MACROINVERTEBRATE ASSEMBLAGES AND BODY SIZE DISTRIBUTION IN RESPONSE TO LAND-USE CHANGES IN HEADWATER STREAMS OF THE SONDU-MIRIU RIVER BASIN, KENYA

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DECLARATION

Declaration by the Candidate

This thesis is my original work and has never been presented for the award of an academic degree in any other university and should not be copied, or reproduced in any format without written authority from the author and/or University of Eldoret.

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Declaration by the supervisors

This thesis is submitted with our approval as University supervisors.

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DEDICATION

I dedicate this work to my lovely father and mother, Mr. Paul and Mrs. Miriam Kuluo for always believing in me and encouraging me throughout my life and study career and to my siblings for their continuous support and motivation.

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ABSRACT

Assemblages of aquatic macroinvertebrates have spatial and temporal variations in structure in response to environmental changes of their habitats, such as streams and rivers. Taxonomic approaches for monitoring the ecological status of aquatic ecosystems using macroinvertebrate assemblages face several limitations, creating a need for ataxonomic methods that are valid, disturbance-sensitive, and cost-effective for freshwater monitoring. This study evaluated the influence of seasonality and land-use changes on macroinvertebrate assemblage structure and water physico-chemical parameters of headwater streams of the Sondu-Miriu River basin, Kenya. Additionally, the study evaluated the utility of macroinvertebrate size-spectrum metrics and abundance-biomass comparison (ABC) curves as ataxonomic methods of evaluating land-use influence on the river's ecological condition. Macroinvertebrate taxonomic abundance (individuals/m^2), wet weight (mg), and physico-chemical variables were measured during the wet and dry seasons in March and September 2024, respectively, from 24 headwater streams distributed across four land-use types: natural forest (NF), tea and tree plantations (TTP), smallholder tea (SHT), and smallholder agriculture (SHA). Results based on water quality showed turbidity, total suspended solids (TSS), total dissolved solids (TDS), electrical conductivity (EC), particulate organic matter (POM), and nitrate (NO_3^--N) identified streams in NF as least disturbed and in SHA as most disturbed; TTP and SHT streams were intermediate in water quality. Taxon richness, diversity, and Ephemeroptera, Plecoptera, and Trichoptera (EPT) indices indicated land-use specific influences on assemblage structure. Redundancy analysis (RDA) showed that small-bodied taxa (<8 mg wet weight) were associated with high disturbance indicators, while large-bodied taxa (>32 mg) were associated with low disturbance indicators and sites. Slopes (λ) of normalized abundance- and biomass-based size-spectrum (a measure of trophic transfer efficiency) deviated from theoretical steady-state conditions (λ=-2.0 and -1.0, respectively), indicating that the sites were disturbed, but the slopes did not vary significantly between land uses or seasons, suggesting size-spectrum slopes had low sensitivity to land-use-based changes in water quality. In contrast, size-spectrum midpoint heights (a measure of ecosystem production) differed significantly between sites, highest at SHT and NF and lowest at SHA and TTP streams. Spectrum midpoint heights were, therefore, more responsive to disturbance than slopes, highlighting their potential as indicators of land-use influence on the Afrotropical streams. Although the ABC curves indicated undisturbed conditions for all sites, Warwick's (W) statistics revealed subtle differences among streams, suggesting variation in local-scale ecological conditions. This study demonstrated that catchment land use significantly influences water quality and macroinvertebrate assemblages in headwater streams of the Sondu-Miriu River basin. Traditional metrics (%EPT and diversity indices) are useful indicators of land use-based disturbance, while size-spectrum midpoint heights are potentially useful ataxonomic indicators of disturbance in the studied streams. It is recommended to integrate both community indices and functional metrics (especially midpoint height) into stream biomonitoring as complementary method for evaluating the ecological status of headwater streams. Management efforts should prioritize riparian buffers and nutrient/sediment control in SHA areas of the basin, while conserving less-impacted NF sites as reference areas. Long-term and broader spatial-scale studies are needed to validate the stability of size-spectrum metrics and ABC curves as rapid tools for monitoring the ecological status of headwater streams in response to anthropogenic influences.

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LIST OF ABBREVIATIONS

ABC - Abundance-biomass comparison

ANOVA - Analysis of variance

AUSRIVAS - Australian river assessment system

DCA - Detrended correspondence Analysis

DO - Dissolved oxygen

EC - Electrical conductivity

EPT - Ephemeroptera, Plecoptera and Trichoptera

FFGs - Functional Feeding Groups

FPOM - Fine particulate organic matter

GLM - Generalized Linear Modeling

H′ - Shannon-Weiner diversity index

HDPE - High-density polyethylene

LULC - Land-use and land cover change

M-IBI - Macroinvertebrate index of biotic integrity

MIBI - Macroinvertebrate-based index of biotic integrity

MMIS - Multimetric Indices

MSE - Mean square error

NASS – Normalized abundance size-spectrum

NBSS – Normalized biomass size-spectrum

POM - Particulate organic matter

RDA - Redundancy analysis

SASS - South African scoring system

SD - Standard deviation

SE - Standard error

SIGNAL - stream invertebrate grade number, average level

SRP - Soluble reactive phosphates

TDS - Total dissolved solids

TSS - Total suspended solids

TVHR - Transparent Velocity Head Rod

VIF - Variance Inflation Factor

W - Warwick's statistic

CHAPTER ONE

INTRODUCTION

1.1 Background information

Freshwater ecosystems such as streams and rivers are increasingly threatened by land-use and land cover changes that alter their ecological structure and functioning (Dudgeon *et al*., 2006; Reid *et al*., 2019). Intensifying anthropogenic pressures, especially from agriculture and urban expansion, have led to elevated nutrient and sediment loads, reduced water quality, and altered community structure and ecosystem functioning in tropical freshwater systems (Gucker *et al*., 2009; Fugère *et al*., 2018; Wanderi *et al*., 2022; Faria *et al*., 2024). Understanding how these changes influence aquatic species distribution, abundance, and trophic dynamics is crucial for effective monitoring and management of surface waters (Hansen and DeFries, 2007; Ramirez *et al*., 2008; Masese *et al*., 2023).

To evaluate anthropogenic and natural impacts on aquatic systems, taxonomic indices involving benthic macroinvertebrates have often been used due to their many inherent advantages, including sensitivity to a wide range of disturbances, site fidelity, and well-characterized life history attributes (Birk *et al*., 2012; Ruaro *et al*., 2020; Masese *et al*., 2024a). Macroinvertebrates exhibit a remarkable diversity of species, each with varying tolerance levels to environmental stressors, making them effective indicators of land-use effects such as water pollution, habitat degradation, altered hydrological regimes, and climate change (Sitati *et al*., 2021a, b). Presence of sensitive groups like Ephemeroptera, Plecoptera, and Trichoptera (EPT) often signal high water quality, but they decline in abundance under increased disturbance, while more tolerant taxa such as certain Diptera, Oligochaeta, and molluscs tend to dominate under degraded conditions (Masese *et al*., 2014a; Wijeyaratne and Bellanthudawa, 2018; Edegbene *et al*., 2022). Functional feeding groups (FFGs) also show distinct responses; for example, shredders are particularly impacted by riparian deforestation, while scrapers are vulnerable to sedimentation, and gatherers display greater resilience to organic enrichment (Masese *et al*., 2014a, b; Sitati *et al*., 2021a, b; Edegbene *et al*., 2022). Their low mobility ensures that macroinvertebrates reflect localized environmental conditions, enabling precise site-specific assessments and facilitating the detection of changes over time (Theodoropoulos *et al*., 2015). Furthermore, their relatively long lifespans allow them to integrate and reflect cumulative impacts, while variations in life history strategies and thermal tolerances provide additional insights into environmental change (Fierro *et al*., 2017). They are abundant, diverse, cost-effective to sample, and particularly valuable in regions where alternative bioindicators, such as fish, are scarce, making them an indispensable component of aquatic biomonitoring programs (Malacarne *et al*., 2016; Masese *et al*., 2023).

Despite their widespread application in biomonitoring, macroinvertebrate-based taxonomic indices have certain limitations. Structural measures, such as taxon richness, diversity indices, and taxonomic composition, may not effectively detect all types of ecological disturbances, particularly those linked to rural land-use and land cover changes (Malacarne *et al*., 2016). These metrics primarily capture changes in species counts and diversity, but may fail to reflect subtle or process-based alterations in ecosystems (Masese *et al.,* 2023). For instance, shifts in energy flow, organic matter quality, or the functional roles of species within food webs might remain undetected when only structural attributes are considered (Basset *et al*., 2004; Minaya *et al*., 2014; Masese *et al*., 2023; Owade *et al*., 2025). Most studies have routinely examined spatio-temporal variation in structural indices (Dalu *et al*., 2017) without relating to land use and land cover change influences.

To gain a more comprehensive understanding of how natural and human-induced disturbances affect rivers and streams, studies have integrated functional approaches alongside structural metrics, which include analyzing the distribution of trophic groups (Merritt and Cummins, 2006). Because they address species heterogeneity, redundancy, and body size structure, functional approaches can uncover ecological changes that may be overlooked by purely structural measures. Nonetheless, a key limitation is that many functional indices are developed for specific regions or ecological contexts, restricting their transferability without recalibration (Masese *et al*., 2023). Furthermore, taxonomic methods are often constrained by the lack of comprehensive identification keys, and they are both costly and time-consuming to implement (de Moor and Day, 2013). These challenges have hindered consistent and effective ecological monitoring of surface waters in East Africa (Ochieng *et al*., 2019; Kitaka *et al*., 2024) and other tropical regions (Sundar *et al*., 2020), underscoring the shortcomings of taxonomic-based approaches (Birk *et al*., 2012; Basset *et al*., 2012). These challenges highlight the need for complementary approaches that offer robust theoretical frameworks for understanding ecosystem processes in aquatic systems, particularly those systems that are data-scarce and vulnerable to human pressures (Guiét *et al*., 2016). The spatio-temporal variation in distribution of organismal abundance and body size is one such ataxonomic approach, providing a valuable means to examine the ecological responses of communities to environmental changes and anthropogenic stressors (Basset *et al*., 2012; Guiét *et al*., 2016). Additional non-taxonomic approaches include the use of Abundance Biomass Comparison (ABC) curves and K-dominance curves (Warwick, 1986), which assess how abundance and biomass are distributed among organisms within a community to help determine the disturbance status of aquatic ecosystems.

Empirical and simulation studies have consistently shown that aquatic communities tend to have roughly equal biomass distributed across logarithmically equal size categories of individuals (Sheldon *et al*., 1972). This consistent relationship between abundance or biomass and body size, usually represented on a log-log scale, is known as the size-spectrum (White *et al*., 2008; Trebilco *et al*., 2013). Size-spectrum analysis is a non-taxonomic approach that focuses on interactions among individual organisms within a community rather than on species identity (Gilljam *et al*., 2011). It provides a straightforward way to understand ecosystem functions, such as productivity and trophic transfer efficiency, by analyzing the shape of the size-spectrum, described through metrics like slope and intercept or midpoint height (Trebilco *et al*., 2013). This analysis can also serve as a framework for assessing the effects of disturbances and changes in ecosystem structure and function (Kerr and Dickie, 2001; Trebilco *et al*., 2013; Blanchard *et al*., 2014) without requiring species-level identification.

Alongside size-spectrum analysis, the ABC curve provides a useful complementary method for assessing how communities respond to environmental change. Originally developed by Warwick (1986), the ABC approach is commonly applied to evaluate the effects of environmental disturbances, especially pollution, on benthic invertebrate communities (Pagola-Carte, 2004). It works by comparing the cumulative distributions of species abundance and biomass within a community, allowing for comparisons across different disturbance levels and helping to determine the extent of disturbance based on patterns observed in community structure (Warwick *et al*., 1987; Warwick and Clarke, 1994; Wijeyaratne and Bellanthudawa, 2018). This study examined the responsiveness of macroinvertebrate assemblage structure to land-use changes, and evaluated the utility of community size-spectrum (abundance and biomass) metrics and ABC curves as indicators of ecological conditions in headwater streams affected by different land-use types in the Sondu-Miriu River basin, Kenya.

1.2 Statement of the problem

The Sondu-Miriu River basin is the fourth largest river draining into Lake Victoria on the Kenyan side The lake is the second largest freshwater body, by surface area, in the world (Okungu *et al*., 2005:Masese *et al*., 2012).). The river basin is exposed to intense land-use and land cover change (LULC) pressures resulting from widespread deforestation, agricultural expansion, and intensification, which increases nutrient and sediment loads into the river (Jacobs *et al*., 2017; 2024; Kroese *et al*., 2020a, b; Koech *et al*., 2023). The LULC change influences have been linked to spatial and seasonal shifts in macroinvertebrate community structure, reduced fish diversity, and compromised ecological integrity in river systems in Kenya (Masese *et al*., 2014a, b; Sitati *et al*., 2021a, b; Achieng *et al*., 2021). Monitoring of the ecological status of rivers and streams have often used the spatio-temporal variation in macroinvertebrate assembalges (Leung *et al*., 2012). Benthic macroinvertebrates are widely used in biomonitoring because they are diverse, sensitive to a range of disturbances, relatively immobile, and cost-effective to sample (Birk *et al*., 2012; Ruaro *et al*., 2020; Masese *et al*., 2023). They effectively indicate land-use effects such as pollution, habitat alteration, and climate change, with sensitive groups (e.g., Ephemeroptera, Plecoptera, Trichoptera) declining under disturbance, and tolerant taxa (e.g., Diptera, Oligochaeta) dominating in degraded conditions (Masese *et al*., 2014a; Sitati *et al*., 2021a; Edegbene *et al*., 2022). However, traditional taxonomic indices, while useful, mainly capture species richness and diversity and may fail to detect subtle ecosystem changes such as shifts in energy flow or functional roles (Basset *et al*., 2004; Masese *et al*., 2023; Owade *et al*., 2025). This limitation highlights the need for complementary approaches like macroinvertebrate size-spectrum and ABC curves, which integrate structural and functional aspects of communities and provide insights into ecosystem processes that taxonomic measures alone may overlook.

1.3 Justification of the study

Based on the results of modelling and field studies, size-spectrum metrics have been used to study the influence of perturbations and exploitation on diverse communities in predominantly temperate aquatic groups, including marine fishes (Rice and Gislason 1996; Bianchi *et al*., 2000), riverine fishes (Fabré *et al*., 2017; McGarvey *et al*., 2019), plankton communities (San Martin *et al*., 2006), and lotic macroinvertebrates (Jennings *et al*., 2002; Martínez *et al*., 2016). However, the paucity of similar studies in tropical aquatic systems, and especially in river systems (Achieng *et al*., 2020), has made it difficult to evaluate the responsiveness and utility of size-spectrum metrics in monitoring and managing tropical freshwater ecosystems. These systems are often data-scarce (Achieng *et al*., 2023) and would benefit immensely from simple and pragmatic frameworks such as size-spectrum metrics (see section 3 for details). This study, therefore, aimed to test the responsiveness of size-spectrum metrics, and ABC curves as alternative and cost-effective tools for monitoring tropical rivers and streams.

The use of ABC curves to assess disturbance effects on aquatic ecosystems is well supported by their proven ability to detect environmental stress through shifts in community structure (Warwick, 1986; Appiah *et al*., 2020). A key advantage of the ABC approach is its internal control mechanism, which removes the requirement for external reference sites (Warwick *et al*., 2025). This feature enhances its applicability across a broad range of ecosystems impacted by different land-use practices (Putro *et al*., 2015; Guerrero *et al*., 2022).

Validation of size-spectrum and ABC methodapproaches has the potential to provide a robust, cost-effective, complementary framework for evaluating the ecological integrity of freshwater systems and can facilitate management and conservation strategies of these ecosystems and similar data-scarce freshwater ecosystems in the tropics.

1.3 Study objectives

1.3.1 General objective

To assess spatio-temporal variation in macroinvertebrate assemblages, and the responsiveness of body size distribution to land-use changes in headwater streams in the Sondu-Miriu River basin in Kenya.

