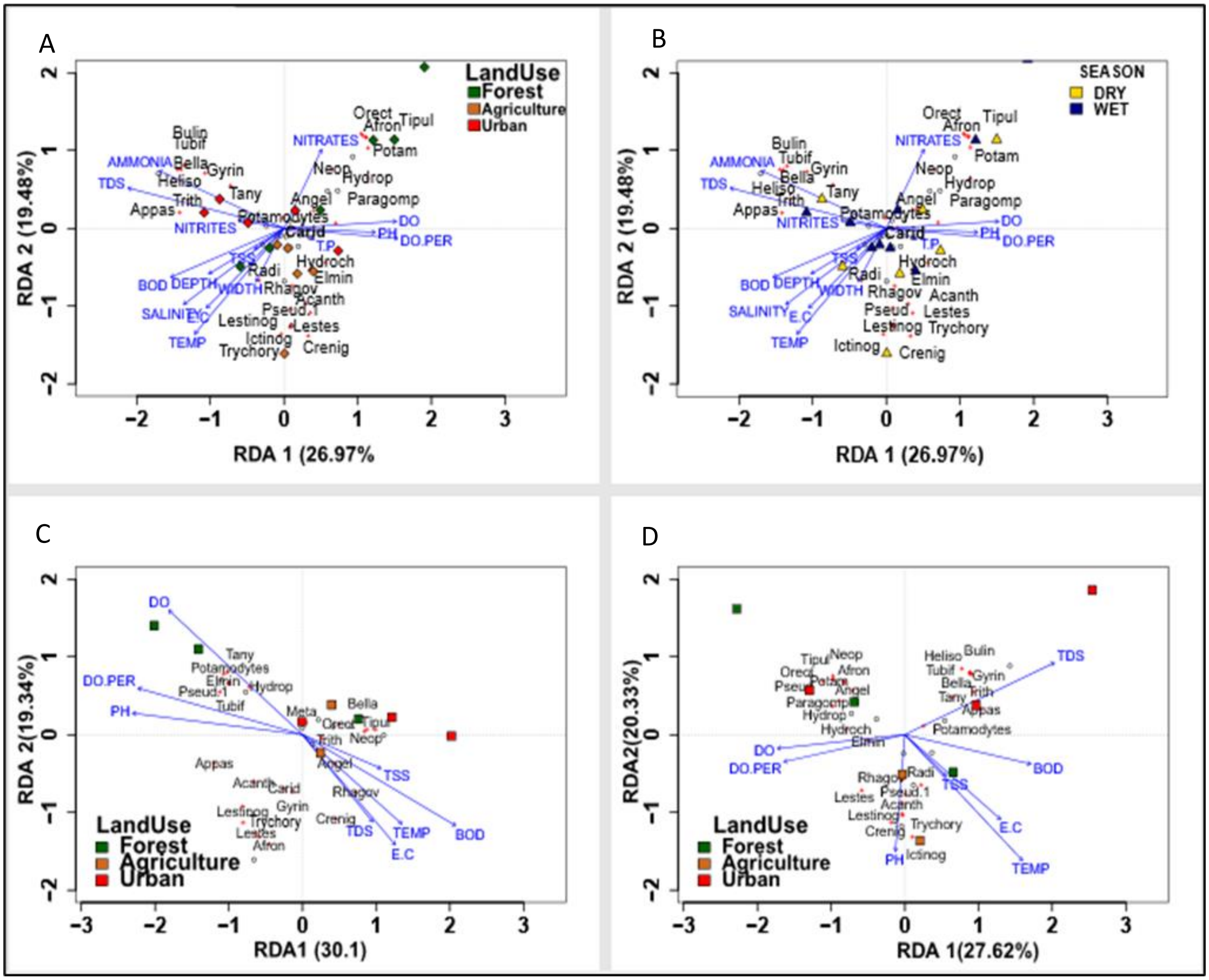


Figure 7. Redundancy Analysis (RDA) biplots showing the relationships between water quality parameters and macroinvertebrates where (A)landuse types, (B)seasonal variations, (C)wet season and (D)dry season.

Table 13. Pearson correlation coefficients between water quality parameters (horizontal) and macroinvertebrate community diversity indices (vertical) with Bonferroni correction for multiple comparisons.

Diversity Indices	**Water Quality Parameters**												
Metric	Ammonium	BOD	Conductivity	DO	DO%	Nitrate	Nitrites	pH	Salinity	TDS	TSS	Temperature	Total Phosphorus
Berger Parker	0.92	0.71	0.19	-0.77	-0.7	-0.39	0.44	-0.6	0.45	0.47	0.61	0.24	0.41
Brillouin	-0.92	-0.77	-0.26	0.79	0.73	0.42	-0.47	0.39	-0.54	-0.45	-0.69	-0.27	-0.36
Chao_1	-0.38	-0.41	-0.15	0.21	0.1	0.17	-0.45	-0.21	-0.36	0.24	-0.84	-0.42	0.31
Dominance_D	0.95	0.6	0.02	-0.71	-0.68	-0.39	0.27	-0.54	0.3	0.52	0.45	0.06	0.54
Equitability J	-0.93	-0.48	0.12	0.62	0.61	0.4	-0.16	0.44	-0.17	-0.48	-0.36	0.09	-0.56
Evenness_eH/S	-0.21	0.07	0.09	0.21	0.33	-0.26	0.7	0.22	0.12	-0.76	0.72	0.3	-0.88
Fisher alpha	-0.67	-0.39	0.05	0.36	0.29	0.15	-0.17	0.08	-0.22	-0.13	-0.58	-0.19	-0.17
Individuals	0.59	0.18	-0.11	-0.5	-0.6	-0.05	-0.42	-0.5	-0.02	0.87	-0.46	-0.33	**0.99***
Margalef	-0.72	-0.54	-0.09	0.48	0.39	0.27	-0.38	0.14	-0.36	-0.12	-0.74	-0.28	-0.09
Menhinick	-0.62	-0.09	0.37	0.2	0.19	0.03	0.23	0.15	0.13	-0.24	-0.14	0.14	-0.43
Shannon_H	-0.92	-0.69	-0.16	0.72	0.67	0.38	-0.37	0.37	-0.44	-0.43	-0.62	-0.2	-0.4
Simpson_1_D	-0.95	-0.6	-0.02	0.71	0.68	0.39	-0.27	0.54	-0.3	-0.52	-0.45	-0.06	-0.54
Taxa_S	-0.59	-0.71	-0.39	0.54	0.42	0.34	-0.68	0.07	-0.61	-0.01	-0.95	-0.52	0.16

CHAPTER FIVE. DISCUSSION

5.1 Spatio-temporal variability in water quality physico-chemical parameters.

The physico-chemical results demonstrate spatio-temporal variations of water quality physico-chemical parameters in the Lunyangwa river during the study period. The enormous differences in parameters such as temperature, dissolved oxygen (DO), DO%, biochemical oxygen demand (BOD), electrical conductivity (EC), pH, salinity, velocity, and discharge across forest, agriculture, and urban land use categories establish the paramount role of land use in determining river health. These results are in harmony with the study objective which was to assess spatial and temporal variability in water quality parameters and reject the null hypothesis (H01) of no significant variability of physico-chemical parameters along the river.