1.3.2 Specific objectives

i. To evaluate the spatio-temporal variation of physico-chemical variables and macroinvertebrate assemblage structure.

ii. To determine the influence of physico-chemical variables on macroinvertebrates' abundance per size class.

iii. To evaluate the responsiveness of macroinvertebrate abundance and biomass size-spectrum metrics to land-use changes.

iv. To evaluate the usefulness of Abundance-Biomass Comparison (ABC) curves as indicators of land-use change effects.

1.4 Hypotheses

H_0: There are no significant spatio-temporal variations in physico-chemical variables or macroinvertebrate assemblage structure in the headwater streams of the Sondu-Miriu River basin.

H_0: Physico-chemical variables have no significant influence on macroinvertebrates' abundance per size class in the headwater streams of the Sondu-Miriu River basin.

H_0: Macroinvertebrate abundance and biomass size-spectrum metrics do not respond significantly to land-use changes in the headwater streams of the Sondu-Miriu River basin.

H_0: Abundance-biomass comparison (ABC) curves are not useful indicators of land-use change effects in the headwater streams of the Sondu-Miriu River basin.

CHAPTER TWO

LITERATURE REVIEW

2.1 Influence of land-use and seasonality on river physico-chemical variables

The physicochemical characteristics of streams arise from a dynamic interplay between natural processes and human activities (Tang *et al*., 2020; Brauns *et al*., 2022; Masese *et al*., 2024b). At broad spatial scales, climate plays a major role in shaping rainfall patterns, runoff, flow volume and permanence, vegetation cover, water temperature, and evaporation rates (Hynes, 1975; Schwarzenbach *et al*., 2010; Lei *et al*., 2014; Michalak, 2016). Geological formations and soil types also influence water chemistry through processes like weathering, solute leaching, infiltration, and runoff (He *et al*., 2023). Hydrological pathways, including both surface runoff and subsurface flow, serve as essential channels for the transport of pollutants and the modification of water chemistry (Jacobs *et al*., 2018). Elevation affects streams both directly and indirectly by influencing climate, geology, vegetation structure, geomorphology, and flow velocity (Masese *et al*., 2024c). In addition, stream size or order (e.g., Strahler order) is a key factor determining the physical and biological characteristics of a system, including how materials originate, move, and are distributed within it (Strahler, 1957; Vannote *et al*., 1980; Wanderi *et al*., 2022).

Land-use changes such as agriculture (Minaya *et al*., 2013; Masese *et al*., 2017; 2021; Jacobs *et al*., 2018; Kroese *et al*., 2020), urban expansion (Elame *et al*., 2023), and livestock grazing (Masese *et al*., 2020; Iteba *et al*., 2021) are major contributors to changes in hydrology, sediment buildup, and nutrient enrichment within river systems. Additional human activities, including wastewater discharge, chemical pollution, dam construction, flow regulation, and excessive water abstraction, further disturb natural flow patterns and interfere with the downstream movement of materials (Winton *et al*., 2019; 2021). At the local scale, in-stream activities like livestock watering, laundry, and bathing can cause short-term spikes in turbidity, nutrient levels, and organic matter (Hamid *et al*., 2020; Iteba *et al*., 2021; Masese *et al*., 2024c). Seasonal variability, particularly the distinct wet and dry periods characteristic, adds an important temporal layer to these dynamics. In the wet season, heavier rainfall and increased runoff accelerate the mobilization and transport of sediments and dissolved substances, often producing marked shifts in water quality (Masese *et al*., 2023).

Land-use change alters the generation, mobilization, and delivery of sediments, nutrients, and organic matter to rivers and streams. Understanding these processes requires a landscape-scale perspective that accounts for the types and proportions of land-use within a catchment, together with its topographic, climatic, and riparian characteristics. Streams draining agricultural areas often exhibit elevated concentrations of nutrients, particularly nitrogen and phosphorus, along with higher total dissolved solids (TDS), turbidity, and electrical conductivity (EC) (Subalusky *et al*., 2018; Kroese *et al*., 2020; Iteba *et al*., 2021; Masese *et al*., 2021; 2024c). These patterns are mainly influenced by fertilizer use, soil disturbance, and the loss of vegetation cover (Jacobs *et al*., 2017; 2018; Nyilitya *et al*., 2020). Reduced riparian shading can also increase water temperatures, which accelerates microbial activity and the breakdown of organic matter, often resulting in oxygen depletion (Minaya *et al*., 2013; Masese *et al*., 2020). In many instances, the conversion of forested or natural land into agricultural fields causes significant erosion, leading to higher suspended sediment loads. Nutrient levels, especially nitrate, ammonium, and soluble reactive phosphorus (SRP), tend to rise due to both fertilizer application and the leaching of nutrients from the soil (Jacobs *et al*., 2018; 2024). The extent of agricultural impacts also depends on management practices such as tillage intensity, crop rotation, and the state of riparian buffers. Well-maintained riparian zones, for example, can trap sediments and nutrients before they reach streams, playing a key role in reducing these impacts (Minaya *et al*., 2013; Sanaullah *et al*., 2020; Kadeka *et al*., 2021).

Forested catchments, especially those found in headwater regions, are generally linked to better water quality (Zhu *et al*., 2019; Sitati *et al*., 2021a, b; Shah *et al*., 2022). Dense forest canopies limit solar radiation, helping to keep water temperatures lower and maintain higher levels of dissolved oxygen (Vannote *et al*., 1980). Streams in these areas usually have low turbidity, minimal suspended solids, and little nutrient enrichment because of reduced disturbance and stable soils (Jacobs *et al*., 2018; Shah *et al*., 2022). They also receive larger amounts of coarse particulate organic matter (CPOM) and high-quality dissolved organic matter (DOM), both essential for supporting diverse aquatic life (Masese *et al*., 2014a, b; Johnson, 2017; Sitati *et al*., 2021b). When forests are converted to agricultural or urban land, these important functions often decline, disrupting nutrient cycling and reducing carbon inputs (Maitama et al., 2009; Seixas *et al*., 2025).

Urbanization places some of the greatest pressures on stream ecosystems and often leads to the poorest water quality conditions. Urban streams tend to have higher temperatures, greater electrical conductivity, and elevated nutrient concentrations, along with lower dissolved oxygen levels (Blaszczak *et al*., 2019; Sitati *et al*., 2021a, b; Brauns *et al*., 2022).

Pollutants such as heavy metals, hydrocarbons, and synthetic organic compounds frequently enter these systems through surface runoff, leaking sewage, and stormwater discharges (Sitati *et al*., 2021a; Masese *et al*., 2024b). The widespread presence of impervious surfaces increases runoff and reduces infiltration, which heightens erosion and leads to rapid, fluctuating flow patterns (Minaya *et al*., 2013; Mwaijengo *et al*., 2020). These hydrological and chemical changes not only reduce water quality but also disturb biological communities and impair ecosystem functions (Violin *et al*., 2011; Sitati *et al.,* 2021a).

Livestock grazing and activities in savanna grasslands similarly affect stream physico-chemistry, particularly in areas where large mammalian herbivores (LMH) such as cattle and hippopotamus are present (Masese *et al.*, 2017; Iteba *et al.*, 2021; Wanderi *et al.*, 2022). Trampling, overgrazing, and unrestricted livestock access to streams increase sediment input, turbidity, and the concentration of major ions and nutrients such as ammonium and phosphorus (Iteba *et al*., 2021). Excretion and egestion by large mammalian herbivores (LMH) directly add organic matter and nutrients to the water, often leading to localized oxygen depletion and changes in primary production (Mallin *et al*., 2006). These conditions can encourage algal blooms and alter the base of the aquatic food web, with cascading effects on macroinvertebrate and fish communities (Mallin *et al*., 2006).

Catchments with mixed land uses generally exhibit intermediate water quality, reflecting the relative influence of each land cover type (Masese *et al*., 2015; Sitati *et al.,* 2021a, b; Wanderi *et al*., 2022). For example, a catchment that includes both agricultural and forested areas may have nutrient and sediment levels higher than those in pristine forest streams but lower than in intensively farmed systems (Kändler *et al*., 2017; Sitati *et al*., 2021a). The spatial arrangement and proximity of different land uses to the stream, particularly at the reach scale, often determine the extent and nature of their impact on water quality. In such cases, effective riparian management becomes crucial for maintaining and moderating in-stream conditions (Minaya *et al*., 2013; Sanaullah *et al*., 2020; Kadeka *et al*., 2021).

Seasonal changes also influence the relationship between land use and stream physico-chemistry. During the wet season, rainfall and runoff enhance the movement and delivery of sediments, nutrients, and organic matter to streams (Muñoz-Villers and McDonnell, 2012; Dewey *et al*., 2020). This typically results in higher concentrations of total suspended solids (TSS), turbidity, and soluble nutrients, especially in catchments dominated by agriculture or urban development (Chua *et al*., 2009; Ouma *et al.,* 2016). In contrast, during the dry season, streamflow is sustained mainly by groundwater and base flow, making water quality more dependent on local geology and in-channel processes (Jacobs *et al.,* 2018; Masese *et al*., 2024c). In rural areas, human and livestock activity in streams often increases during the dry season when alternative water sources are limited, leading to localized declines in water quality (Yillia *et al*., 2008a, b; Iteba *et al*., 2021). Conversely, in the wet season, the availability of harvested rainwater reduces direct dependence on streams for domestic and livestock use, temporarily easing pressure on these water systems (Yillia *et al*., 2008a, b).

2.2 Influence of land-use change and seasonality on macroinvertebrate assemblage structure

Land-use change exerts a significant influence on the structural and functional, composition, of macroinvertebrate assemblages in freshwater ecosystems (Dalu and Masese, 2025; Owade *et al.*, 2025). Anthropogenic pressures alter stream water quality, in-stream habitat complexity, and food resource availability, ultimately reshaping macroinvertebrate communities (Dudgeon *et al.*, 2006; Raburu *et al.*, 2009; Lubanga *et al.*, 2021; Sitati *et al.*, 2021a, b). The Ephemeroptera, Plecoptera, and Trichoptera (EPT) orders are among the most sensitive taxa, with richness and abundance typically declining under conditions of pollution and habitat disturbance (Masese *et al.*, 2014a; b). However, some EPT taxa, such as members of the Baetidae, Caenidae, and Hydropsychidae, are capable of persisting in organically enriched or seasonally disturbed systems due to adaptive life-history traits (Yegon *et al.*, 2021; Sitati *et al.*, 2024). Other macroinvertebrate groups, such as Coleoptera, Odonata, Diptera, and Oligochaeta, also show distinct responses to land-use change. Coleoptera are moderately sensitive and play varied ecological roles, functioning as shredders, predators, and other feeding types (Masese *et al.,* 2024a). Odonata, which rely on both aquatic and terrestrial habitats, respond strongly to alterations in riparian vegetation and in-stream structure (Clausnitzer *et al*., 2012). Their numbers often rise with disturbance, reflecting their adaptability and predatory behavior. Diptera, especially Chironomidae, along with Oligochaeta, are tolerant to pollution and tend to dominate in heavily disturbed environments, as they can survive in low-oxygen and organically enriched conditions (Masese *et al*., 2014a).

Land-use practices affect macroinvertebrate communities at both the catchment and reach scales. Deforestation reduces canopy cover, raises stream temperatures, and decreases leaf litter inputs, which directly impacts shredder guilds and taxa sensitive to temperature changes (Masese *et al.,* 2014a). Agricultural activities contribute nutrients, sediments, and agrochemicals to aquatic systems, degrading habitat quality and favoring generalist collector-gatherers and scrapers (Lubanga *et al*., 2021). Urbanization introduces major hydrological and chemical disturbances, often leading to biotic homogenization and the dominance of tolerant taxa (Masese *et al*., 2021; Sitati *et al*., 2021a, b). Interestingly, predator richness may increase in such settings, as seen in the higher abundance of Odonata within disturbed habitats (Sitati *et al*., 2024a, b).

The impacts of land use are further influenced by seasonal dynamics. During the wet season, macroinvertebrate abundance typically increases due to enhanced flow and improved habitat connectivity, although nutrient and sediment levels also rise, especially in agricultural and urban catchments (Mwaijengo *et al*., 2020; Masese *et al.,* 2024b). In contrast, the dry season is marked by reduced flow, lower oxygen availability, and higher water temperatures, which place added stress on sensitive taxa and lower overall diversity. These seasonal patterns underscore the importance of conducting multi-seasonal assessments to accurately evaluate how land-use changes affect aquatic invertebrate communities.

2.3 Macroinvertebrates as bioindicators of stream and river health

Two complementary approaches are widely used to assess stream and river conditions through invertebrates. Taxonomic approach, identifies organisms to determine species richness or diversity, and supports the development of metrics such as the Index of Biotic Integrity (IBI) (Karr and Chu, 1999). The second functional approach focuses on the ecological roles of species (Cummins and Merritt, 2005). Taxonomic methods can be applied qualitatively, based on the presence or absence of indicator species, or quantitatively, measuring their abundance or diversity (Basset *et al.*, 2008).

Benthic macroinvertebrates have long been recognized as reliable indicators of water quality (Hynes, 1959), and their effectiveness has been confirmed in many regions. This has led to the development of a variety of biotic indices, each adapted to local conditions. Examples include the biological monitoring working Party (BMWP) score system in Europe (Bonada *et al.*, 2006), the hilsenhoff biotic index in North America (Rosenberg and Resh, 1993; Barbour *et al.*, 1999), the benthic macroinvertebrate index of biotic integrity (BM-IBI) in South America (Buss and Vitorino, 2010), the South Korean Biotic Index in Asia (Hartmann *et al.*, 2010), In Australia, the stream invertebrate grade number, average level (SIGNAL) and the Australian river assessment system (AUSRIVAS) are widely used in (Chessman, 1995, 2003), the Macroinvertebrate Community Index in New Zealand (Stark, 1993), and systems such as South African scoring system (SASS) and the macroinvertebrate index of biotic integrity (M-IBI) in Africa (Dickens and Graham, 2002; Masese *et al.*, 2009a; Masese and Raburu, 2017; Masese *et al.*, 2023).

Macroinvertebrates are widely regarded as key bioindicators in freshwater ecosystems due to their varied responses to multiple short-term environmental stressors, making them valuable for assessing stream and river health (Rosenberg and Resh, 1993; Barbour *et al.*, 1999; Birk *et al.*, 2012; Ruaro *et al.*, 2020). Their largely sedentary behavior enables spatial assessment of pollutants (Hellawell, 1986), and their relatively long life cycles allow evaluation of temporal changes caused by disturbances. Their broad distribution across streams and rivers, coupled with the ease of sampling and relatively low cost of equipment, further supports their practicality for bio assessment (Rosenberg and Resh, 1993; Barbour *et al.*, 1999; Bonada *et al.*, 2006). In the region, where fish diversity is comparatively low, macroinvertebrates serve as the primary organisms for biomonitoring headwater streams (Masese *et al.*, 2023).

Although diversity, richness, biotic, and multimetric indices are widely applied in Africa, they face limitations in assessing ecological conditions under multiple stressors (Masese *et al.*, 2023). Many have not been standardized or tested beyond the regions where they were developed (Masese *et al.*, 2023), which restricts their broader applicability. Furthermore, the absence of legal provisions in most African countries for incorporating biological criteria into national surface water quality assessments hampers the development of standardized biomonitoring protocols (Masese *et al.*, 2023). While indices such as SASS and Multimetric Indices (MMIs) have been successfully applied in some regions without modification, their unvalidated use across diverse ecosystems can introduce bias and uncertainty in accurately reflecting ecological health (Achieng *et al.*, 2021).

2.4. Size-spectrum applications

Body size is a fundamental trait in ecology that correlates with metabolic rate, trophic level, and energy transfer across food webs (Brown *et al.*, 2004). Size-based approaches, particularly community size-spectrum analysis, have gained prominence as tools for understanding ecological structure and function (Bianchi *et al.*, 2000; Basset *et al.*, 2004;

Marin *et al*., 2023). The size-spectrum, typically expressed as the relationship between the abundance or biomass of organisms and their body size on a log-log scale, reflects the underlying energetic and trophic dynamics within ecosystems (Sheldon *et al*., 1972; Sprules and Barth, 2015). The slope of the size-spectrum is generally negative, indicating a decrease in abundance with increasing body size. This parameter reflects the efficiency of energy transfer from smaller to larger organisms, with steeper slopes often suggesting reduced energy flow or the removal of top predators (Jennings and Blanchard, 2004; Trebilco *et al*., 2013). The intercept or midpoint height typically corresponds to overall community biomass or productivity and increases with higher resource availability, such as from nutrient enrichment (Murry and Farrell, 2014). Deviations from linearity in the size-spectrum are increasingly viewed as indicators of ecosystem stress or alteration (Arranz *et al*., 2019). Size-spectrum approaches are particularly useful in ataxonomic contexts, where detailed species identification is challenging. They bypass taxonomic resolution and allow for standardized comparisons across ecosystems and stressors (Petchey and Belgrano, 2010). This makes them powerful complementary tools to traditional biomonitoring, especially in regions like the Afrotropics, where taxonomic expertise and resources may be limited.

Size-spectrum offer several benefits: they are ataxonomic, cost-effective, scalable, and rooted in robust theoretical frameworks such as metabolic theory (Brown *et al*., 2004). They integrate information from individuals to whole communities, reflecting ecosystem-level processes such as energy flux, productivity, and disturbance responses (Woodward *et al*., 2005; Martínez *et al*., 2016). Despite their promise, challenges remain. Methodological inconsistencies in data binning, variable sensitivity across taxa, and limited mechanistic understanding of cumulative stressor effects constrain broader application (Riseng *et al*., 2011).

2.5. Influence of land-use and seasonality on macroinvertebrate size-spectrum

Changes in forest cover significantly affect the macroinvertebrate size-spectrum by modifying the quantity and quality of available food resources. A reduction in allochthonous inputs (e.g., leaf litter) resulting from deforestation leads to lower biomass of large detritivores and steeper size-spectrum slopes (Martinez *et al*., 2016; Estévez *et al*., 2020). Conversely, intact riparian zones maintain diverse feeding guilds and wider size distributions through consistent detrital inputs and stabilized temperature regimes (Gregory *et al*., 1991; Sweeney, 1993). Macroinvertebrate communities may show compensatory changes in size structure; for instance, omnivores can adjust their density without altering body size, whereas detritivores may display body size reductions through taxonomic replacement (Estévez *et al*., 2020). These variations influence trophic transfer efficiency and can impact the resilience of stream food webs.

Land-use changes associated with agriculture, urbanization, and mining strongly modify stream ecosystems through elevated nutrient inputs, sedimentation, pesticide runoff, hydrological alterations, flow regime changes, and habitat fragmentation (Walsh *et al*., 2005; Jacobs *et al*., 2018; Sitati *et al*., 2021a, b; Oltramare *et al*., 2023). Such disturbances affect macroinvertebrate community structure, frequently decreasing size diversity and shifting biomass toward smaller taxa (Martínez *et al*., 2016). Rising nutrient inputs can raise the intercept, or midpoint, of the size-spectrum, signifying increased primary productivity and higher consumer biomass (Achieng *et al*., 2020). In subtropical streams, urbanization has been associated with steeper size-spectrum slopes, which reflect the decline of larger taxa and shifts in trophic transfer (Benejam *et al*., 2018). Progress in size-based indicators, including size diversity, together with image-based high-throughput methods, is promoting broader use of size-spectrum approaches in macroinvertebrate monitoring programs.

Mining activities significantly alter the size distribution of macroinvertebrates. Streams that are heavily affected often become dominated by small, metal-tolerant species and lose many of their large predators, resulting in shallower slopes that resemble those found in detritivore-dominated communities (Pomeranz *et al*., 2019). These structural changes can be difficult to identify using traditional taxonomic methods but are clearly revealed through size-spectrum analysis. Recent studies in stream environments have linked seasonal shifts in size-spectrum to variations in water temperature and flow patterns (McGarvey *et al.,* 2019). In contrast, research in transitional systems such as Mediterranean and Black Sea lagoons has shown little seasonal variation, with size distributions and functional guilds remaining fairly stable throughout the year (Barbone *et al*., 2012; Gjoni *et al*., 2019).

Size-spectrum are becoming reliable indicators of macroinvertebrate community structure. They serve as a valuable complement to traditional taxonomic assessments and can offer early warnings of human-induced impacts, including those linked to land-use change. As environmental stressors become increasingly complex, incorporating size-based approaches into regular macroinvertebrate monitoring, especially in regions with limited data, provides a promising path for improving the assessment and management of streams and rivers.

2.6 Abundance-Biomass Comparison (ABC) curve

The Abundance Biomass Comparison (ABC) method, first introduced by Warwick (1986) and further developed by Clarke and Warwick (2001), is a graphical approach for detecting and interpreting environmental disturbance in aquatic ecosystems. It uses K-dominance plots to compare ranked cumulative abundance and biomass curves, revealing shifts in community structure and ecological condition. The method is grounded in *r/k* selection theory, which links species life-history strategies to environmental stability. In undisturbed systems, communities are generally dominated by *k*-selected species, large-bodied, slow-growing, and long-lived, resulting in the biomass curve lying above the abundance curve (Sprules & Munawar, 1986; Xu *et al.*, 2021). Disturbed systems, in contrast, are often dominated by *r*-selected species, small-bodied, fast-growing, and opportunistic, producing an abundance curve above the biomass curve (Warwick and Clarke, 1994; Sabbeel and Vanreusel, 2015). Moderately disturbed systems often show intersecting or closely aligned curves (Wijeyaratne and Bellanthudawa, 2018). The degree and direction of curve separation can be quantified using Clarke's Warwick (W) statistic, which ranges from +1 (undisturbed) to -1 (heavily disturbed), with values near zero indicating intermediate disturbance (Yemane *et al.*, 2005).

The ABC method has been applied across diverse aquatic systems. In coastal lagoons such as the Ebrié Lagoon, it has detected disturbance gradients linked to agricultural runoff, untreated sewage, and industrial effluents (Appiah *et al.*, 2020). In Mediterranean rivers, ABC curves have reflected the impacts of urban sewage, agricultural waste, and industrial emissions (Guerrero *et al.*, 2022). Studies in Brazilian streams have differentiated rural from urban disturbance patterns, revealing nutrient enrichment effects invisible to simple visual inspection (Mise *et al.*, 2018). Other applications include hyporheic zones (Di Lorenzo *et al.*, 2022), wetlands (Wijeyaratne & Bellanthudawa, 2018), and aquaculture-impacted estuaries (Tran *et al.*, 2018; Putro *et al.*, 2015; Table 1).

Several features make the ABC method particularly suitable for tropical headwater streams. Its internal control design compares abundance and biomass distributions within the same sample, removing the need for pristine reference sites (Tweedley *et al.*, 2015; Das *et al.*, 2021). The method is also adaptable to varying taxonomic resolutions; although originally developed for species-level data, family, or genus-level analyses can yield similar results, offering cost savings in resource-limited settings (Guerrero *et al.*, 2022). Its proven application in tropical systems such as Sudanese mangroves and Brazilian reservoirs supports its transferability (Sabbeel and Vanreusel, 2015).

However, interpretation requires ecological context. Dominance by large-bodied or exotic species can mask disturbance signals, while natural stressors such as organic matter inputs or hydrodynamic variability can mimic pollution effects (Yemane *et al.*, 2005). Sampling effort must be sufficient to capture dominant biomass species but avoid over representing rare, large individuals (Dauer *et al.*, 1993). Combining ABC outputs with knowledge of local species ecology, statistical indices, and complementary tools such as size-spectrum improves reliability and ecological relevance (Mise *et al.*, 2018; Di Lorenzo *et al.*, 2022).

Table 1: Global applications of the abundance-biomass Comparison (ABC) methods, highlighting ecosystem types, primary stressors, taxa studied, and key findings from various aquatic environments.

Ecosystem / Location	Main Stressors	Taxa Studied	Key Findings	References
Ebrié Lagoon, Côte d'Ivoire	Agricultural runoff, untreated sewage, industrial effluents	Benthic macrofauna	Biomass curves above abundance curves in low-disturbance zones; reversed pattern in heavily impacted areas	Appiah *et al.*, 2020
Mediterranean rivers	Urban sewage, agricultural waste, industrial emissions	Macroinvertebrates	ABC curves reflected disturbance gradients; intersecting curves in moderately disturbed sites	Guerrero *et al.*, 2022
Brazilian streams and reservoirs	Nutrient enrichment, rural vs. urban land-use	Macroinvertebrates	Rural streams showed higher biomass dominance; urban streams had abundance curves above biomass curves	Mise *et al.*, 2018
Hyporheic zones, Italy	Flow regulation, sedimentation	Benthic invertebrates	Detected shifts in community structure not evident through traditional indices	Di Lorenzo *et al.*, 2022
Wetlands, Sri Lanka	Agricultural runoff, organic enrichment	Aquatic invertebrates	Patterns revealed subtle impacts of nutrient enrichment	Wijeyaratne and Bellanthudawa, 2018
Aquaculture-impacted estuaries, Vietnam & Indonesia	Organic loading from aquaculture	Benthic macrofauna	Biomass dominance in less impacted sites; abundance dominance in impacted sites	Tran *et al.*, 2018; Putro *et al.*, 2015
Sudanese mangroves	Organic loading, habitat alteration	Benthic fauna	Demonstrated sensitivity of ABC to tropical mangrove disturbances	Sabbee*l* and Vanreusel, 2015

The ABC approach represent robust, theory-driven indicators for assessing ecological disturbance. Its incorporation into Afrotropical headwater stream monitoring can yield nuanced insights into shifts in community structure, particularly in contexts where pristine reference conditions are absent. When applied alongside complementary metrics and interpreted within appropriate ecological contexts, these tools can enhance the accuracy and depth of freshwater bioassessment.

CHAPTER THREE

METHODOLOGY

3.1. The Study Area

The study was conducted in 24 headwater streams in the Chemosit sub-basin, which forms part of the 3451 km^2 Sondu-Miriu River Basin (SMRB), Kenya (Figure 1), during the wet season (March 2024) and the dry season (September 2024). The headwater streams originate from a key Afromontane Forest, the South-West block within the greater Mau Forest Complex, an important water tower in Kenya (UNEP 2008). Four dominant land-use types characterize the catchment and riparian zones: Natural Forest (NF), Smallholder Agriculture (SHA), Smallholder Tea (SHT), and Tea and Tree plantations (TTP) (Jacobs *et al*., 2018a). The rainfall regime is bimodal, with long rains from April to July and short rains from October to December (Koech *et al*., 2023). The study area receives an average annual rainfall of approximately 1,752 mm (Jacobs *et al*., 2018). Temperatures range from approximately 16 °C in the headwaters to 24 °C in the lower elevations. The Chemosit sub-basin is part of the upper Sondu-Miriu catchment, where streams are mostly first- to third-order according to Strahler's classification (1957). The area has a population density of about 300 persons/km^2 and faces increasing pressures from land-use and land-cover change, including deforestation, agricultural expansion, and grazing, which contribute to elevated sediment and nutrient loads in streams (Weeser *et al*., 2018; Jacobs *et al*., 2017; Kroese e*t al*., 2020a, b). Geologically, the upper areas are dominated by phonolitic nephelinites interspersed with Tertiary tuffs (Jennings, 1971), while the lower areas are mainly characterized by phonolites (Binge, 1962). The predominant soils are humic Nitisols (ISRIC, 2007).

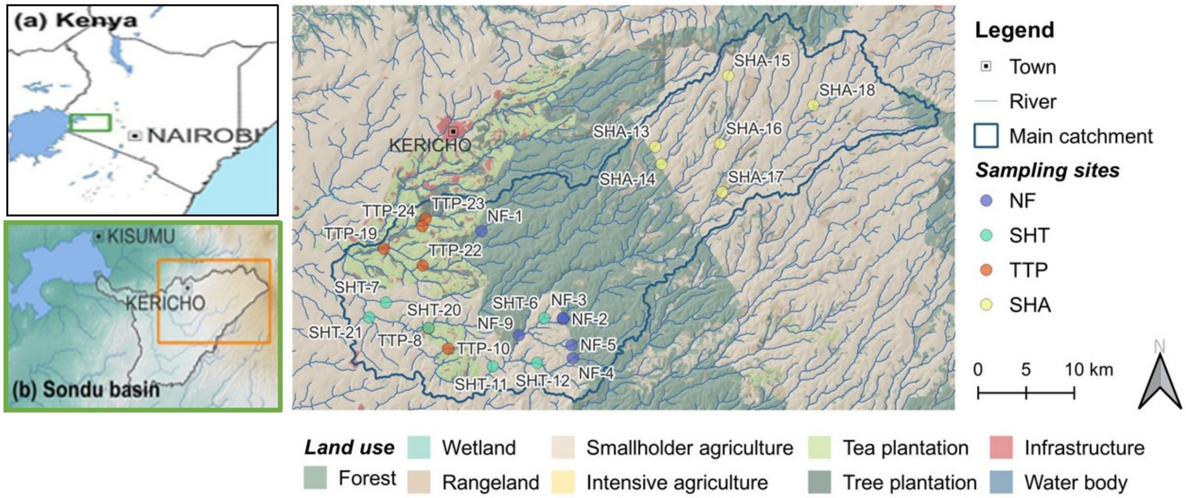

Figure 1: Location of the study area in the south-west Mau region, Kenya, showing: (a) the map of Kenya; (b) the Sondu-Miriu River basin; and (c) land-use distribution in the Chemosit sub-basin, which forms part of the Sondu-Miriu River Basin (SMRB), derived from Landsat imagery (2013; swart, 2016).: NF = natural forest, SHT = smallholder tea, TTP = tea and tree plantations, and SHA = smallholder agriculture.

3.2 Study design

To capture the influence of land-use effects on river macroinvertebrate assemblage structure and physico-chemical characteristics, the Chemosit sub-basin was divided into four groups of study sites, each corresponding to the four land-use types in the basin as: natural forest (NF), smallholder tea (SHT), smallholder agriculture (SHA), and tea and tree plantations (TTP) (Figure 1, Plate 1). The studied headwater streams drained each of the four land-use types, with six individual streams selected per land-use, giving a total of 24 streams sites. The characteristics of each of the four land-use types forming sampling sites are summarized in Table 2.

Table 2: Summary of the characteristics of land-use categories and streams in headwater in the Sondu-Miriu River basin sampled in this study. Stream locations are as shown on Figure 1 and Plate 1.