Forested plots always recorded water quality parameter values that were superior to the agricultural and urban plots, having higher dissolved oxygen (DO) and lower biochemical oxygen demand (BOD), conductivity, and salinity. These results corroborate with the recommended standard values and with other research results that indicated forested sites often act as reference sites due to minimal human impacts and for having intact riparian zones (Raphahlelo et al. 2022). These areas typically have dense vegetation cover which limits solar radiation, leading to lower water temperatures (Tela and Masayi, 2023). Colder water naturally holds more dissolved oxygen (Omary et al. 2023). In contrast, urban and agricultural areas showed degradation, likely due to wastewater discharge, agricultural runoff, and disturbance of land use.

Higher Biochemical Oxygen Demand (BOD) in urban and agricultural areas means greater organic pollution since it is a measure of the sum of the oxygen utilized by bacteria in decomposing organic matter (Omary et al. 2023). With high BOD, microorganisms consume dissolved oxygen more rapidly, leading to possible oxygen depletion that stresses or kills aquatic organisms (Adesakin et al. 2023). In urban areas for instance, this is caused by sewage effluent, domestic waste, and industrial effluents (Lina, 2016). Agricultural lands contribute via runoff with fertilizers, animal wastes, and landfills. Forested areas have lower BOD due to minimal disturbance and cleaner water, while agricultural and urban areas show higher conductivity and salinity from fertilizer runoff and waste, indicating human activity (Lubanga et al. 2021). For example, Omubira Stream had high salinity and conductivity within the town, reducing towards forested areas (Mzungu et al. 2022).

Seasonal changes play a major role in water quality, either by amplifying or reducing the effects of different land use. Discharge and velocity were much higher in the wet season on all the land uses due to more rainfall and runoff, so the material transport to rivers is higher. Higher flow also consists of more turbulence and with lower temperatures makes possible higher levels of dissolved oxygen (DO). On the other hand, the dry season is typified by low stream flow levels, where pollutants are concentrated and water has a limited capacity to dilute them. The pH also varied with land use and season, especially in town and forest locations as indicated in this study and elsewhere. For example, in Omubira Stream, pH transitioned smoothly from alkaline in town to more neutral in forest, perhaps due to fewer disturbances and relatively more stable conditions (Mzungu et al. 2022). Seasonal pH changes are regulated by dilution through rain and biological processes like photosynthesis during warm months raises the pH. In some cases, pH was higher during the rainy season since dilution took place resulting from higher amounts of water (Onwona Kwakye et al. 2021).

The PCA biplots clearly show distinct clustering of agricultural and urban sites, which always group along pollution indicator gradients such as Biochemical Oxygen Demand (BOD), electrical conductivity (EC), salinity, temperature, Total Suspended Solids (TSS), and Total Dissolved Solids (TDS). This is because the same type and magnitude of pollution are brought about by human activities. These areas' high BOD is due to organic waste, including sewage, household runoff, and agricultural waste, which impose oxygen demands during microbial breakdown. Fertilizer runoff accounts for high EC and salinity, particularly at agricultural sites, while urban areas have intermediate levels due to a mix of sewage and stormwater contaminants. In one study, agricultural sites recorded the highest EC during the dry season, while forested sites had the lowest. High EC values in agricultural and urban streams during the wet season are attributed to runoff from farmlands and urban areas (Ochieng et al. 2021). Increased temperatures in such areas are often a result of forest destruction and riparian vegetation loss, which creates the exposure of water bodies to the sun and reduced dissolved oxygen. The highest temperature levels are typically recorded in agricultural and urban sites. For instance, highest mean temperatures were observed in the town area of Omubira stream, while the lowest were recorded in the forested area (Mzungu et al. 2022). The higher mean temperature in agricultural and urban streams is largely attributed to open canopy cover along the riparian zones (Lubanga et al. 2021). Increased TSS and turbidity are the outcomes of runoff, erosion, and sand mining operations. In the Omubira stream, turbidity dropped from the town area to the forest area (Mzungu et al. 2022). High TSS values in disturbed sites result from erosion of unprotected banks and siltation (Lubanga et al. 2021). Runoff from agricultural lands and overused unpaved tracks is a major cause of erosion and surface runoff into rivers (Wanderi et al. 2022). In agricultural and urbanization zones, high organic and inorganic sediments from farm and urban waste runoffs contribute to high suspended solids and turbidity (Tela and Masayi, 2023). In contrast, forested sites consistently separate from agricultural and urban groups in the PCA plots with elevated dissolved oxygen levels and reduced pollution levels. These sites are more seasonally stable with intact riparian corridors that buffer temperature and pollution fluctuations, further enhancing their role as water quality maintainers. The first PCA axis typically characterizes a pollution gradient strongly distinguishing degraded agricultural-urban sites from less degraded forested sites.

Although seasonality influenced water quality, its impact was less than that of land use in this research. According to the results, seasonal variation had a minimal impact on water quality parameters with a non-significant PERMANOVA p-value for season of 0.59 and no significant interaction with land use. This means land use was the dominant factor impacting the trends in water quality. In contrast to this study on Mara River in Kenya it was found that both seasonality and land use significantly influenced water quality variables (Wanderi et al. 2022). Furthermore, a significant interaction between season and land use was observed ($p = 0.026$), implying that the influence of land use on water quality in the Mara River depended on the season (Wanderi et al. 2022). This study explicitly identified land use as the main driver of water quality, followed by seasonality as it was eclared elsewhere in Africa (Wanderi et al. 2022). But other studies have shown that land-use impacts could be compounded by season, most notably in the wet season when increased runoff, erosion, and leaching enhance pollution burdens. For instance, research on a study on Sosiani-Kipkaren river showed significant seasonal fluctuation in physico-chemical and nutrient parameters such that most variables had maximum values in the dry season whereas dissolved oxygen (DO), and Total Suspended Solids (TSS), were higher in the wet season (Sitati, 2021). Particularly, whereas seasons were less significant in impacting water quality in some cases, they played a larger role than land use in impacting macroinvertebrate communities, highlighting the intricacy of seasonal dynamics within aquatic ecosystems.

The WQI results revealed clear spatial and temporal variation along the Lunyangwa River, where the forested locations (Sites 1–3) were all in excellent to good condition with respect to water quality, testifying to the buffering role of intact riparian canopy and low disturbance in maintaining ecological health. The urban and agricultural (Sites 5–9) were in poor to very poor condition, with the most degraded value at Site 6 (Chinese). This trend is anticipated, as untreated domestic wastewater, agro-industrial effluent, and diffuse urban runoffs are common stressors in developing urban environments. The low WQI values at the water treatment plant (Site 4) reflect operating inefficiencies or treatment processes' failure to meet increasing loads of pollutants. The observed WQI gradient from forested to agricultural areas aligns with findings by studies in Lake Chaohu Basin identified Group I, located in upstream areas of rivers like Nanfei and Hangbu, as having "good" water quality with a mean Water Quality Index (WQI) of 76.6, and 82.9% of its sites classified as "good"(Wu et al. 2021).

Seasonal variation was significant, with higher WQI values indicating poorer quality mostly observed in the wet season. This likely reflects increased runoff, erosion, and pollutant wash-in during rainfall events, which dilute point-source inputs but mobilize large amounts of nonpoint-source pollutants such as sediments, nutrients, and organic matter and direct them to the river. High rainfall during the wet season significantly contributes to runoff from agricultural fields and other polluted terrestrial areas (Balaka and Chagoma, 2022). The lack of a significant interaction between site and season suggests that while both factors affect water quality independently, their combined influence does not alter the overall spatial hierarchy, forested sites remain consistently better, while urban and industrial sites remain degraded.