Land-use	Stream sites	Stream-order	Elevation (m.a.s.l.)	Location(Latitude, Longitude)	Land-use features
NF	1	5	2173	0.46404° S, 35.30833° E	Streams draining catchments in predominantly natural forest; reference sites
	2	5	2227	0.54660° S, 35.38455° E	
	3	5	2229	0.54541° S, 35.38452° E	
	4	5	2105	0.58323° S, 35.39364° E	
	5	5	2073	0.57090° S, 35.39258° E	
	6	5	1967	0.56153° S, 35.34323° E	
SHA	7	3	2368	0.38461° S, 35.47060° E	Streams in areas with smallholder agriculture of small farms of up to 2 ha and mixed subsistence farming (maize, beans, cabbage, and potatoes). Riparian zones are often degraded.
	8	3	2428	0.40105° S, 35.47644° E	
	9	3	2579	0.31801° S, 35.53888° E	
	10	3	2441	0.38171° S, 35.53128° E	
	11	3	2395	0.42727° S, 35.53336° E	
	12	3	1564	0.34556° S, 35.61846° E	
SHT	13	4	2040	0.54570° S, 35.36689° E	Streams draining smallholder tea catchments. Riparian zones are often degraded due to water fetching and animal watering points.
	14	4	1821	0.53096° S, 35.21884° E	
	15	4	2127	0.59103° S, 35.31897° E	
	16	4	2174	0.58755° S, 35.36027° E	
	17	4	2127	0.55485° S, 35.25880° E	
	18	4	2007	0.54507° S, 35.20312° E	
TTP	19	4	1883	0.55530° S, 35.25875° E	Streams in catchments with commercial tea and tree plantations, approximately 20,000 ha. In this catchment, the commercial tea is alternated with eucalyptus and cypress.
	20	4	1973	0.57436° S, 35.27696° E	
	21	4	1778	0.48052° S, 35.21682° E	
	22	4	1882	0.49627° S, 35.25323° E	
	23	4	1901	0.45235° S, 35.25602° E	
	24	4	2125	0.45928° S, 35.25260° E	

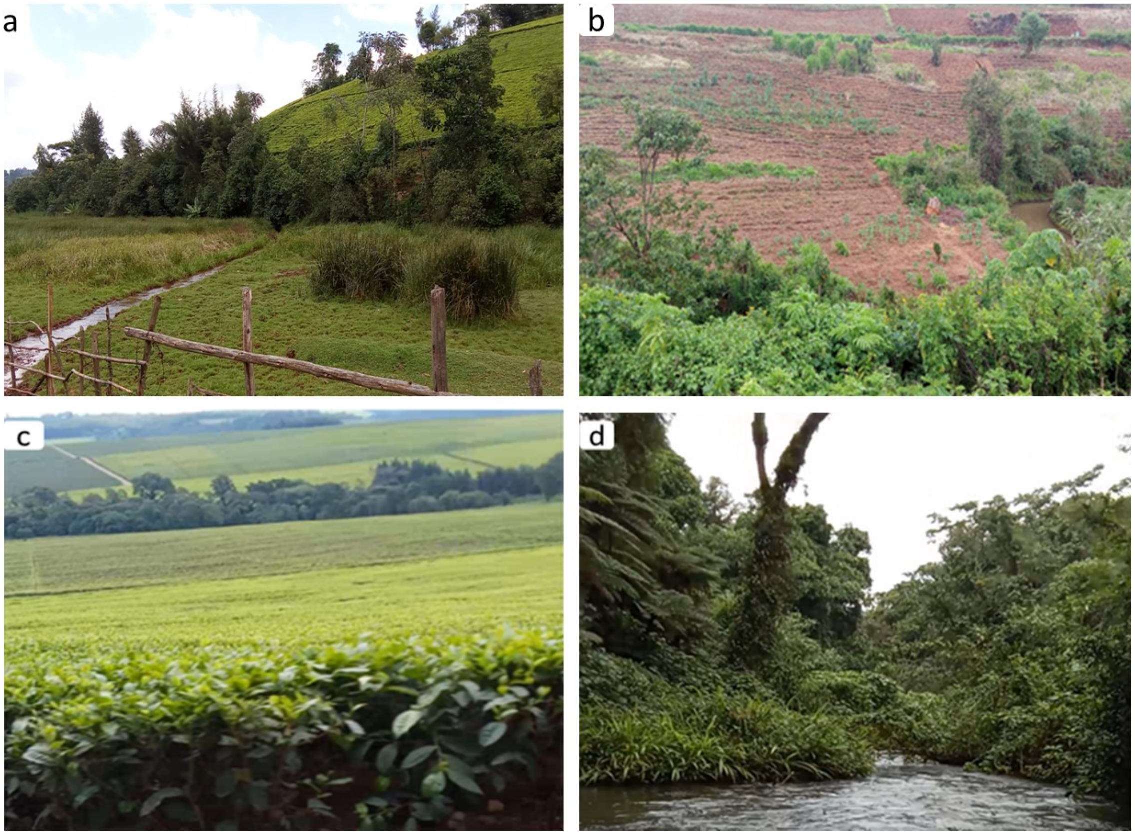

Plate 1: Land-use types in the headwater stream of the Sondu-Miriu River basin: (a) stream flowing through smallholder tea (SHT), (b) stream flowing through disturbed smallholder agriculture (SHT), (c) commercial tea and tree plantations (TTP) with blocks of eucalyptus plantations and dense riparian woodlands, (d) stream flowing through natural forest (NF).

3.3 Field sampling

For dissolved nutrients, water samples were collected using a bucket from the deepest and fastest-flowing part of the stream (the thalweg) and subsequently filtered using GF/F filters (Whatman, 0.70 µm pore size, 47 mm in diameter). Similarly, pre-weighed GF/F filters were used to filter known volumes of water samples for determination of total suspended solids (TSS) and particulate organic matter (POM). Filtered water samples were stored in 500 ml High-density polyethylene (HDPE bottles and placed in a cooler box (4 °C) and transported to the laboratory, where they were analyzed for soluble reactive phosphorus (SRP), ammonium (NH_4+-N), nitrate (NO_3^--N), and nitrite (NO_2^--N). Unfiltered water samples were also collected from the sampling sites and stored in 150 ml HDPE bottles for analysis of total phosphorous (TP). Water physical quality parameters, including temperature, dissolved oxygen (DO), pH, electrical conductivity (EC), and total dissolved solids (TDS), were measured directly in the field using a multi-probe water quality meter (Multi 3420, WTW GmbH, Weilheim, Germany). Turbidity was also measured on-site with a portable meter (HACH LANGE GmbH, Berlin, Germany). All measurements were taken in triplicate along a 100-meter stream reach at each sampling site prior to collecting macroinvertebrate samples. Stream width was recorded at 10 transects spaced 10 meters apart along the study reach. At each transect, water depth was measured at a minimum of five evenly spaced points across the stream channel using a 1-meter ruler. Flow velocity was then measured at the same points using a Transparent Velocity Head Rod (TVHR, Water Research Commission, 2017). Using the velocity-area technique, stream discharge (m^3/s) was then determined (Wetzel and Likens, 2000).

At each of the four land-use sites, macroinvertebrate sampling was carried out along a randomly selected longitudinal stretch or stream reach of about 100 m using a semi-quantitative kick-net sampling method (Dickens and Graham, 2002). Along the 100 m stream reach of the six streams per site, the following biotopes were identified and sampled to obtain four replicate samples per biotope: gravel, sand and mud (GSM; standing water in pools and backwaters), stones (bedrock, boulders, cobbles, and pebbles) in and out of the current; marginal vegetation (submerged and emergent marginal vegetation); and leaf packs or coarse particulate organic matter (Dickens and Graham, 2002; Masese *et al.*, 2024a). The sampling method involved disturbing the riverbed (approximately 1 m^2) upstream while holding a South African Scoring System (SASS) kick net (500-µm mesh size) in place for about 60 seconds per biotope, allowing dislodged macroinvertebrates to be carried into the net by the water currents (Dickens and Graham, 2002). Large substrate types such as boulders and cobbles were hand-picked and washed into the net. To minimize the effects of physical disturbance while kicking-sampling and thus trigger macroinvertebrate drift, sampling of the four biotopes started at the downstream end of the identified study reach and progressed upstream (Dickens and Graham, 2002; Masese *et al.*, 2023). Macroinvertebrates were sorted in the field and preserved in labelled vials (site, stream number, replicate number, date) using 75 % ethyl ethanol and stored in a cooler box for transportation to the laboratory for further processing.

3.4 Laboratory analysis

Macroinvertebrate samples were identified to the lowest taxonomic level possible (mainly genus), using taxonomic keys (Fry *et al.*, 2021; de Moor *et al.*, 2003a, b; Stals and de Moor, 2007) and counted. Subsequently, their wet mass was measured using an analytical balance (Sartorius Secura 124-1S; 0.001 g). The concentration of SRP in the water samples was determined using the ascorbic acid method (APHA, 2005), while TP was measured following acid persulfate digestion and analyzed using the same method. The NH_4^+-N was analyzed using the salicylate-isocyanurate method (APHA, 2005). Nitrite (NO_2^--N) and nitrate (NO_3^--N) were both measured colorimetrically following the APHA (2005) protocols, with NO_3^--N determined after the cadmium reduction method. For TSS and POM, GF/F filters with embedded sediments were oven-dried at 60 °C for 48 hours to achieve a constant weight. The filters were then weighed to determine TSS gravimetrically (APHA, 2005). The TSS concentration (mg L^{-1}) was calculated using the equation (APHA, 2005):

$$TSS = \left(\frac{A-B}{V}\right) * 10^6 \text{.....................Equation 1}$$

Where, A is the combined mass of the filter and dried residue (g), B is the mass of the clean dry filter (g), and V is the volume of water filtered (L). Subsequently, the filters were combusted at 450 °C for 4 hours in a muffle furnace to remove organic matter. The filters were then weighed again, and POM was calculated as the difference between TSS and the ash-free dry mass. The concentration of POM (mg L^{-1}) was obtained using (APHA, 2005):

$$POM = \left(\frac{C-B}{V}\right) * 10^6 \text{.................. Equation 2}$$

Where B is the dry mass of the filter (g), C is the mass of the ashed filter (g), and V is the volume of the sample filtered (L).

3.5 Size-spectrum analysis

Size-spectrum can be defined as the variation of a community attribute with body size (Rice and Gislason, 1996). To model the abundance (individuals per square meter, ind./m^2) and biomass (milligrams per square meter, mg/m^2) size-spectrum of macroinvertebrate communities at each site, macroinvertebrate replicate samples were pooled and individuals assigned to logarithmic size-classes based on body wet mass (in mg) (Sheldon *et al.*, 1972; Edward *et al.*, 2017). Logarithmic binning was used because it maintains consistent resolution across the size range, allowing equitable comparison of the distribution of small-and large-bodied organisms (Blanco *et al.*, 1994; Sprules and Barth, 2016). A total of 13 size classes were defined, ranging from ≤2 mg (the smallest size class) to 4096-8192 mg (the largest size class). Macroinvertebrate abundance and biomass per size-class were first normalized by dividing each biomass or abundance in the size- class by the width of the size-class interval in order to minimize the bias associated with logarithmically increasing size-class width or weights (White *et al.*, 2008). Normalized abundance and biomass values were then $\log_2$-transformed and regressed against the $\log_2$-centered midpoints of the respective size classes using least-squares linear regression to give the normalized abundance and normalized biomass size-spectrum. This double-logarithmic approach is widely used in aquatic food web studies to quantify the slope (λ) and intercept of the size-spectrum (White *et al.*, 2008; Sprules and Barth, 2016), which describes the relationship between size and either biomass or abundance as:

$$\mathrm{Log}_2(y) = a\,\mathrm{Log}_2(x) + b \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{Equation 3}$$

where: x = body size (mg), y = normalized biomass or abundance, a = slope (λ), b = intercept.

In size-spectrum analysis, slope and intercept are often statistically correlated, which complicates the interpretation of each parameter independently. To address this, the correlation was reduced by using a midpoint height (“H”) defined as the normalized abundance or biomass at the center of the spectrum - instead of the traditional y-intercept (Daan *et al.*, 2005). Midpoint height serves as a robust proxy for total community production or richness, and facilitates unbiased comparisons of community structure across spatial and temporal scales (Daan *et al.*, 2005).

Modelling and field studies have determined the normalized size-spectrum slope (λ) of communities at equilibrium (undisturbed) to approximate -1.0 and -2.0 for normalized biomass- and abundance-based size-spectrum, respectively (White *et al*., 2008; Sprules and Barth, 2016). The steepening of the size-spectrum slope (λ) (being more negative) in time or space has been hypothesized to reflect relative dominance of small-bodied organisms due to greater size-dependent mortality leading to a lower trophic transfer efficiency in the ecosystem while Shallower λs are taken to represent communities with larger-sized individuals (Rice and Gislason, 1996). Intercepts or midpoint heights of the size-spectrum are taken to reflect productivity of the community, with lower intercepts/midpoint heights taken to reflect an overall reduction in the food-web capacity or productivity of a community (Guiét *et al*., 2016).

Abundance Biomass Comparison (ABC) curves were constructed by superimposing k-dominance curves of abundance and biomass on the same plot. A k-dominance curve shows the cumulative abundance or biomass of species as a function of their rank (Clark and Warwick, 2001). To construct the k-dominance curves, all identified genera were first arranged in descending order based on their relative importance, which was determined from their abundance and biomass values separately (Lambshead and Platt, 1985; Clark and Warwick, 2001; Warwick *et al*., 2008). The genus contributing the most individuals or biomass occupied the top rank, followed sequentially by those with lesser contributions. For each genus in the ranked list, the proportional dominance was calculated as the percentage share of the total abundance or total biomass of the assemblage. These proportional values were then cumulatively summed, beginning with the most dominant genus and progressing through the ranks, to yield the cumulative proportional dominance, or “k-dominance,” for each genus rank (Warwick *et al*., 2008). The resulting data for biomass and abundance were plotted on the same plot with genus rank (k) along the horizontal axis, represented on a logarithmic scale to compress lower-ranked genera, and cumulative proportional abundance or biomass (expressed as percentages from 0% to 100%) along the vertical axis (Lambshead *et al*., 1983; Clarke, 1990). The relative positions of the two curves were then examined to assess community disturbance levels. This method follows the approach described by Warwick (1986), where curve separation provides insight into the dominance structure and the ecological status of the community. The ecological status of each site was interpreted based on the relative positions of the biomass and abundance curves as; i) Undisturbed condition: The biomass curve lies entirely above the abundance curve, suggesting dominance by a few large-bodied genera, ii) Moderately disturbed condition: The biomass and abundance curves intersect or run closely together, indicating an intermediate level of disturbance, and iii) Grossly disturbed condition: The abundance curve lies entirely above the biomass curve, typically indicating dominance by small-bodied macroinvertebrates (Warwick, 1986). To quantify the difference between the abundance and biomass curves, Warwick’s W-statistic (Warwick, 1986) was calculated using the formula:

$$W = \left(\Sigma\,(\mathrm{Bi} - \mathrm{Ai})\right) \div \left(50 * (S - 1)\right) \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{Equation 4}$$

Where:

- B_i = cumulative percentage biomass for genus i

- A_i = cumulative percentage abundance for genus i

- S = total number of genus

A positive W-statistic indicates the biomass curve is above the abundance curve reflecting unstressed community while, a negative W-statistic indicates the abundance curve is above the biomass curve reflecting a stressed community. A W-statistic near zero value obtains when the curves overlap or intersect, indicating a moderately disturbed community (Warwick, 1986).

3.7 Data treatment and statistical analyses

Physico-chemical stream variables were first tested for normality of distribution using the Kolmogorov-Smirnov test and for homogeneity of variances using Levene’s test (Zar, 2010). As variables violated the assumptions of normality, land-use comparisons were conducted using the non-parametric Kruskal-Wallis analysis of variance (ANOVA) test, followed by Dunn’s *post hoc* test for pairwise differences among means (Zar, 2010) to evaluates the effects of land use on water physical-chemico parameters. Generalized linear models (two-way ANOVA) were used to evaluate the effects of land-use, season, and their interaction on water physico-chemical variables. All variables were $\log_{10}(x+1)$-transformed to meet model assumptions. Macroinvertebrate order-level relative abundances were calculated per site and season, summarized by land-use, and plotted to visualize dominant assemblage patterns.

To evaluate the macroinvertebrate community structure across sites and land-use types, several community structural indices were calculated. These included: taxon richness, Simpson's index of diversity (1-D), and Shannon-Weiner diversity index (H′, Magurran, 2013). The percentage of Ephemeroptera + Plecoptera + Trichoptera individuals in a sample (%EPT index) was calculated for each site as an indicator of disturbance, with a higher index reflecting a low disturbance regime (Barbour *et al*., 1996). The EPT orders are considered to be among the most sensitive among macroinvertebrates to different forms of disturbance in streams and rivers (Barbour *et al*., 1999).

Detrended Correspondence Analysis (DCA) was first conducted to determine the appropriate ordination method for analyzing the relationship between macroinvertebrate assemblage structure and physico-chemical variables. The DCA produced short gradient lengths (<3 SD units), indicating linear species–environment relationships. Based on these results, Redundancy Analysis (RDA) was selected as the most suitable method to examine the associations between macroinvertebrate communities and physico-chemical parameters during the wet and dry seasons (Ter Braak, 1986). Sequential generalized linear modeling (GLM; Zar, 2010) was used to evaluate the contribution of predictor variables, including body size, season, land-use, and water physico-chemical variables, to macroinvertebrate abundance and biomass per size class. Model performance was assessed using mean square error (MSE) and the coefficient of determination (R^2) (Neter *et al*., 1996). Multi-collinearity among water quality variables was examined using the Variance Inflation Factor (VIF) (Zurr *et al*., 2007). Only variables with VIF values less than 5, considered the threshold for low collinearity, were retained in the model. Water quality variables were entered into the model as a single block in the final step before testing interaction terms.

Analysis of covariance (ANCOVA) was applied to assess the effects of land use, seasonality, and their interactions on the slope and midpoint height of the normalized abundance and biomass size spectrum, as well as Warwick's W-statistics. Linear regression analyses were conducted to examine the relationships between size-spectra metrics (slope and midpoint height) and log-transformed water physico-chemical variables and community structural indices. All statistical analyses and figure plots were carried out in R version 4.5.1 (R Core Team, 2025) using the vegan package (Oksanen *et al*., 2013).

CHAPTER FOUR

RESULTS

4.1 Variation of physico-chemical variables of streams between land-use and seasons

Both land-use type and seasonality influenced the physico-chemical properties of the studied streams (Table 3). Turbidity, TDS, EC, POM, and NO_3^--N varied significantly among streams in different land-use types. Smallholder agriculture (SHA) generally showed higher values of the physico-chemical variables in both wet and dry seasons. In contrast, natural forest (NF) stream sites recorded relatively lower values for most variables, including turbidity, EC, TDS, and TSS, considered indices of water pollution in the study area (Table 3). Total suspended solids (TSS) exhibited substantial variability across land-uses and seasons ($p < 0.05$). In the wet season, TSS concentrations were highest in SHA streams (103.80 ± 24.03 mg/L), followed by NF streams (86.50 ± 6.85 mg/L), and lowest in SHT streams (60.50 ± 3.66 mg/L). In the dry season, TSS values were consistently lower across all land uses but remained significantly elevated in SHA streams (28.46 ± 6.15 mg/L), compared to TTP (14.93 ± 3.88 mg/L), SHT (13.86 ± 0.44 mg/L), and the lowest in NF (8.35 ± 0.45 mg/L). This sharp seasonal contrast underscores the influence of rainfall and land-use practices on sediment loading, with SHA consistently contributing the highest suspended solids load across both hydrological periods.

Similar to TSS, the concentrations of POM were highest at SHA streams (wet: 98.10 ± 15.20; dry: 16.82 ± 2.71 mg/L) and lowest in SHT streams during the wet season (58.96 ± 6.26 mg/L) and NF streams during the dry season (6.09 ± 0.40 mg/L).

Table 3: Mean (±SD) variation of physico-chemical variables in the different land-use categories in the Sondu-Miriu River basin. Turb = turbidity, Temp = temperature, Natural Forest (NF), Smallholder Agriculture (SHA), Smallholder Tea (SHT), and Tea and Tree Plantation (TTP). The rest of the abbreviations and acronyms for variables are described in the text. H = Kruskal-Wallis ANOVA test statistic.

Variables	Season	NF	SHA	SHT	TTP	H	p-value
Turb (NTU)	Wet	10.0±3.9^{a}	52.4±34.2^{b}	16.4±23.8^{ab}	24.1±31.6^{ab}	9.69	0.020*
	Dry	8.75±0.37^{a}	53.00±10.30^{b}	24.17±7.19^{ab}	22.11±7.47^{ab}	11.51	0.009*
Temp (° C)	Wet	17.2±0.49^{a}	17.40±0.94^{a}	18.93±0.46^{a}	17.52±0.28^{a}	5.83	0.120
	Dry	16.02±0.54^{a}	16.35±0.42^{a}	17.47±0.53^{a}	17.12±0.41^{a}	5.09	0.166
DO (mg/l)	Wet	7.51±0.13^{a}	6.61±0.45^{a}	7.17±0.13^{a}	7.55±0.11^{a}	6.89	0.076
	Dry	7.65±0.16^{a}	7.08±0.18^{b}	7.37± 0.18^{a}	7.69±0.11^{a}	7.29	0.063
DO saturation (%)	Wet	97.85±0.84^{a}	89.65±5.56^{a}	96.20±1.91^{a}	103.67±4.92^{a}	3.74	0.291
	Dry	98.45±1.16^{a}	96.87±1.40^{a}	96.52±1.89^{a}	97.80±0.91^{a}	0.38	0.945
TDS (mg/l)	Wet	27.95±0.43^{b}	58.30±14.30^{a}	37.00±1.48^{ab}	47.67±3.52^{ab}	13.34	0.004*
	Dry	28.50±0.56^{c}	67.00±7.14^{a}	38.83±2.33^{bc}	47.17±4.03^{b}	18.51	0.0003*
pH	Wet	7.07±0.16^{a}	7.36±0.47^{a}	6.46±0.15^{a}	7.19±0.70^{a}	6.44	0.092
	Dry	7.43±0.53^{a}	7.71±0.45^{a}	6.58±0.14^{a}	7.77±0.68^{a}	6.37	0.095
EC (µS/cm)	Wet	27.85±0.46^{b}	58.10±14.40^{a}	36.88±1.53^{ab}	47.53±3.57^{ab}	13.43	0.004*
	Dry	28.55±0.53^{c}	65.10±7.16^{a}	36.78±3.16^{bc}	47.23±3.97^{b}	16.82	0.0008*
TSS (mg/l)	Wet	91.8±0.46^{a}	140.70±24.30^{a}	80.38±9.48^{a}	134.50±26.40^{a}	4.97	0.174
	Dry	13.73±0.97^{b}	48.89±7.88^{a}	21.18±5.54^{bc}	30.17±6.61^{ab}	11.61	0.009*
POM (mg/l)	Wet	75.67±7.58^{ab}	98.10±15.20^{a}	58.96±6.26^{b}	75.82±5.61^{ab}	8.21	0.042*
	Dry	6.09±0.40^{b}	16.82±2.71^{a}	8.68±1.73^{b}	10.95±1.88^{b}	12.03	0.007*
TP (mg/l)	Wet	0.07±0.02^{a}	0.22±0.06^{a}	0.21±0.10^{a}	0.14±0.04^{a}	4.43	0.218
	Dry	0.02±0.001^{a}	0.04±0.01^{a}	0.03±0.003^{a}	0.03±0.01^{a}	5.86	0.119
SRP (µg/l)	Wet	0.03±0.003^{a}	0.05±0.03^{a}	0.07±0.06^{a}	0.02±0.01^{a}	0.39	0.942
	Dry	0.02±0.001^{a}	0.02±0.003^{a}	0.02±0.002^{a}	0.02±0.003^{a}	4.57	0.206
NH_4^+-N (µg/l)	Wet	2.66±0.23^{a}	1.83±0.64^{a}	3.52±1.02^{a}	2.72±0.83^{a}	2.04	0.564
	Dry	0.01±0.002^{a}	0.02±0.006^{a}	0.01±0.001^{a}	0.01±0.001^{a}	6.98	0.072
NO_2^--N (mg/l)	Wet	0.93±0.58^{a}	1.21±0.19^{a}	0.84±0.42^{a}	0.62±0.32^{a}	5.94	0.115
	Dry	0.03±0.01^{a}	0.02±0.004^{a}	0.03±0.01^{a}	0.08±0.04^{a}	3.37	0.338
NO_3^--N (mg/l)	Wet	0.42±0.09^{b}	0.82±0.12^{b}	1.52±0.65^{ab}	3.31±0.89^{a}	6.33	0.001*
	Dry	0.04±0.01^{b}	0.03±0.01^{b}	0.07±0.01^{b}	0.19±0.04^{a}	6.98	0.001*
Depth (m)	Wet	0.17±0.01^{b}	0.36±0.04^{a}	0.34±0.05^{ab}	0.24±0.06^{ab}	9.15	0.027
	Dry	0.16±0.02^{b}	0.21±0.02^{ab}	0.31±0.04^{a}	0.20±0.05^{ab}	6.40	0.094
Width (m)	Wet	5.52±1.09^{a}	4.02±1.16^{a}	4.10±1.65^{a}	3.95±1.03^{a}	1.56	0.668
	Dry	5.35±2.70^{a}	3.17±1.87^{a}	3.93±4.27^{a}	3.63±1.82^{a}	3.05	0.383
Discharge (m^3/s)	Wet	0.29±1.82^{a}	0.12±0.15^{a}	0.20±0.27^{a}	0.12±0.13^{a}	1.41	0.702
	Dry	0.05±0.02^{a}	0.08±0.06^{a}	0.06±0.09^{a}	0.05±0.06^{a}	3.05	0.623

NB: Groups with different superscripts differ significantly, as determined by Dunn *post hoc* tests. * indicate significant differences among land-uses at $p < 0.05$.

During the wet season, the NF streams also recorded high POM concentration (75.67 ± 7.58 mg/L) that did not differ from the highest value reported in SHA streams. Concentrations of NO_3^--N were highest in the TTP streams during both seasons (wet:

3.31 ± 0.89 mg/L; dry: 0.19 ± 0.04 mg/L), likely due to intensive fertilizer application in commercial tea plantations. In contrast, lower values were recorded in NF streams during the wet season (0.42 ± 0.09 mg/L) and in SHA during the dry season (0.03 ± 0.01 mg/L, Table 2). The EC, often an indicator of water pollution when elevated (increases in dissolved ions), was highest at the SHA streams (wet: 58.10±14.40 μS/cm; dry: 65.10±7.16 μS/cm) and lowest in the NF streams (wet: 27.85±0.46 μS/cm; dry: 28.55±0.53 μS/cm), with the SHT and TTP streams showing intermediate values (Table 3).

Following GLM (two-way ANOVA) analysis, a significant effects of land use were observed for several variables, including Turbidity, TSS, POM, and NO3--N, as also noted in (Table 3). Seasonal variation also significantly affected Temperature ($F = 6.72$, $p = 0.012$), TSS ($F = 11.35$, $p < 0.001$), POM ($F = 11.62$, $p < 0.001$), Discharge ($F = 6.72$, $p = 0.012$), TP ($F = 12.47$, $p < 0.001$), NH4+-N ($F = 15.44$, $p < 0.001$), NO2--N ($F = 20.33$, $p < 0.001$), and NO3 -N ($F = 26.01$, $p < 0.001$). A significant interaction between land use and season was detected for NO3--N ($F = 6.80$, $p = 0.001$), indicating that seasonal shifts in NO_3^--N concentrations varied depending on land use type.

4.2 Macroinvertebrate community composition

Macroinvertebrate assemblage composition based on relative abundance varied across land-use, with few taxa dominating assemblage composition (Figure 2). In NF land-use, the most sensitive orders to water pollution and disturbance - Trichoptera, Ephemeroptera, and Plecoptera - recorded high relative abundances (mean ± SD%) in both seasons (wet: 27.4 ± 4.4, 25.2 ± 4.6, and 15.4 ± 4.4; dry: 30.3 ± 2.6, 22.1 ± 3.6, and 22.5 ± 4.5, respectively) potentially reflecting relatively good water quality at the forested streams that were less influenced by human activities during both dry and wet seasons. In contrast, Hirudinea, Spongillida, and Platyhelminthes were the least represented orders at all land-use sites. The SHA land-use showed higher relative seasonal abundance of Ephemeroptera and Diptera (wet: 38.5 ± 7.1 and 29.9± 12.1; dry: 28.1 ± 4.8 and 34.4 ± 8.9, respectively), while Plecoptera, Spongillida, and Hirudinea contributed lower percentages.

In SHT land-use, Ephemeroptera and Trichoptera orders recorded high relative seasonal abundance (mean ± SD%: wet: 29.9 ± 7.9 and 27.0 ± 5.1; dry: 23.6 ± 6.9 and 26.0 ± 4.9), whereas Hirudinea, Spongillida, and Nematomorpha were among the least abundant (Figure 2). Plecoptera had lower relative abundance at SHT and SHA land-use sites. The TTP land-use showed relatively high proportions of Trichoptera, Ephemeroptera, and Plecoptera, (mean ± SD %: wet: 30.6 ± 8.7, 24.5 ± 8.3, and 23.2 ± 6.7; dry: 29.9 ± 5.9, 10.5 ± 3.1 and 17.1 ± 3.0%) closely resembling the structure of the NF land-use, while Nematomorpha and Hirudinea remained low in both seasons (Figure 2). Groups like Diptera and Coleoptera showed relatively high abundance at all land-use sites.

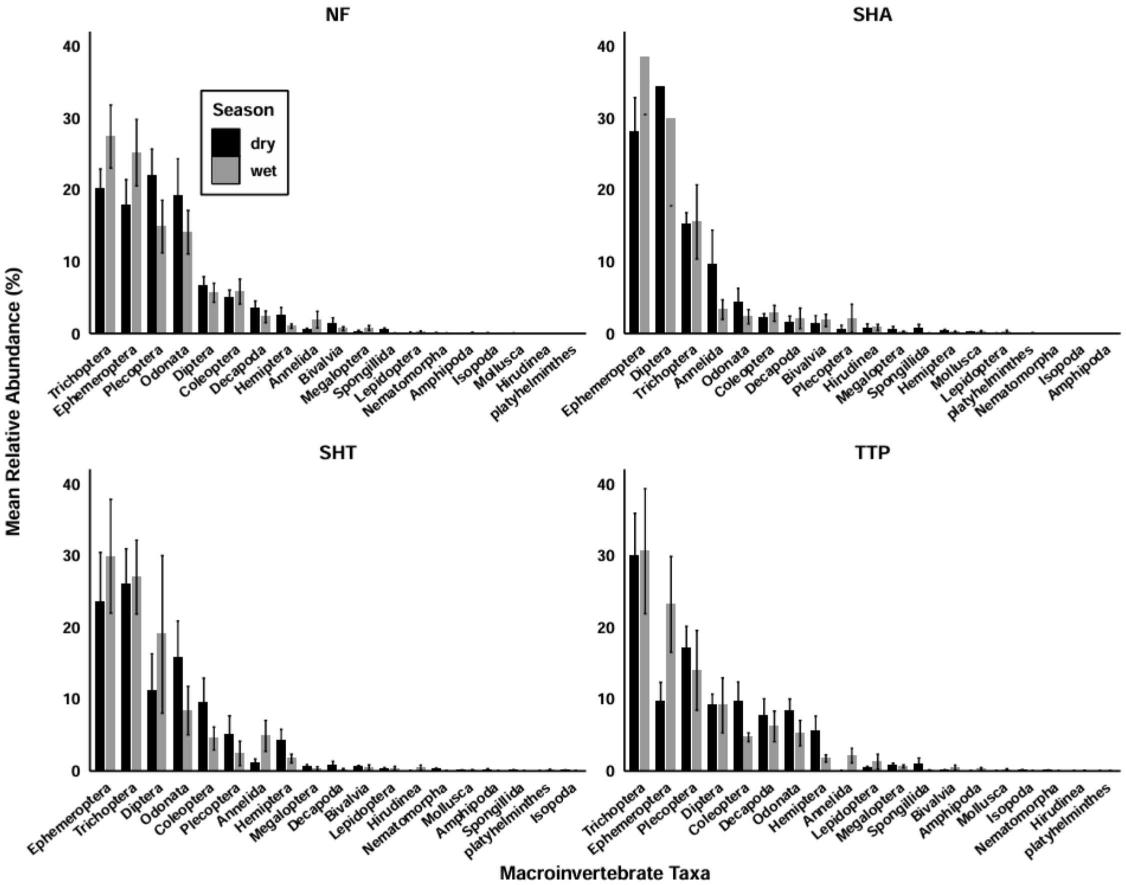

Figure 2: Mean seasonal relative abundance (%) of macroinvertebrate taxa across four land-use streams in the Sondu-Miriu River basin during wet and dry seasons: Natural Forest (NF), Smallholder Agriculture (SHA), Smallholder Tea (SHT), and Tea and Tree Plantation (TTP). Error bars represent standard error of the mean.