5.2 Spatio-temporal variability in composition, distribution and diversity of benthic macroinvertebrates

The study assessed the spatio-temporal dynamics of benthic macroinvertebrate assemblages along the Lunyangwa River and to examine how their composition, distribution, and diversity are influenced by anthropogenic land-use changes. The results indicate a very distinct and highly significant gradient of ecological impairment in relation to catchment land use. Forest sites supported the most stable and diverse communities, while urban sites supported the most diverse communities, with agricultural sites having an intermediate level of disturbance. This trend was strongly observed in both dry and wet seasons, as well as further highlighting land use as a more significant factor than variation in seasonality in organizing the macroinvertebrate community.

Macroinvertebrate diversity, as measured by indices such as taxa richness (Taxa_S), Shannon-H, and Margalef, was highest in forested sites during both wet and dry seasons (Table 7), indicating more equitable and species-rich communities. This can be attributed to the structural complexity of forested riparian zones, which offer diverse microhabitats, stable water flows, and reduced pollution inputs, fostering a wider array of sensitive taxa. They provide a mosaic of different habitats, including various types of biotopes like stones-in-current (SIC), marginal vegetation, aquatic vegetation, and different sediment types (Matomela et al. 2021). They are also a significant source of allochthonous organic matter, such as dead wood, leaf litter, and coarse particulate organic matter (CPOM) (Arimoro and Keke, 2017). The structural complexity afforded by forested riparian zones is indispensable for maintaining high macroinvertebrate diversity. These zones create and sustain high-quality habitats by providing diverse physical structures, abundant food resources, stable temperatures, improved water quality, and stable substrates.

Urban localities consistently demonstrate impaired macroinvertebrate communities across both wet and dry seasons, characterized by low taxa richness, high dominance, and low diversity indices such as Shannon-H and Margalef, and a high Berger-Parker index, which collectively indicate that these communities are dominated by a few, highly tolerant taxa. Reduced species diversity and richness coupled with high dominance are classic indicators of environmental degradation and pollution in aquatic systems (Dabessa et al. 2021). For example, a study on Ozomu Lake, Southern Nigeria, found that the most stressed environments, had the lowest Shannon-Wiener diversity and the highest Simpson's dominance index (0.1362) compared to less disturbed stations (Enabulele et al. 2024). The observation of a counterintuitive surge in urban macroinvertebrate abundance during the dry season (from 101 to 585 individuals) is a pattern frequently observed in severely degraded systems. This phenomenon, where high numbers of individuals coexist with low diversity and high dominance, suggests an ecosystem under significant stress rather than a healthy one (Dabessa et al. 2021). This pattern is likely driven by low flow conditions during the dry season, which reduce water levels and confine aquatic organisms to smaller, limited habitats. As a result, species that can tolerate such stressful conditions appear denser within the remaining areas (Medeiros et al. 2021). And, the lack of strong water flow or floods during the dry season allows pollution-tolerant species to reproduce and flourish without physical disturbance, leading to a "boom" in their populations (Medeiros et al. 2021).

Agricultural sites typically exhibit an intermediate but variable ecological condition, often falling between forest and urban sites in terms of diversity indices like Shannon-H and Margalef. This suggests that some level of biodiversity can be maintained. This moderate diversity in agricultural settings could stem from the presence of buffer strips or less intensive farming practices. Streams with minimally disturbed riparian vegetation often create diverse habitats that support higher biodiversity and abundance of macroinvertebrates (Sitati et al. 2021), along with the persistence of moderately tolerant species. Research highlights the presence of moderately tolerant taxa in areas with some agricultural influence. For instance, in the Upper Awash River, moderately tolerant taxa such as Baetidae, Caenidae, and Hydropsychidae were numerically dominant in sites with less human impact and agricultural activities (Dabessa et al. 2021). However, a key observation for these sites is the consistently and extremely low abundance of macroinvertebrates, which was the lowest among all land-use types in the dry season. This very low abundance strongly implies periodic, and severe stressors that suppress overall population sizes, such as pesticide runoff, fertilizer-induced eutrophication and siltation from erosion (Sitati et al. 2021). This indicates that while the habitat may support a moderate number of species, its overall carrying capacity and health are severely compromised.

Application of the SASS5 biomonitoring tool indicated that land use type was the primary driver of macroinvertebrate-based water quality assessment in the Lunyangwa River. Forest sites repeatedly displayed the best ecological health, with 'good' to 'excellent' water quality scores. At the other end of the spectrum, urban sites were consistently the most impaired, with ASPT scores indicating severe degradation. Agricultural sites displayed intermediate and highly variable conditions. Notably, seasonal variation had a minor statistically significant influence on the bioassessment metrics compared to the overriding influence of land use. Studies corroborate the finding that land use and anthropogenic disturbances are crucial in shaping spatial water quality patterns and macroinvertebrate communities in rivers (Wu et al. 2021). In contrast, other research in tropical Southeast Asian countries, such as Vietnam, Thailand, and Malaysia, noted that seasonality did not affect the ecological status when using multimetric indices (MMI) (Sripanya et al. 2023). Similarly, a long-term study in Kruger National Park rivers observed generally minimal temporal effects on SASS scores and ASPT, with only one out of four rivers showing significant temporal variation (Sithole et al. 2025).

Seasonal patterns were less clear-cut indicating forest sites to be more highly scored in the wet season, suggesting recruitment of sensitive species and dilution of pollutants by enhanced flows. Urban sites decreased further in the wet season, perhaps due to increased runoff bringing contaminants with it as rainfall increased. Urbanization leads to increased impervious surfaces, which prevent rainwater infiltration and result in greater runoff volumes (Barasa et al. 2025). This runoff often carries a high load of contaminants, including chemicals, bacteria, nitrates, total phosphorus, and organic waste, from roads, buildings, and untreated sewage, directly into rivers (Barasa et al. 2025). These conditions are highly detrimental to macroinvertebrate communities, leading to a reduction in sensitive taxa and a dominance of tolerant species, thus lowering ASPT scores (Getachew et al. 2023). Agricultural sites exhibited variable responses seasonally, both capturing dilution benefits and adverse effects of soil erosion and wash-off of fertilizers. The increased runoff during the wet season transports large quantities of fine sediments, increasing turbidity and suspended solids in the water (Barasa et al. 2025). The unstable nature of substrates due to stormwater influx during the rainy season can also lead to a reduction in macroinvertebrate diversity (Arimoro and Keke, 2017).

The Average Score Per Taxon (ASPT) is generally considered as a more consistent and repeatable measure of river health assessment compared to the SASS Score (Dickens and Graham, 2002). SASS scores are designed to reflect the presence of taxa with varying sensitivities to pollution, making them responsive to environmental degradation. However, studies strongly indicate that biotic indices, including SASS5, can be very sensitive to seasonality and flow conditions, often performing poorly or showing declines in discriminatory ability during the wet season (Masese et al. 2023). Unlike the SASS Score, ASPT is relatively unaffected by sampling effort. This consistency makes it a preferred indicator, especially for assessing good quality rivers (Dickens and Graham, 2002). While SASS scores can differentiate land use impacts, their seasonal variability can be high (Sithole et al. 2025). ASPT, by accounting for taxa richness, offers greater consistency and sensitivity, providing a more reliable measure of ecological health (Dickens and Graham, 2002).

The significant variation in macroinvertebrate abundance among land-use types during the dry season indicates that habitat conditions and stressors differ strongly across forested, agricultural, and urban streams when water levels are low. Forested sites supported the highest number of individuals (mean = 283), followed by agricultural sites (mean = 197), while urban sites supported far fewer (mean = 42). The macroinvertebrates community structure is affected by physical, chemical, and biological conditions, making them reliable indicators of recent environmental events (Sitati et al. 2021). The abundance and diversity of macroinvertebrates respond to various stressors like toxic pollutants, sedimentation, habitat disturbance, nutrient runoff, and hydrologic regimes (Abeke Ayoade and Adewumi Adeyemi, 2022).