Structural community indices, including taxon richness, Simpson's index of diversity (1-D), and the Shannon-Weiner diversity index (H′), varied across land-uses and seasons (Table 4). During the wet season, differences among land uses were not statistically significant ($p > 0.05$). Nevertheless, SHA land-use recorded the lowest values for taxon richness, H′ and %EPT whereas, NF and TTP exhibited comparatively higher values for these metrics. In the dry season, significant variation was observed in the indices among land-uses. Taxon richness was highest in SHT land-use (50.2 ± 8.8) and lowest in SHA land-use (30.0 ± 6.5) while, Simpson's index of diversity (1 - D) was lowest in SHA land-use (0.78 ± 0.12), while NF, TTP, and SHT land-uses had similar higher values (0.88 ± 0.03, 0.88 ± 0.05, and 0.88 ± 0.05, respectively). Similarly, the H′ varied among land-uses during the dry season, with NF, SHT, and TTP land-use showing similar diversity values (2.60 ± 0.31, 2.45 ± 0.38, and 2.48 ± 0.45, respectively), while SHA land-use had the lowest (2.08 ± 0.42) value. The %EPT index showed no significant differences across land-uses ($p > 0.05$; Table 4). However, mean values indicated some variation in EPT contribution to macroinvertebrate composition across land-uses, including changes in community responses to disturbance, especially during the wet season. During this period, the highest and comparable %EPT index values were recorded at the TTP and NF land-uses (71.2 ± 13.6 and 69.9 ± 8.2, respectively), while the lowest value was observed in the SHA land-use (60.2 ± 26.5), indicating poorer water quality.

Table 4: Community structural indices, and percentage of Ephemeroptera, Plecoptera, and Trichoptera (%EPT, mean ± SD) of macroinvertebrate assemblages across four land-uses in the headwater streams of Sondu-Miriu River basin: Natural Forest (NF), Smallholder Agriculture (SHA), Smallholder Tea (SHT), and Tea and Tree Plantation (TTP), during wet and dry seasons.

Metrics	Season	NF	SHA	SHT	TTP	F-value	*P*-value
Taxon richness (S)	Wet	41.0 ± 11.3	37.0 ± 15.3	41.5 ± 14.1	44.2 ± 12.5	0.29	0.830
	Dry	48.0 ± 10.5^{a}	30.0 ± 6.5^{b}	50.2 ± 8.8^{a}	42.2 ± 6.3^{ab}	7.24	0.002*
Simpson index (1-D)	Wet	0.88 ± 0.03	0.75 ± 0.17	0.84 ± 0.10	0.84 ± 0.08	1.46	0.257
	Dry	0.88 ± 0.03	0.78 ± 0.12	0.88 ± 0.05	0.87 ± 0.04	3.06	0.052
Shannon index (H′)	Wet	2.60 ± 0.31	2.07 ± 0.64	2.45 ± 0.38	2.48 ± 0.45	1.51	0.243
	Dry	2.69 ± 0.29^{a}	2.08 ± 0.42^{b}	2.76 ± 0.35^{a}	2.62 ± 0.23^{a}	5.37	0.007*
%EPT index	Wet	69.9 ± 8.2	60.2 ± 26.5	63.6 ± 27.8	71.2 ± 13.6	0.61	0.618
	Dry	61.8 ± 15.2	52.3 ± 17.8	56.9 ± 21.5	58.0 ± 8.3	0.40	0.752

NB: Groups with different superscripts differ significantly, as determined by Tukey *post hoc* tests. Asterisks indicate significant differences among land-uses at $p < 0.05$

4.3 Variation of macroinvertebrate biomass and abundance with size-class

Macroinvertebrate size distribution differed among land-uses and between seasons, displaying unimodal patterns in abundance and multimodal patterns in biomass in some land-uses (Figure 3). In the wet season, SHA land-use exhibited the highest abundance in the smallest size class (<2 mg), reaching approximately 900 individuals in this class (Figure 3a). In contrast, the other land-uses showed relatively low abundances in the smaller size classes (< 8 mg) of < 370 individuals per size class. The TTP land-use had peak macroinvertebrate abundance in the 4-8 mg size class (420 individuals) which then reduced progressively in the larger size classes (Figure 3a). During the dry season, the abundance-size distribution showed a peak at between 4 and 16 mg for all land-uses, indicating a predominance of small-sized individuals at the land-uses compared to the wet season (Figure 3b). Except for the SHA land-use, the dry season abundance of individuals was

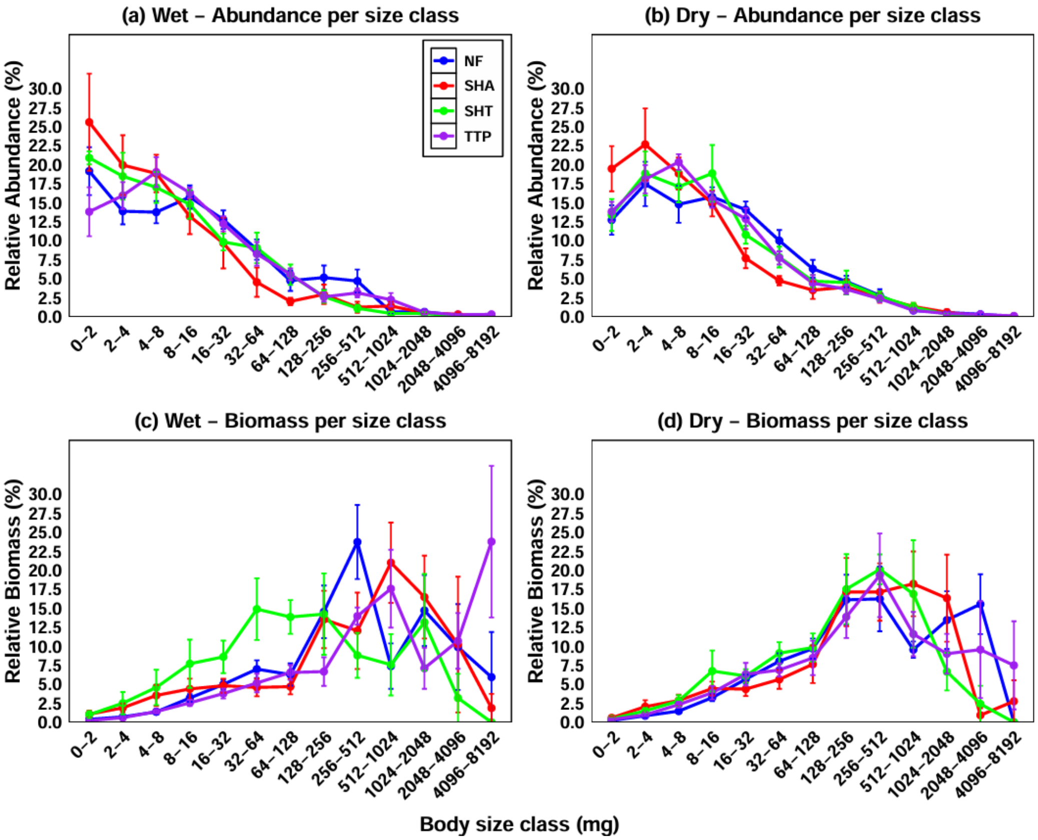

Figure 3: Seasonal distribution of macroinvertebrate abundance and biomass (mg) across body size classes among the stream sites in the headwater stream in the Sondu-Miriu River basin; Natural Forest (NF), Smallholder Agriculture (SHA), Smallholder Tea (SHT), and Tea and Tree Plantation (TTP).

more than twice the wet season abundance for the 2-64 mg size classes. The SHA land-use recorded the lowest modal abundance of below 550 individuals in the small size classes (<2 mg, Figure 3b). In contrast, other land-uses exhibited higher modal abundances ranging from 600 individuals in the 2-4 mg size class and 700 individuals in the 4-8 mg size class for the streams in the NF and TTP land-uses, respectively (Figure 3b). Biomass distribution tended to be multimodal in nature compared to the abundance distribution. However, the wet season showed a mostly unimodal distribution in most land-uses, except for the NF land-use sites, which were bimodal (Figure 3c and 3d). During the dry season, the land-uses had a largely bimodal distribution of sizes, except for the SHT land-use that had a unimodal biomass peaking at between 250 and 500 mg.

Sequential GLM revealed macroinvertebrates' body size (mg) alone accounted for 51% of the variation in macroinvertebrate abundance per size class (MSE = 0.25) (Table 5), underscoring its strong predictive role in size-spectrum structure. Adding season as a factor slightly improved the model, increasing the explained variance to 53% (MSE = 0.24; $p < 0.001$), indicating some influence of temporal variability on abundance distributions. Incorporating land-use produced a modest improvement, maintaining R^2 at 53% (MSE = 0.23; $p = 0.029$), highlighting the role of anthropogenic factors in shaping size-based macroinvertebrate community patterns. Addition of water physico-chemical variables (Table 4) resulted in a slight gain in explained variance ($R^2 = 54\%$, MSE = 0.23; $p = 0.495$). The inclusion of interaction effects between body size (mg), season, and land-use provided minimal but insignificant further improvement ($R^2 = 54\%$, MSE = 0.23; $p = 0.162$) (Table 5). Similarly, sequential GLM results for biomass per size class (Table 5), showed that macroinvertebrate body size alone accounted for 24% of the variation in biomass distribution across size classes (MSE = 1.10), indicating a moderate but significant influence on size structure. Adding season as a predictor led to a slight improvement, increasing the explained variance to 26% (MSE = 1.07; $p < 0.001$), suggesting some temporal shifts in biomass allocation. The inclusion of land-use further enhanced the model, raising the R^2 to 28% (MSE = 1.04; $p < 0.001$). Incorporating water physico-chemical variables (VIF < 5) improved the model fit modestly ($R^2 = 31\%$, MSE = 0.99; $p = 0.002$). Finally, interaction terms between body size and both season and land-use brought the total explained variance to 35% (MSE = 0.95; $p < 0.001$). The remaining 46%

and 65% of the variation in abundance and biomass per size class, respectively, are likely attributable to unexplained ecological processes (e.g., habitat heterogeneity, species interactions) or limitations of the model.

Table 5: Results of sequential generalized linear modeling (GLM) analysis on macroinvertebrate abundance per size class across all stream sites and seasons. All variables were entered sequentially into the model. MSE = Mean square error, R^2=coefficient of determination.

		Abundance			Biomass		
Steps	**Variables added**	**R^2 (%)**	**MSE**	**p-value**	**R^2 (%)**	**MSE**	**p-value**
1	Body size (mg)	51	0.25	0.001	24	1.10	0.001
2	+ Season	53	0.24	0.001	26	1.07	0.001
3	+ Land use	53	0.23	0.029	28	1.04	0.001
4	+ Physico-chemical variables (VIF < 5)	54	0.23	0.495	31	0.99	0.002
5	+ Interactions (Body size × Season × Land use)	54	0.23	0.162	35	0.95	0.001

4.4 Relationship between macroinvertebrate abundance and physico-chemical variables

Redundancy analysis (RDA) revealed distinct associations between macroinvertebrate abundance in the size classes and physico-chemical variables across the wet and dry seasons (Figure 4). During the wet season (Figure 4a), the SHA and SHT streams were strongly aligned with elevated levels of SRP, turbidity, EC, and TDS, while TTP streams were associated with high NO_3^--N concentrations and turbidity. These factors were closely associated with higher abundances of smaller macroinvertebrate size classes (<8 mg). Conversely, stream sites in NF land-use were weakly associated with these factors and tended to be located toward the origin or opposite direction of nutrients, EC, and turbidity vectors. The POM showed a moderate association with NF and SHT land-uses, suggesting a possible detrital influence on macroinvertebrate communities. Larger size classes (>32 mg) appeared opposite the nutrient vectors, indicating reduced abundance in nutrient-enriched or eutrophic streams.

In the dry season, the SHA and SHT land-uses were again associated with higher concentrations of POM, TSS, turbidity, EC, and TDS (Figure 4b), indicating physico-chemical disturbance during low-flow conditions. These land uses showed increased representation of small size classes (<8 mg). In contrast, NF land-use was located away from most disturbance vectors and showed weak or negative associations with SRP and NO_3^--N. Larger macroinvertebrate size classes (>32 mg) were again positioned furthest from these factors or located near the plot center, reflecting moderate or generalist responses across environmental gradients.

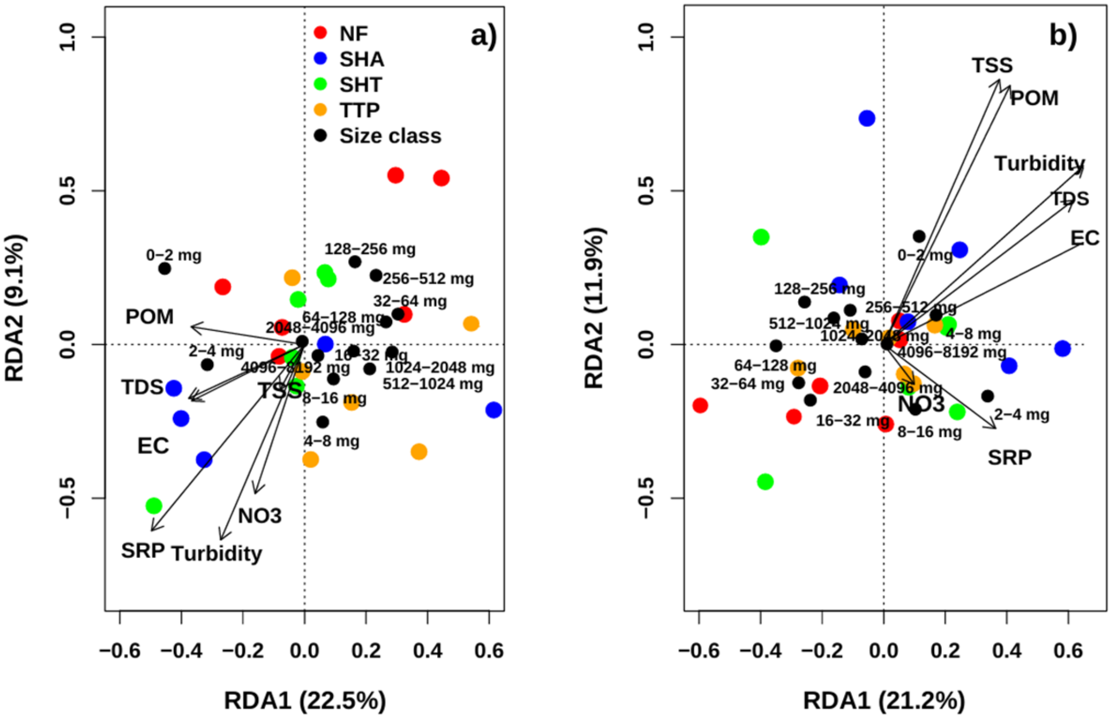

Figure 4: Redundancy Analysis (RDA) showing the relationships between macroinvertebrates abundance per size class and water physicochemical variables during the (a) wet and (b) dry seasons across the stream sites; Natural Forest (NF), Smallholder Agriculture (SHA), Smallholder Tea (SHT), and Tea and Tree Plantation (TTP).

4.5 Abundance and biomass size-spectrum patterns

The relationship between the slopes and midpoint heights of the size-spectrum was weak or marginal during the wet and dry seasons for both NASS (wet: r = 0.12, p = 0.58; dry: r = 0.40, p = 0.06) and NBSS (wet: r = 0.01, p = 0.96; dry: r = 0.21, p = 0.33). This allowed for the use of the two metrics (slope and midpoint heights) as independent measures of land-use effects on stream condition. Size-spectrum slopes and midpoint heights varied

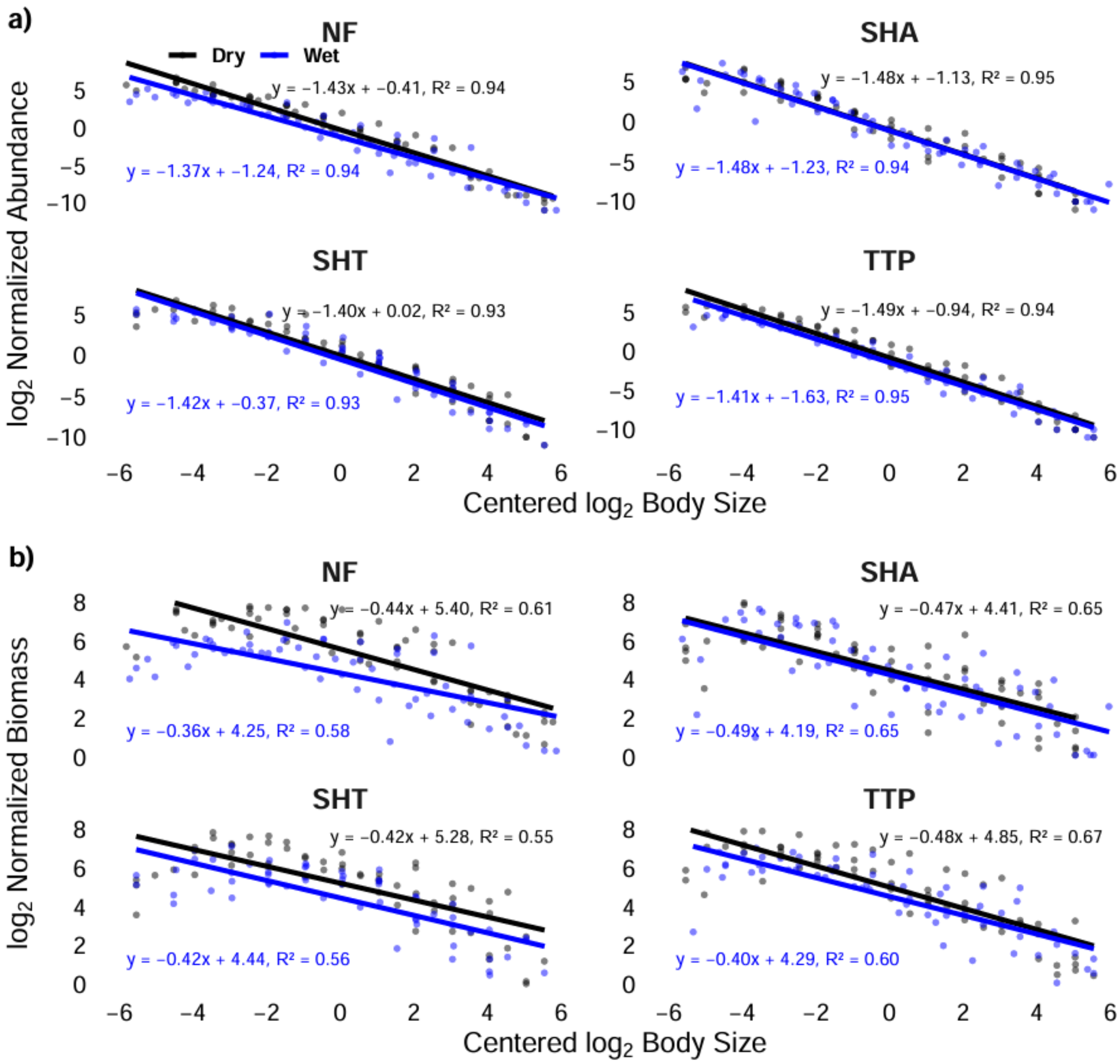

Figure 5: Normalized (a) abundance and (b) biomass size-spectra for macroinvertebrates in four land-use types: Natural Forest (NF), Smallholder Agriculture (SHA), Smallholder Tea (SHT), and Tea and Tree Plantation (TTP) in both wet and dry seasons. Slopes and heights for the plots are already described in the text.

across land-use types and seasons, indicating spatio-temporal differences in community size structure and energy transfer efficiency (Figure 5 and 6; Appendix 1). The normalized abundance size-spectrum (NASS) and normalized biomass size-spectrum (NBSS) slopes were consistently negative across all land-uses in both wet and dry seasons (Figure 5, Appendix 1). All mean slopes were less steep than the expected equilibrium value of -2.0 for NASS and -1.0 for NBSS Appendix 1), indicating a greater representation of large-bodied macroinvertebrates per size class than predicted from theory.

4.5.1. Variation of size-spectrum slopes with land-use and season

During the wet season; the shallowest (least negative) slopes were observed in the NF, TTP, and SHT land-uses for both NASS (-1.37 ± 0.14, -1.41 ± 0.12, and -1.42 ± 0.08, respectively), and NBSS (-0.37 ± 0.13, -0.40 ± 0.12, and -0.42 ± 0.09, respectively). In contrast, the steepest slopes occurred in SHA land-use for both NASS and NBSS (1.48 ± 0.19, and -0.49 ± 0.19, respectively; Figure 5 and 6). These differences in macroinvertebrate size-spectrum slopes may indicate variations in energy transfer efficiency associated with size-distributions and linked to land-use intensity.

During the dry season, the steepest slopes were observed in SHA and TTP land-uses for both NASS (-1.48 ± 0.16 and -1.49 ± 0.05, respectively) and NBSS (-0.47 ± 0.15 and -0.48 ± 0.04, respectively) (Figure 5 and 6, Appendix 1), indicating a dominance of small-sized macroinvertebrates, likely contributed by Ephemeroptera (family: Baetidae, Figure 2). In contrast, NF and SHT land-uses displayed shallower slopes in both NASS (-1.43 ± 0.09 and -1.40 ± 0.15, respectively) and NBSS (-0.44 ± 0.08 and -0.42 ± 0.15, respectively) (Figure 5 and 6), which suggest presence of larger macroinvertebrates per size class, depicting a more efficient trophic transfer at these sites during this season.

The ANCOVA analysis indicated no significant effects of land-use on slopes based on both NASS (F = 0.92, df = 3, p = 0.44) and NBSS (F = 0.89, df = 3, p = 0.46). Similarly, there was no significant effect of season on slopes based on both NASS (F = 0.72, df = 1, p = 0.40) and NBSS (F = 0.77, df = 1, p = 0.39) analysis. The interaction between land-use and season had no significant effect on slope in either spectrum: NASS (F = 0.40, p = 0.76) or NBSS (F = 0.54, p = 0.66) (Table 7).

Table 6: The ANCOVA results testing the effects of land-use change, season, and their interaction on macroinvertebrates biomass and abundance size-spectrum slopes in headwater streams in the Sondu-Miriu River basin, Kenya.

		Biomass		**Abundance**	
Factor	**DF**	**F**	**p**	**F**	**p**
Land use	3	0.89	0.456	0.92	0.440
Season	1	0.77	0.387	0.72	0.402
Land use × Season		0.54	0.656	0.40	0.756

4.5.2. Variation of size-spectrum midpoint heights with land-use and season

During the wet season; higher midpoint heights (mean ± SD) were observed in SHT land-use for both NASS and NBSS (-0.37 ± 1.09 and 4.44 ± 0.31, respectively), suggesting greater relative abundance and biomass in this land-use, possibly due to increased nutrient (SRP) availability (Table 3). Lower values of midpoint heights were recorded in TTP (-1.63 ± 0.48) for NASS and in SHA (4.19 ± 0.79) for NBSS (Figure 5 and 6, Appendix 1).

During the dry season; midpoint heights were higher in SHT land-use (0.02 ± 0.94) in NASS and NF land-use (5.40 ± 0.43) in NBSS. These results indicate ecosystem richness, as NF and SHT also exhibited higher taxon richness (Table 4). Lower midpoint heights were recorded in SHA land-use for both NASS and NBSS (-1.13 ± 0.63 and 4.41 ± 0.43, respectively), further showing synchrony in the estimation of system production by the two size-spectrum midpoint height (Figure 6). The SHT land-use showed the highest variability in midpoint height among seasons being ±1.09 (SD) in the wet season and ±0.94 (SD) in the dry season for NASS, indicating greater ecological heterogeneity in these systems (Figure 6).

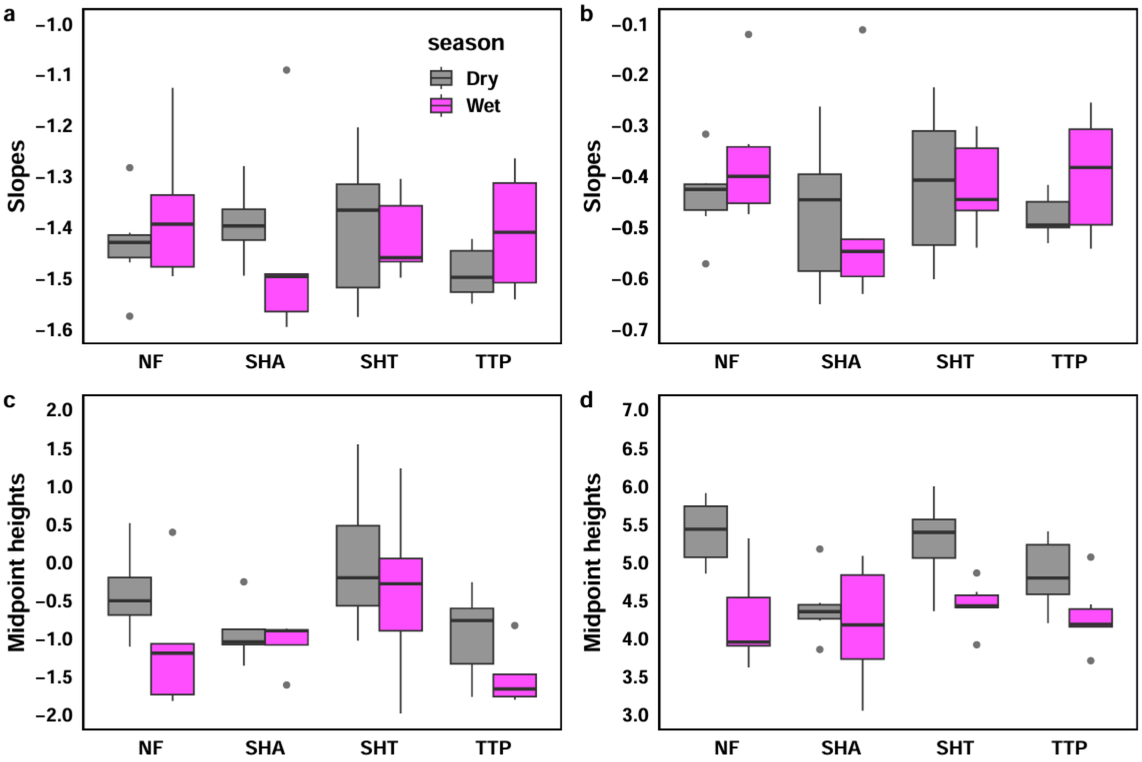

Figure 6: Boxplots showing the distribution of macroinvertebrates normalized abundance size-spectra (a) slopes and (c) midpoint heights, and normalized biomass size-spectra (b) slopes and (d) midpoint heights across land-use types: Natural Forest (NF), Smallholder Agriculture (SHA), Smallholder Tea (SHT), and Tea and Tree Plantation (TTP), during the wet and dry seasons.

The relationship between NASS and NBSS midpoint heights showed a significant positive correlation ($r = 0.72$, $p = 0.001$), suggesting congruence between abundance and biomass per size class across land-use sites in the study area.

The ANCOVA showed a significant difference effect of land-use on midpoint heights for both NASS ($F = 5.53$, $df = 3$, $p = 0.003$) and NBSS ($F = 2.94$, $df = 3$, $p = 0.045$). Similarly, season had a significant effect on midpoint height values for NASS ($F = 5.68$, $df = 1$, $p = 0.022$) and NBSS ($F = 20.63$, $df = 1$, $p < 0.001$). The interaction between land-use and season showed no significant effect on midpoint heights in both NASS ($F = 0.59$, $p = 0.63$) and NBSS ($F = 1.72$, $p = 0.18$) analyses (Table 8).

Table 7: The ANCOVA results testing the effects of land-use change, season, and their interaction on macroinvertebrates biomass and abundance size-spectrum midpoint-heights in headwater streams in the Sondu-Miriu River Basin, Kenya.

		Biomass		**Abundance**	
Factor	**DF**	**F**	**p**	**F**	**p**
Land use	3	2.94	0.045	5.53	0.003
Season	1	20.63	0.001	5.68	0.022
Land use × Season		1.73	0.177	0.59	0.627

There was a strong positive correlation between the slopes of NASS and NBSS across land-use sites ($r = 0.98$, $p < 0.001$), Similarly, the midpoint height showed a strong positive relationship with community structural patterns ($R^2 = 0.72$, $p = 0.001$) (Figure 7) indicating consistent prediction of community structural patterns by both metrics.

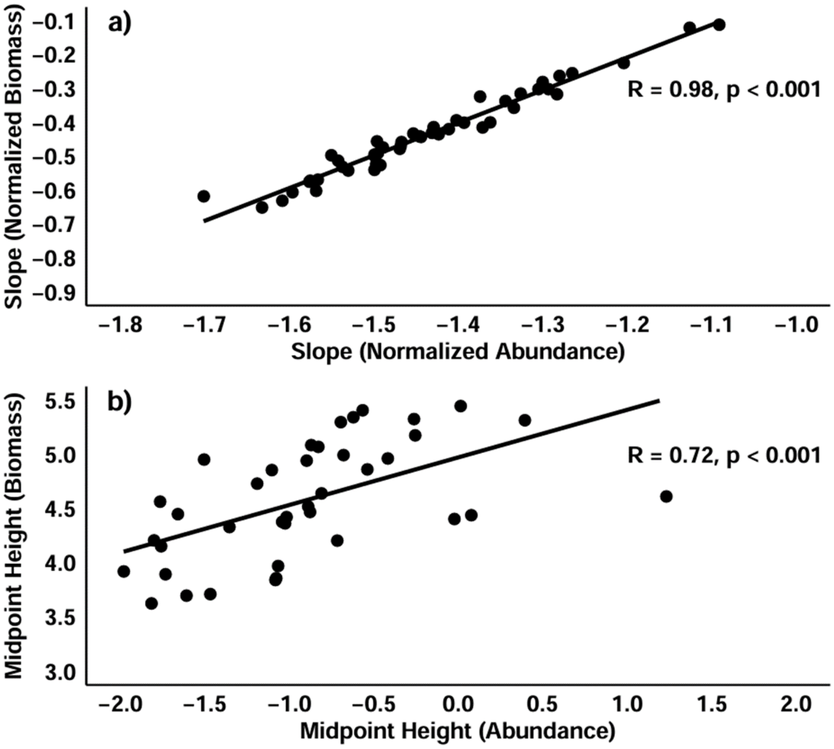

Figure 7: Pearson correlation plots illustrating the relationships between (a) slopes and (b) intercepts of normalized abundance and biomass size-spectrum across four land use types. Each point represents a stream. Coefficient of determination (r2) and significance level (p = 0.01).

4.5.3. Size-spectrum metric sensitivity and performance

Overall, NF land-use streams consistently exhibited the most favourable size-spectrum: shallower slopes and the highest NBSS midpoints (Figure 5), indicating communities with well-represented large-bodied macroinvertebrates. The SHT land-use streams demonstrated high variability but generally favourable values, suggesting intermediate disturbance, while the TTP streams showed moderate values across all metrics, indicative of partially altered systems. In contrast, the SHA streams consistently exhibited the steepest slopes and lowest midpoint heights for both NASS and NBSS (Figure 5), indicating simplified, stress-prone macroinvertebrate communities.

Table 8: Summary performance of slopes and midpoint heights as metrics of effects of land-use change on macroinvertebrate communities in headwater streams in the Sondu-Miriu River Basin, Kenya.

Metric	Responsiveness to Land-use	Responsiveness to Seasonality	Statistical Significance	Indicator Suitability
NASS Slope	Low	Low	No	Low
NBSS Slope	Low	Low	No	Low
NASS Midpoint	High	Moderate	Yes	High
NBSS Midpoint	High	High	Yes	High

The ANCOVA tests confirmed that land-use had a significant effect on NASS midpoints (p <0.001) and NBSS midpoints (p = <0.05). Seasonal effects were also significant for NBSS midpoints ($p < 0.001$). These results highlight that midpoint height was a more responsive and ecologically informative indicator of environmental disturbance compared to slope, which remained statistically stable across conditions (summarized in Table 9).

4.7 Relationship between size-spectrum metrics and community predictors

The relationships between slope and midpoint height from normalized abundance size-spectrum (NASS) and normalized biomass size-spectrum (NBSS) with selected water physico-chemical variables and community indices that showed statistically significant relationships ($p < 0.05$) are presented in Figure. 8. The slopes were negatively correlated with TDS and EC in both NASS ($R^2 = 0.18$, $p = 0.04$ and $R^2 = 0.19$, $p = 0.03$, respectively) and NBSS, ($R^2 = 0.19$, $p = 0.04$ and $R^2 = 0.19$, $p = 0.03$, respectively) (Figure 8a-f and Figure 8g-l), indicative of their responsiveness to polluted water and reflecting reduced efficient transfer of energy to higher trophic levels. There was a significantly positive relationship for both Simpson diversity and Shannon diversity index with slopes in NASS ($R^2 = 0.17$, $p = 0.043$ and $R^2 = 0.17$, $p = 0.046$) and marginally significant in NBSS ($R^2 = 0.16$, $p = 0.05$ and $R^2 = 0.16$, $p = 0.056$), respectively (Figure 8a-f and 8g-l), implying that shallower slopes, indicative of increased efficient transfer of energy, was associated with reduced dominance and increased diversity of macroinvertebrates.