This EPT analysis clearly demonstrates that land use type, particularly urbanization, is a primary determinant of EPT community structure in these streams, overwhelming any potential seasonal variation. Urbanization is consistently identified across the sources as a primary driver of damage to stream ecosystems and, specifically, to Ephemeroptera, Plecoptera, and Trichoptera (EPT) communities (Addo-Bediako, 2021). Urban areas are major contributors to point source pollution through domestic sewage, industrial effluents, and municipal wastewater treatment plant discharges (Balaka and Chagoma, 2022). These discharges introduce a variety of harmful substances directly into rivers. For instance, untreated wastes reduce the water's ability to selfpurify, threatening sensitive aquatic taxa (Barasa et al. 2025). Industrial effluents often contain heavy metals and sulphates, which contribute to water quality deterioration and create unfavorable conditions for aquatic organisms. EPT taxa are widely recognized as sensitive indicators of good water quality and healthy habitat conditions, meaning their presence signifies less pollution and more stable environments, while their decline indicates environmental stress. Studies in River Kiminini and other Afrotropical streams found a decrease in EPT richness with increasing human activity and pollution from agriculture, urban runoff, and domestic wastes (Barasa et al. 2025).

The Analysis of Similarities (ANOSIM) provides robust statistical confirmation that land-use type is the primary driver structuring benthic macroinvertebrate community composition in the Lunyangwa River. Anthropogenic land-use activities, such as agriculture, urbanization, and industrialization, are implicated in impairing freshwater ecosystem health and functioning worldwide (Akamagwuna et al. 2022). These activities alter streams' hydrological features and water chemistry, ultimately changing the complex biotic and abiotic processes that shape biological communities (Akamagwuna et al. 2022). Land-use change degrades river health by polluting water (favoring tolerant species), altering habitats (shifting food webs), and removing riparian buffers, which together reduce biodiversity and ecosystem function aligning with the study done by Munyai et al. 2025. The same was reported in the Ethiopian river basins, where land use and spatial factors significantly contributed to macroinvertebrate community composition. Environmental characteristics were the most important component (42% of explained variation), with land use accounting for 35%. This research emphasized land use planning as a crucial strategy for improving stream conditions and aquatic macroinvertebrate community composition (Getachew et al. 2023).

This land-use impact, which degrades river health by polluting water, altering habitats, and removing riparian buffers, was quantified by the SIMPER analysis, which identified the specific taxa responsible for the observed compositional shifts. During the dry season Bellamya capillata accounted for 24.95% of dissimilarity, being nearly absent in forests but highly abundant in urban streams. Tanypodinae (chironomids) showed a similar pattern, while Hydropsyche was more abundant in forests. Snails (Gastropoda) are categorized as pollution-tolerant taxa that can thrive in a wide range of water quality conditions, including low oxygen and high nutrient concentrations (Mouton et al. 2022). On the other hand, Chironomidae were identified as the most abundant family in some river systems, with a very high number of individuals (Pinna et al. 2024). Chironomids are consistently classified as tolerant taxa that can survive in highly polluted or degraded water conditions. Chironomids are known to "favor" anthropogenically modified flow, increased nutrient enrichment, and sedimentation (Patrick et al. 2015). Their ability to tolerate depleted oxygen concentrations is attributed to the possession of hemoglobin, which traps oxygen within their bodies (Edegbene et al. 2022). This indicates that tolerant taxa such as snails and chironomids thrive under polluted or nutrient-rich urban conditions, while sensitive caddisflies prefer clean, oxygenated waters typical of forested streams. Further on Elassoneuria, was completely absent in forests but abundant in agricultural streams, while Hydropsyche remained more common in forests. One study on the Omubiru River, found to have the highest abundance of hydropsyche in forested reaches in some studies, with specific observations of 28.65 individuals at forested sites (Mzungu et al. 2022). Elassoneuria in agricultural areas, where they are associated with high-nutrient habitats, suggests a shift in the community structure and is a common trend observed in degraded. This family is generally classified among pollution-sensitive macroinvertebrate taxa. This suggests that agriculture introduces moderate disturbance, which favors some taxa but reduces the abundance of sensitive species.

While, during the wet seasons, potamonautes contributed most to dissimilarity, being more abundant in forested streams. Hydropsyche again dominated in forests, while Bellamya capillata was absent in forests but common in urban streams. Research reports, potamonautes (Freshwater Crabs) to be common across both first-order and second-order stream systems in Afrotropical streams, with substantial numbers found at higher elevation and forested streams (Sitati et al. 2024). For example, Potamonautes elgonensis is an endemic crab species found in the upper reaches of rivers in the highlands of western Kenya and eastern Uganda. The increase in crabs in urban areas may reflect higher organic matter availability, but the consistent presence of Hydropsyche in forests reinforces their role as indicators of good water quality. Furthermore, across both seasons, tolerant taxa such as Bellamya capillata and Tanypodinae were strongly associated with urban streams, while sensitive taxa such as Hydropsyche and Elassoneuria were more abundant in forests. Agricultural sites consistently showed intermediate conditions. The consistent dominance of tolerant taxa in urban streams highlights the effects of organic pollution, low oxygen, and habitat alteration. On the other hand, the presence of Hydropsyche in forests indicates the presence of fast-flowing, well-oxygenated waters. These taxa can therefore be trusted as good bioindicators for determining stream health in tropical rivers.

NMDS ordination demonstrated clear differences in community composition between seasons and among land-use types. While NMDS did reveal the occurrence of seasonally bunched patterns, ANOSIM shows that land use is more and more robust in its effect compared to seasonal influences. This implies that although hydrology shifts the pattern of distribution of taxa between wet and dry seasons, human disturbance impacts more and permanently on community composition. Furthermore, the separation of urban sites in the NMDS corresponds to the dominance of tolerant taxa such as Bellamya capillata and Tanypodinae in SIMPER analysis, while forest clusters align with sensitive taxa such as Hydropsyche and stoneflies. Agricultural sites consistently occupied intermediate positions, reflecting mixed communities. This means human activities are fundamentally reorganizing freshwater biodiversity, leading to distinct macroinvertebrate communities aligned with the dominant land-use type.

5.3 Relationship between water quality physico-chemical parameters and benthic macroinvertebrates.

Water quality parameter-macroinvertebrate community relationships provide evidence of specific environmental drivers of community patterns. The redundancy analysis revealed that physico-chemical water quality parameters explained 46.45% of benthic macroinvertebrate community structure variation, indicating strong environmental condition-biological assemblage relations. Such significant explanatory power supports the central ecological generalization that abiotic conditions play key filtering roles in community assembly processes. The clear separation of forest, agricultural, and urban sites, along with distinct wet and dry season patterns, indicates that both spatial and temporal factors interact with water chemistry to structure aquatic communities.

Dissolved oxygen (DO) concentrations were in the range of good water quality for aquatic life condition indicating superior water quality. Aquatic systems with ample DO generally support a greater diversity and abundance of pollution-sensitive species, reflecting a healthier ecosystem (Ouma et al. 2025). These sites were indeed associated with pollution-sensitive macroinvertebrates like Hydropsyche and other EPT taxa, aligning with their known requirements for well-oxygenated, pristine conditions. DO levels regulate the metabolic activities of organisms and manages the metabolism of the entire biological community, serving as a key indicator of a water body's trophic status (Omary et al. 2023). While dissolved oxygen is a vital indicator, another critical parameter, pH, also plays a major role as a critical environmental driver, influencing macroinvertebrate communities and determining water chemistry and biochemical processes (Getachew et al. 2023). Macroinvertebrates exhibit contrasting sensitivity levels to pollution based on pH (Munyai et al. 2024). For instance, slight increases in pH have been associated with a deterioration of the functional diversity of stream macroinvertebrates (Sotomayor et al. 2023). While Odonata may show relative insensitivity to pH, Plecoptera taxa are particularly sensitive to variations in pH levels (Barasa et al. 2025). Conversely, some taxa like Chironomidae and certain crustaceans have been linked to environments with low pH (Odountan et al. 2019). Nutrients, especially nitrogen compounds (nitrates, nitrites, ammonium, and total organic nitrogen), are repeatedly identified as significant environmental factors that influence macroinvertebrate community composition (Getachew et al. 2023). These compounds, particularly at high concentrations, are associated with eutrophication and oxygen depletion, which severely affect macrobenthic invertebrate assemblages (Arimoro and Keke, 2017). Tolerant taxa like Oligochaeta increase in abundance with elevated nutrient enrichment. High nitrate concentrations are often associated with lower EPT richness, and Trichoptera taxa are influenced by nitrate levels in the water. Other parameters that influenced macroinvrtabrate composition and structure in L. River are temperature and salinity. Temperature also influences chemical and bacterial activity, as well as water evaporation (Hammoumi et al. 2024). Pollution-sensitive taxa, such as Ephemeroptera, Plecoptera, Trichoptera, and Odonata (EPTO), require habitats with cooler water temperatures (Musonge et al. 2020). Salinity levels can vary both seasonally and spatially and then impact biotic and abiotic parameters in water body systems. It can be correlated with the distribution of certain macroinvertebrate groups, such as Gastropoda. High salinity values can exceed permissible limits for brackish water and can reduce dissolved oxygen levels in water leading to unsuitable water quality condition in the river 's ecosystem (Yazdian et al. 2014).

The profound influence of these parameters, as shaped by land use and season, is clearly demonstrated in the Redundancy Analysis (RDA) ordination. The RDA biplots (Figure 7) visually confirm this relationship between water quality physico-chemical parameters and macroinvertebrates, showing a clear separation of sites based on land use type. Forested sites cluster tightly with high dissolved oxygen (DO) vectors and are associated with pollution-sensitive taxa. These pollution-sensitive species require habitats with cooler water temperatures and higher DO levels to survive. Barasa et al. (2025) indicated that ephemeroptera (mayflies) were found in high numbers in natural vegetation, benefiting from pollutant filtration, reduced sediments, abundant organic matter, and regulated temperatures due to shade. Moving along this gradient, agricultural sites align strongly with nutrients like phosphorus and nitrates, supporting a community of moderately tolerant taxa. Agricultural practices, including crop cultivation and livestock farming, are identified as major contributors to nutrient pollution (nitrogen and phosphorus) in river systems (Balaka and Chagoma, 2022). This pollution often occurs through runoff and leaching of fertilizers and manure, leading to increased nutrient concentrations and potential eutrophication in aquatic environments (Masese et al. 2023). Macroinvertebrate communities in areas dominated by agriculture tend to include moderately tolerant taxa. Higher abundances of Elmidae and certain Odonata and Hemiptera species in agricultural zones, which can be indicators of water quality deterioration (Mzungu et al. 2022). Finally, urban sites are positioned near vectors for ammonium, BOD, and TSS, demonstrating dominance by highly tolerant taxa such as *Tubifex* and *Bulinus*. Urbanization and rapid industrial development are major drivers of environmental degradation, significantly impacting water quality through the discharge of untreated or poorly treated industrial and domestic effluents (Gbedzi et al. 2022). Urban environments foster conditions where highly pollution-tolerant macroinvertebrate taxa dominate, replacing sensitive species that cannot adapt to degraded water quality, low oxygen, and high pollutant loads. Ochieng et al. (2021) reported that, *Bulinus sp*. was explicitly identified in a canonical correspondence analysis (CCA) as being correlated with environmental variables typically elevated in urban settings. Another study by Tela and Masayi, (2023) demonstrated that Chironomidae (midge larvae) and Oligochaeta (aquatic earthworms) such as tubifex were frequently found to be the most abundant taxa in highly polluted urban areas, even thriving in very low oxygen or anoxic conditions due to their adaptations, such as high hemoglobin content. This progression from forested to urban via agricultural land use visually represents a trajectory of increasing anthropogenic impact and declining ecological integrity.

Seasonality plays a significant role in influencing water and habitat quality variables and macroinvertebrate community structure. During the dry season, community structure showed tighter clustering and stronger environmental determinism, suggesting that water quality constraints become more pronounced under low-flow conditions (Figure 7). Studies in the Mara River Basin also noted that electrical conductivity and temperature were higher during the dry season, and that reduced flows lead to the accumulation of organic matter and ammonia, increased concentration of solutes, and an overall decline in DO concentration (Masese et al. 2023). In contrast, the wet season showed greater dispersion of communities in ordination space, indicating that the dilution effect of increased rainfall and higher discharge may weaken the direct relationships between water quality parameters and community composition. Dissolved oxygen (DO) levels often increase during the wet season due to increased turbulence and higher oxygen solubility following lower temperatures (Ochieng et al. 2021). Runoff and leaching during the wet season can increase the delivery of sediments, nutrients, and dissolved organic carbon into streams and rivers (Masese et al. 2023). Biotic indices have been observed to perform poorly during the wet season, failing to differentiate between disturbance levels, likely due to temporary improvements in environmental conditions caused by dilution and flushing (Masese et al. 2023). The dry season tends to concentrate pollution impacts, leading to more stressed and predictably structured communities dominated by tolerant taxa, while the wet season's dilution and flushing effects can ameliorate some of these impacts, resulting in more dispersed and varied community assemblages. This underscores the importance of multi-seasonal monitoring for a comprehensive understanding of riverine ecosystem health. The primary highlight is the clear and distinct seasonal patterns in how water quality parameters influence macroinvertebrate communities. This demonstrates that ecological responses are not static but vary significantly with the time of year.

The Pearson correlation analysis revealed strong functional relationships between water chemistry and macroinvertebrate community structure along the river gradient. Ammonium emerged as the most influential stressor, with a very strong positive correlation with dominance indices (Berger-Parker and Dominance_D) and equally strong negative correlations with diversity indices such as Shannon_H and Brillouin. This indicates that ammonium enrichment promotes the proliferation of a few tolerant taxa while excluding sensitive species, leading to low community diversity. High levels of nutrients, including ammonia, can lead to a reduction in species richness and diversity, causing a shift in species composition. This involves the selective elimination of less tolerant species, allowing more tolerant species to increase in abundance due to reduced competition and predation. Specifically, this shift was observed in the Urban Funa Stream, where areas with high ammonium levels showed a marked increase in the abundance of tolerant taxa such as

Chironomids and Oligochaeta, which are known to thrive in disturbed, nutrient-enriched conditions (University of Kinshasa and Patrick, 2015). Total phosphorus showed a very strong positive correlation with community, indicating that nutrient enrichment drives higher biomass. However, this increase in numbers was associated with reduced evenness, suggesting that phosphorus enrichment benefits certain dominant taxa at the expense of community balance. High levels of organic nutrients, which include phosphorus, can lead to increased abundance of macroinvertebrates, particularly in sites affected by agriculture and sewage (Addo-Bediako, 2021). The evenness index, which increases with a decrease in ecosystem stress, is described as decreasing with increased anthropogenic perturbations in streams (Ouma et al. 2025). Nitrates and nitrites also influenced community composition, particularly in agricultural sites, consistent with nutrient inputs from fertilizer runoff. High nitrate levels, often exceeding permissible limits, have been recorded in rivers flowing through agricultural regions (Barasa et al. 2025). Nitrites, in particular, have been linked to cattle grazing and are significant factors in structuring macroinvertebrate communities (Odountan et al. 2019). Elevated nitrate concentrations are frequently associated with lower Ephemeroptera, Plecoptera, and Trichoptera (EPT) richness or diversity (Kaboré et al. 2017). Barasa et al. (2025) found sensitive taxa including Ephemeroptera and Trichoptera, to show strong negative correlations with nitrates, indicating that high levels of these parameters are detrimental to them.

Suspended solids were another critical stressor, showing a strong negative correlation with richness. This reflects how increased turbidity and siltation reduce habitat heterogeneity, clog interstitial spaces, and limit oxygen penetration, which leads to the exclusion of sensitive taxa. Urban and agricultural catchments are particularly prone to sediment loading, which may explain the lower richness observed in these sites compared with forested areas. Suspended solids, often manifesting as turbidity and leading to siltation, are consistently identified as significant stressors on aquatic ecosystems and macroinvertebrate communities (Munyai et al. 2024). Research consistently demonstrates that elevated turbidity and sediment load negatively impact macroinvertebrate communities, notably by reducing the density of sensitive taxa such as mayflies (Ephemeroptera) and caddisflies (Trichoptera) (Choi et al. 2024). Similarly, in a study in South Africa, the lowest diversity index values were found at downstream sites impacted by agricultural and mining activities, where suspended solids were higher, causing a decrease in the EPT index with increasing human impact (Addo-Bediako, 2021). Dissolved oxygen was positively correlated with diversity and negatively with dominance, confirming its role as a key determinant of stream health. Sites with higher DO supported sensitive taxa such as Hydropsyche and Potamodytes, while oxygen-poor environments favored tolerant taxa like Tubifex. Forested reaches, with greater canopy cover and lower organic loading, maintained higher oxygen levels, which explains their higher diversity. Dissolved oxygen is identified as one of the most important water quality variables influencing macroinvertebrate community composition (Getachew et al. 2023). Research shows clear association between low functional diversity (FD) values and reduced DO concentration, aligning with observed study results. This implicitly demonstrates a positive correlation between DO and diversity metrics (Sotomayor et al. 2023). Another study explicitly noted increasing abundance and diversity of pollution-sensitive macroinvertebrates with increasing DO concentrations (Ouma et al. 2025). On the other hand, Oxygen depletion, resulting from processes like the decomposition of organic content by microorganisms, leads to a reduction in species richness, evenness, and phylogenetic diversity, and results in the community being heavily dominated by a few species (Adesakin et al. 2023). This finding aligns with previous research. For example, in their study, Makumbe et al. (2022) demonstrated that macroinvertebrate communities at sites with high dissolved oxygen (e.g., 9.4 mg/L) exhibited significantly lower dominance values than sites with low oxygen levels (e.g., 3.3 mg/L), confirming the inverse relationship between DO and dominance shown in our results.

CHAPTER SIX. CONCLUSION AND RECOMMENDATION

6.1 CONCLUSIONS

This study shows that land use is the dorminant driver of water quality variation in the Lunyangwa River, overwhelmingly more than seasonal effects. A clear and consistent gradient of ecological degradation was established with forested sites experiencing excellent to good water quality, while agricultural sites noting intermediate degradation, and urban sites being in the range from poor to very poor condition. The improved water quality of the forested watersheds characterized by higher dissolved oxygen and lower BOD, conductivity, and salinity is attributed to restricted human impact and intact riparian areas. Urban and agricultural watersheds, in contrast, are strongly degraded due to wastewater discharge, runoff, and land disturbance. Statistical analysis confirms the dominant impact of land use, with seasonal change as a secondary factor. While the Water Quality Index (WQI) did register strong seasonal change with the wet season enhancing the delivery of pollution through higher runoff, this effect was secondary to the overriding and chronic effect of land use. The broad spatial pattern of water quality is ultimately controlled by anthropogenic land use, with seasonality acting as a transient modulator of pollutant concentration.

The composition, distribution, and diversity of benthic macroinvertebrate communities in the Lunyangwa River are overwhelmingly structured by land use types. Forest stands have richest and most stable macroinvertebrate assemblages, and urban stands are most degraded with lowest diversity and dominance by tolerant species like Bellamya capillata and Tanypodinae. Agricultural stands have intermediate conditions but persistently low density due to periodic stressors like pesticides and eutrophication.

Forested stations retain maximum diversity in both seasons due to structural complexity and reduced pollution, while urban sites exhibit degraded communities characterized by low taxa richness and high-influence dominance. SASS5 biomonitoring and ANOSIM analysis confirm land use as the dominant driver of ecological integrity, and EPT taxa serve as appropriate water quality indicators whose densities decline strongly with urbanization. While seasonal effects mold community patterns, land use effects uniformly overwhelm temporal variation in structuring macroinvertebrate assemblages.

There exists a strong, and quantifiable relationship between physico-chemical water quality parameters and benthic macroinvertebrate community structure. Principal water quality drivers included ammonium, nutrients (phosphorus, nitrates), suspended solids, and dissolved oxygen and explained the majority (46.45%) of variance in biological communities in the L. River. These land use-driven factors act as environmental filters: tolerant taxa thrive in high nutrients and low oxygen in urban/agricultural areas, while sensitive EPT taxa are nourished by high oxygen in forests. Seasonality moderates this relationship so that dry seasons tighten pollutants and maximize environmental limitation, while wet seasons impose dispersion through dilution, highlighting the value of multi-seasonal monitoring.

6.2 RECOMMENDATIONS

Based on the study findings addressing each specific objective, the following recommendations are proposed

i. Reduce pollution from urban/agricultural sources through improved wastewater treatment, organic farming, and stricter control to lower BOD, nutrient, and conductivity levels that are currently beneficial to tolerant organisms.
ii. Establish a regular biomonitoring program using macroinvertebrates as ecological indicators across the land-use gradient, conducting biannual assessments using SASS5

methodology to provide early warning of ecological decline and inform adaptive management decisions.

iii. Develop an integrated assessment framework, such as a Multimetric Index (MMI), that combines water quality and macroinvertebrate data to provide a clear and comprehensive measure of the Lunyangwa River's ecological health.

6.2.1 Management and Policy Recommendations

i. Establish mandatory 30-meter riparian buffer zones along river sections traversing urban and agricultural land uses to enhance pollutant filtration capacity and mitigate sediment erosion into aquatic systems.

ii. ii. Upgrade wastewater treatment infrastructure within Mzuzu City to reduce organic pollutant loading and enhance dissolved oxygen concentrations in downstream river reaches.

iii. iii. Integrate macroinvertebrate-based biomonitoring protocols into Malawi's national river health assessment framework, aligning with Sustainable Development Goal 6 targets for clean water and sanitation.

iv. iv. Implement capacity-building initiatives targeting riparian communities on sustainable agricultural practices, emphasizing reduced synthetic fertilizer application and soil conservation techniques to minimize nutrient and sediment export from agricultural catchments.

v. Implement best management practices (BMPs) in agricultural areas. This includes reducing reliance on chemical fertilizers and pesticides, implementing soil conservation techniques to prevent erosion, and creating fenced buffers to keep livestock away from waterways.

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APPENDICES

APPENDIX I: Mzunirec Approval

MZUZU UNIVERSITY

DIRECTORATE OF RESEARCH

Mzuzu University
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MZUZU UNIVERSITY RESEARCH ETHICS COMMITTEE (MZUNIREC)

Ref No: MZUNIREC/DOR/24/110 **14/10/2024.**

Aisha Athumani Lali.
Mzuzu University,
P/Bag 201, Luwinga,
Mzuzu 2.

Email: aishalali521@gmail.com

Dear Aisha,

RESEARCH ETHICS AND REGULATORY APPROVAL AND PERMIT FOR PROTOCOL REF NO. MZUNIREC/DOR/24/110: SPATIO-TEMPORAL VARIABILITY IN WATER QUALITY AND MACROINVERTEBRATES ASSEMBLAGE OF LUNYANGWA RIVER, MALAWI.

Having satisfied all the relevant ethical and regulatory requirements, I am pleased to inform you that the above referred research protocol has officially been approved. You are now permitted to proceed with its implementation. Should there be any amendments to the approved protocol in the course of implementing it, you shall be required to seek approval of such amendments before implementation of the same.

This approval is valid for one year from the date of issuance of this approval. If the study goes beyond one year, an annual approval for continuation shall be required to be sought from the Mzuzu University Research Ethics Committee (MZUNIREC) in a format that is available at the Secretariat. Once the study is

1

finalised, you are required to furnish the Committee with a final report of the study.

The Committee reserves the right to carry out compliance inspection of this approved protocol at any time as may be deemed by it. As such, you are expected to properly maintain all study documents including consent forms.

Wishing you a successful implementation of your study.

Yours Sincerely,

Wanangwa K. Msowoya
MZUNIREC Secretariat
FOR: MZUNIREC CHAIRPERSON

MZUZU UNIVERSIT
RESEARCH ETHICS COM... EE
14 OCT 2024
PRIVATE BAG 201, LUWINGA
MZUZU 2

Mzuzu University Research Ethics Committee
Directorate of Research
Mzuzu University
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2

APPENDIX II: Forest Access Permission Letter

REF.No.09/05
Ministry of Natural Resources and Climate Change,

(Department of Forestry)

All correspondences to
the Zone Forestry Office (N)
P.O Box 223, Mzuzu
Tel: +265 0888895825/0999792427
Email: pcunorth@gmail.com

22nd November, 2024

Head, Department of Fisheries and Aquatic Sciences
Mzuzu University
Private Bag 201
Luwinga, Mzuzu 2

Attention: Mr Kumbukani Mzengereza PhD

Dear Sir,

AUTHORIZATION TO CONDUCT DATA COLLECTION EXERCISE IN KANING'INA FOREST RESERVE FOR ACADEMIC RESEARCH PURPOSE

Reference is made to your letter dated 20th November, 2024 requesting for permission to access Lunyangwa River in Kaning'ina Forest Reserve for research by MSc student namely Aisha Lali.

I am glad to inform you that your request has been granted. Please, notify this office of the intended commencement and completion dates for the data collection period. You are also advised to work closely with this office for guidance and direction to ensure that all forest protection and conservation procedures are followed during this period.

You are further requested to furnish this office with the outcome of your research study for review and consideration. We anticipate that your research will yield valuable insights that will enhance sustainable management and protection of Kaningina Forest Reserve and its natural resource base.

Please feel free to contact our office if you require any further assistance.

Gloria Makhambera
THE DEPUTY ZONE FORESTRY MANAGER (N)

ZONE FORESTRY MANAGER (N)
22 NOV 2024
P.O BOX 223 MZUZU

APPENDIX III: Images of Macroinvertebrate Specimens Sampled

Afronurus sp

Potamodytes sp (larvae)

Helisoma duryi

Ranatra sp

Appasus sp

Potamonautidae

APPENDIX IV: SASS Score Sheet

African Journal of Aquatic Science 27:1-10.

SASS Version 5 Score Sheet

Version date: Sept 2005

Date (dd:mm:yr):			(dd.ddddd)	**Biotopes Sampled (tick & rate)**	**Rating (1 - 5)** / **Time (min)**
RHP Site Code:		Grid reference (dd mm ss.s) Lat:	S	Stones In Current (SIC)	
Collector/Sampler:		Long:	E	Stones Out Of Current (SOOC)	
River:		Datum (WGS84/Cape):		Bedrock	
Level 1 Ecoregion:		Altitude (m):		Aquatic Veg	
Quaternary Catchment:		Zonation:		MargVeg In Current	
	Temp (°C):	Routine or Project? (circle one)	Flow:	MargVeg Out Of Current	
Site Description:	pH:	Project Name:	Clarity (cm):	Gravel	
	DO (mg/L):	Thukela survey @ Mandini	Turbidity:	Sand	
	Cond (mS/m):		Colour:	Mud	
	Riparian Disturbance:	Limited - some subsistence farming		Hand picking/Visual observation	
	Instream Disturbance:	None			

Taxon	QV	S	Veg	GSM	TOT
PORIFERA (Sponge)	5				
COELENTERATA (Cnidaria)	1				
TURBELLARIA (Flatworms)	3				
ANNELIDA					
Oligochaeta (Earthworms)	1				
Hirudinea (Leeches)	3				
CRUSTACEA					
Amphipoda (Scuds)	13				
Potamonautidae* (Crabs)	3				
Atyidae (Freshwater Shrimps)	8				
Palaemonidae (Freshwater Prawns)	10				
HYDRACARINA (Mites)	8				
PLECOPTERA (Stoneflies)					
Notonemouridae	14				
Perlidae	12				
EPHEMEROPTERA (Mayflies)					
Baetidae 1sp	4				
Baetidae 2 sp	6				
Baetidae > 2 sp	12				
Caenidae (Squaregills/Cainfles)	6				
Ephemeridae	15				
Heptageniidae (Flatheaded mayflies)	13				
Leptophlebiidae (Prongills)	9				
Oligoneuridae (Brushlegged mayflies)	15				
Polymitarcyidae (Pale Burrowers)	10				
Prosopistomatidae (Water specs)	15				
Teloganodidae SWC (Spiny Crawlers)	12				
Tricorythidae (Stout Crawlers)	9				
ODONATA (Dragonflies & Damselflies)					
Calopterygidae ST,T (Demoiselles)	10				
Chlorocyphidae (Jewels)	10				
Synlestidae (Chlorolestidae)(Sylphs)	8				
Coenagrionidae (Sprites and blues)	4				
Lestidae (Emerald Damselflies/Spreadwings)	8				
Platycnemidae (Stream Damselflies)	10				
Protoneuridae (Threadwings)	8				
Aeshnidae (Hawkers & Emperors)	8				
Corduliidae (Cruisers)	8				
Gomphidae (Clubtails)	6				
Libellulidae (Darters/Skimmers)	4				
LEPIDOPTERA (Aquatic Caterpillars/Moths)					
Crambidae (Pyralidae)	12				

Taxon	QV	S	Veg	GSM	TOT
HEMIPTERA (Bugs)					
Belostomatidae* (Giant water bugs)	3				
Corixidae* (Water boatmen)	3				
Gerridae* (Pond skaters/Water striders)	5				
Hydrometridae* (Water measurers)	6				
Naucoridae* (Creeping water bugs)	7				
Nepidae* (Water scorpions)	3				
Notonectidae* (Backswimmers)	3				
Pleidae* (Pygmy backswimmers)	4				
Veliidae/M...veliidae* (Ripple bugs)	5				
MEGALOPTERA (Fishflies, Dobsonflies & Alderflies)					
Corydalidae (Fishflies & Dobsonflies)	8				
Sialidae (Alderflies)	6				
TRICHOPTERA (Caddisflies)					
Dipseudopsidae	10				
Ecnomidae	8				
Hydropsychidae 1 sp	4				
Hydropsychidae 2 sp	6				
Hydropsychidae > 2 sp	12				
Philopotamidae	10				
Polycentropodidae	12				
Psychomyiidae/Xiphocentronidae	8				
Cased caddis:					
Barbarochthonidae SWC	13				
Calamoceratidae ST	11				
Glossosomatidae SWC	11				
Hydroptilidae	6				
Hydrosalpingidae SWC	15				
Lepidostomatidae	10				
Leptoceridae	6				
Petrothrincidae SWC	11				
Pisuliidae	10				
Sericostomatidae SWC	13				
COLEOPTERA (Beetles)					
Dytiscidae/Noteridae* (Diving beetles)	5				
Elmidae/Dryopidae* (Riffle beetles)	8				
Gyrinidae* (Whirligig beetles)	5				
Haliplidae* (Crawling water beetles)	5				
Helodidae (Marsh beetles)	12				
Hydraenidae* (Minute moss beetles)	8				
Hydrophilidae* (Water scavenger beetles)	5				
Limnichidae (Marsh-Loving Beetles)	10				
Psephenidae (Water Pennies)	10				

Taxon	QV	S	Veg	GSM	TOT
DIPTERA (Flies)					
Athericidae (Snipe flies)	10				
Blepharoceridae (Mountain midges)	15				
Ceratopogonidae (Biting midges)	5				
Chironomidae (Midges)	2				
Culicidae* (Mosquitoes)	1				
Dixidae* (Dixid midge)	10				
Empididae (Dance flies)	6				
Ephydridae (Shore flies)	3				
Muscidae (House flies, Stable flies)	1				
Psychodidae (Moth flies)	1				
Simuliidae (Blackflies)	5				
Syrphidae* (Rat tailed maggots)	1				
Tabanidae (Horse flies)	5				
Tipulidae (Crane flies)	5				
GASTROPODA (Snails)					
Ancylidae (Limpets)	6				
Bulininae*	3				
Hydrobiidae*	3				
Lymnaeidae* (Pond snails)	3				
Physidae* (Pouch snails)	3				
Planorbinae* (Orb snails)	3				
Thiaridae* (=Melanidae)	3				
Viviparidae* ST	5				
PELECYPODA (Bivalvles)					
Corbiculidae (Clams)	5				
Sphaeriidae (Pill clams)	3				
Unionidae (Perly mussels)	6				
SASS Score					
No. of Taxa					
ASPT					
Other biota:					
Comments/Observations:					

9

Procedure:
Kick SIC & bedrock for 2 mins, max. 5 mins. Kick SOOC & bedrock for 1 min. Sweep marginal vegetation (IC & OOC) for 2m total and aquatic veg $1m^2$. Stir & sweep gravel, sand, mud for 1 min total. * = airbreathers
Hand picking & visual observation for 1 min - record in biotope where found (by circling estimated abundance on score sheet). Score for 15 mins/biotope but stop if no new taxa seen after 5 mins.
Estimate abundances: 1 = 1, A = 2-10, B = 10-100, C = 100-1000, D = >1000 S = Stone, rock & solid objects; Veg = All vegetation; GSM = Gravel, sand, mud SWC = South Western Cape, T = Tropical, ST = Sub-tropical
Rate each biotope sampled: 1=very poor (i.e. limited diversity), 5=highly suitable (i.e. wide diversity) Rate turbidity: V low, Low, Medium, High, Very High
Rate flows: Zero, trickle, low, medium, high, flood Rate colour: transparent, tea brown, light brown, dark brown, light green, dark green, yellow, red, grey, milky white, black

APPENDIX V: Macroinvertebrate Presence/Absence Data

Taxa			Land use types					
			Dry season			Wet season		
Order	**Family**	**Genus**	**Forest**	**Urban**	**Agriculture**	**Forest**	**Urban**	**Agriculture**
Arachnida	Angelenidae	*Angelena*	+	+	+	+	+	+
Annelida	Oligochaeta	*Tubifex*	-	+	-	+	+	+
Platyhelminthes	Tubellaria	-	+	-	-	+	-	-
Crustacea	Potamonautidae	*Potamonautes*	+	+	+	+	+	+
Crustacea	Aytidae	*Caridina*	+	-	-	+	-	-
Odonata	Coenagrionidae	*Pseudagrion*	+	+	+	-	+	+
Odonata	Gomphidae	*Crenigomphus*	+	+	+	+	-	+
Odonata	Libellulidae	*Trithemis*	+	+	+	+	+	+
Odonata	Gomphidae	*Ictinogomphus*	+	-	+	+	+	+
Odonata	Gomphidae	*Lestinogomphus*	+	-	+	-	-	-
Odonata	Gomphidae	*Paragomphus*	+	+	+	+	+	+
Odonata	Lestidae	*Lestes*	+	+	+	-	-	-
Odonata	Calopterygidae	*Phaon iridipennis*	+	-	+	-	-	+
Coleoptera	Elmidae	*Potamodytes*	+	+	+	+	-	+
Coleoptera	Seritidae	-	+	+	-	+	+	-
Coleoptera	Gyrinidae	*Orectogyrus*	+	+	-	+	+	-
Coleoptera	Gyrinidae	*Gyrinus sp*	-	+	-	-	+	-
Coleoptera	Hydrochidae	*Hydrochus*	+	+	+	-	-	+
Coleoptera	Elmidae	*Pseudancyronyx*	-	+	-	-	-	+
Diptera	Athericiade	-	+	+	+	+	-	+
Diptera	Tipulidae	*Tipula*	+	+	-	+	+	-
Diptera	Chironomidae	*Tanypodinae*	-	+	-	-	+	-
Ephemeroptera	Heptageniidae	*Afronurus*	+	-	-	+	-	+

Ephemeroptera	Dicercomyzidae	*Dicercomyzon*	+	-	+	-	-	+
Ephemeroptera	Trichorythidae	*Trychorythus*	+	-	+	+	+	+
Ephemeroptera	Baetidae	*Acanthiops*	+	-	+	-	-	-
Ephemeroptera	Oligoneuriidae	*Elassoneuria*	+	+	+	+	+	+
Hemiptera	Veliidae	*Rhagovelia sp*	-	-	+	+	-	+
Hemiptera	Belostomastidae	*Appasus sp*	+	+	-	-	-	+
Hemiptera	Saldidae	-	-	+	-	+	+	+
Hemiptera	Gerridae	*Neogerris*	+	+	-	-	-	+
Hemiptera	Nepidae	*Ranatra sp*	+	+	+	+	+	+
Tricoptera	Hydropsychidae	*Hydropsyche sp*	+	+	+	+	+	+
Plecoptera	Perlidae	*Neoperla sp*	+	-	-	+	-	-
Lepidoptera	Crambidae	*Nymphulinae*	+	+	-	-	-	-
Mollusca	Planorbidae	*Helisoma duryi*	+	+	-	-	+	-
Mollusca	Viviparidae	*Bellamya capillata*	+	+	+	-	+	+
Mollusca	Lymnaeidae	*Radix natalensis*	+	+	-	-	+	+
Mollusca	Bulinidae	*Bulinus africanus*	-	+	-	-	-	-
Mollusca	Pisidiidae	*Pisidium sp*	-	+	-	-	-	-