The midpoint height exhibited a significantly positive relationship with SRP and taxon richness in NASS ($R^2 = 0.34$, $p = 0.003$ and $R^2 = 0.20$, $p = 0.03$), respectively. The NBSS showed significantly positive relationship with taxon richness ($R^2 = 0.50$, $p < 0.001$) and non-significant positive relationship with SRP (Figure 8a-f and 8g-l). This indicates that increased nutrient availability leads to greater overall biomass or ecosystem carrying capacity, which tends to support a higher number of different taxa, as observed in NF and SHT land-uses during the dry season and in TTP during the wet season (Table 4).

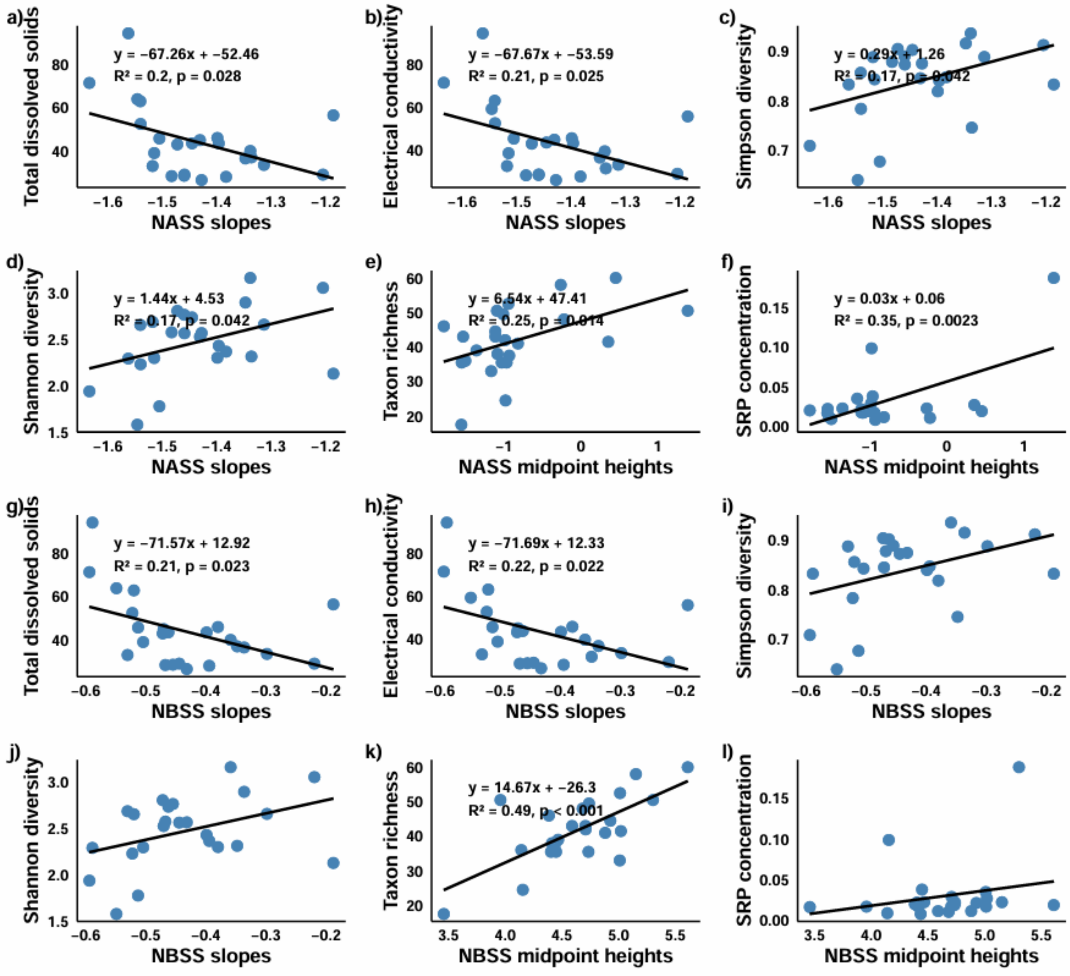

Figure 8: Regression analysis of water physico-chemical variables and community structural indices with slopes and midpoint height of normalized abundance size-spectra (NASS, e-f) and normalized biomass size-spectra (NBSS, g-l) for macroinvertebrates in the headwater streams in the Sondu-Miriu River Basin.

4.8 Abundance-biomass comparison (ABC) curves

Across all four land-uses, the Biomass Curve remained consistently above the Abundance Curve, indicating ecological conditions typical of undisturbed systems (Figure 9). This pattern likely reflects communities dominated by K-selected species, large-bodied, slow-growing organisms that contribute substantially to biomass without numerical dominance.

The Warwick's (W) statistics reveal subtle differences among land-uses, suggesting variation in local-scale ecological condition despite the overall health status. During the wet season, W-statistics were all positive, indicating undisturbed stream conditions (Figure 9). The TTP land-use recorded the highest W-statistic (0.27 ± 0.08), followed closely by NF (0.25 ± 0.05). The SHA land-use recorded the lowest W-statistic (0.15 ± 0.10), with SHT land-use having an intermediate value (0.18 ± 0.13). In the dry season, all W-statistics were positive, with the highest value recorded in TTP land-use (0.18 ± 0.04, Figure 9 and 10). The SHT and SHA had the lowest values (0.15 ± 0.08; 0.16 ± 0.07, respectively). These values were generally lower than those recorded in the wet season, particularly for NF and TTP land-uses, possibly due to reduced dilution from lower flow (Figure 9 and 10).

A two-way ANOVA analysis revealed a significant effect of season on W-statistic (F =4.75; p = 0.04), with higher values recorded during the wet than in the dry season. However, land-use showed no significant effect on the W-statistic (F = 1.86; p = 0.15). The interaction between land-use and season was also not significant (F = 1.05; p = 0.38).

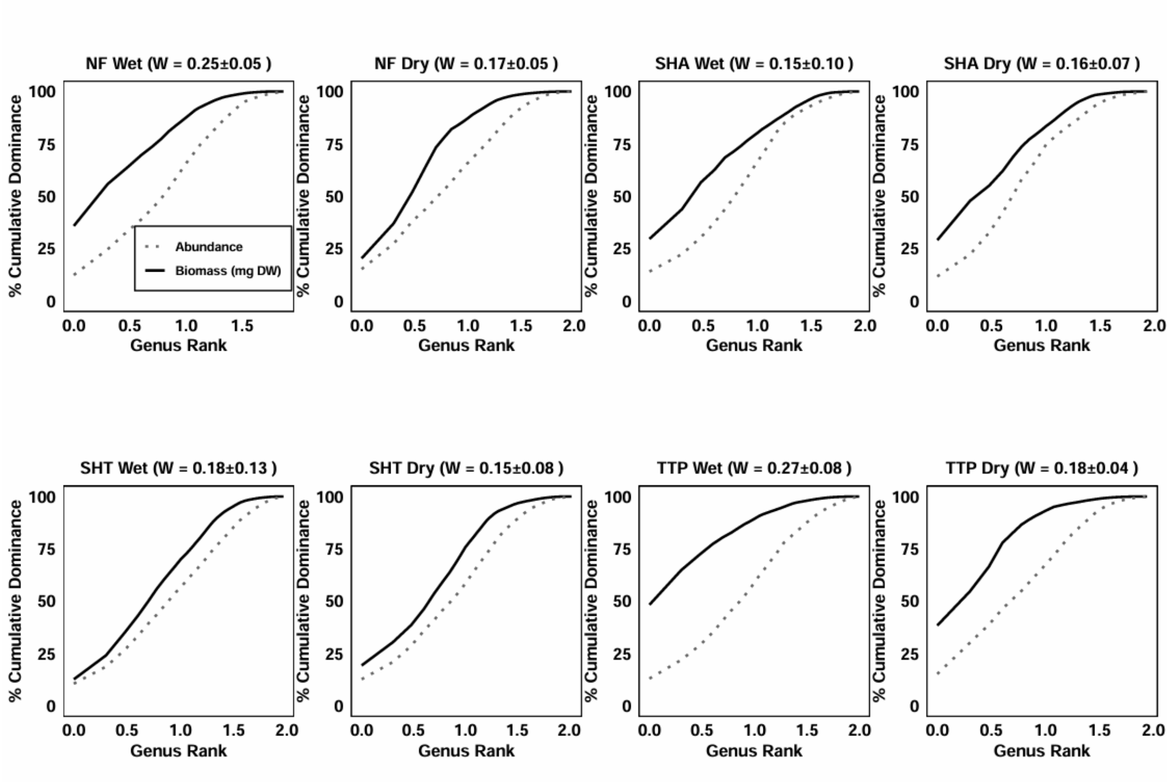

Figure 9: Abundance-Biomass Comparison (ABC) curves for macroinvertebrates in headwater streams in the Sondu-Miriu River basin during wet and the dry seasons across different land-uses types: natural forest (NF), smallholder agriculture (SHA), smallholder tea (SHT), and tea and tree plantation (TTP).

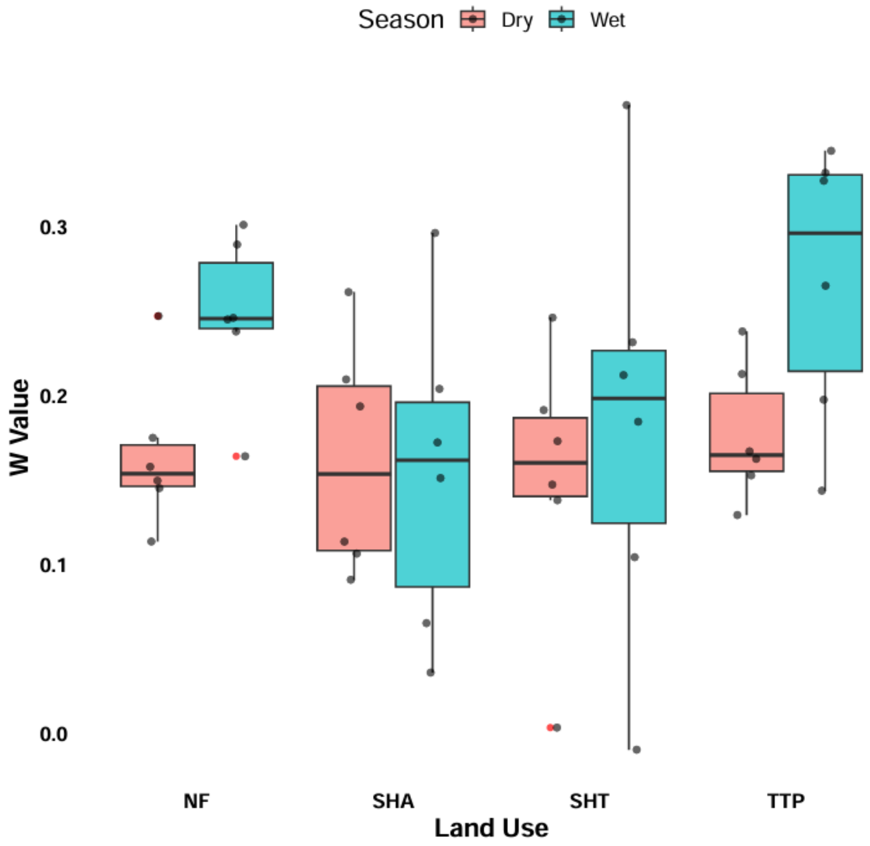

Figure 10:Boxplots showing the distribution of Warwick's (W) statistics in headwater stream in the Sondu-Miriu River basin across land-use types: Natural Forest (NF), Smallholder Agriculture (SHA), Smallholder Tea (SHT), and Tea and Tree Plantation (TTP), during the wet and dry seasons.

CHAPTER FIVE

DISCUSSION

This study aimed to evaluate the responsiveness of macroinvertebrate assemblages to water quality changes attributed to different land-use activities, and if size-spectrum metrics and Abundance Biomass Comparison (ABC) curves can track water quality differences at seasonal and intra-basin scales. Size-spectrum metrics and ABC curves, if responsive to perturbations, can then be used as a simple deterministic framework for monitoring and managing river basins. Land-use exerted a strong influence on stream water quality, with natural forest streams that acted as reference sites maintaining the lowest levels of nutrients and suspended solids, while smallholder agriculture streams showed pronounced physico-chemical degradation. These gradients were reflected in macroinvertebrate assemblages, where forested streams hosted more diverse and sensitive taxa, in contrast to agriculturally impacted sites dominated by pollution-tolerant, small-bodied taxa. The changes in water quality and assemblage structure were also reflected in size-spectrum metrics used as indicators of ecological condition. Although the slopes remained statistically invariant across land-uses and seasons, midpoint heights varied significantly, effectively capturing differences in food web capacity or ecosystem productivity caused by land-uses changes.

5.1 Variation of water physico-chemical variables between sites

To characterize the water quality of the streams of the Sondu-Miriu River basin, physico-chemical variables showed results that were consistent with their traditional application in evaluating water quality changes in streams and rivers influenced by anthropogenic activities including land-use types (Minaya *et al*., 2014; Masese *et al*., 2017; Jacobs *et al*., 2018b). The physico-chemical variables of the streams showed consistent deterioration along a gradient of increasing anthropogenic influence spanning the relatively undisturbed natural forest (NF) land-use to the more intensively used smallholder agriculture (SHA) sites land-use. Streams draining NF land-use exhibited low values of physico-chemical indicator variables (turbidity, EC, POM, NO_3^--N TDS, DO) consistent with ecologically good water quality (Jacobs *et al*., 2017; Kroese *et al*., 2020a, b; Masese *et al*., 2024c) as opposed to the SHA land-use that displayed highly elevated values of these variables, suggesting reduced water quality. The smallholder agricultural activities within the SHA land-use likely enhanced erosion and reduced land cover resulting into increased sediment load, nutrient and organic matter/fertilizer inputs into the streams leading to poor water quality as measured by the variables (Defersha *et al*., 2012; Jacobs *et al*., 2018a, b; Kroese *et al*., 2020a, b; Iteba *et al*., 2021; Masese *et al*., 2021). Smallholder tea (SHT) and TTP streams appear to be intermediate in water quality, with the higher NO_3^--N concentrations observed in TTP land-use streams likely due to the intensive use of inorganic fertilizers for top-dressing prevalent in commercial tea plantations (Jacobs *et al* 2018b; Nyilitya *et al.,* 2020). Although, the evaluation of land-use influence using physico-chemical water properties is robust, it does not account for species-specific interactions, and are costly for continuous monitoring (Bonada *et al*., 2006; Masese *et al*., 2024b). Furthermore, these variables only provide a snapshot of water quality during sampling or measurement, as most of the variables respond rapidly to changes in flow conditions, such as rainfall events (Jacobs *et al*., 2018b; Jacobs and Breuer, 2024).

5.2 Macroinvertebrate assemblage structure

Although we found no significant differences in macroinvertebrate structural indices between land-uses during the wet season, there were high taxa richness (S) and diversity indices (H' and 1-D) at the NF and TTP land-uses, while SHA land-use streams registered low values thereby validating the findings from physico-chemical assessment as being the most impacted streams. Additionally, the distribution of the pollution-sensitive EPT taxa across land-uses, showed higher %EPT values in NF and TTP land-uses during both seasons while, lower values were noted in SHA and SHT land-uses, thereby further validating the findings of physico-chemical analysis. The uniformity in structural indices between land-uses during the wet season likely reflects a homogenizing effect of elevated water levels and increased discharge, which can redistribute macroinvertebrate taxa and mask localized disturbances. The limited discriminatory power of diversity metrics under such hydrological conditions has been noted previously (Masese *et al*., 2014b; 2023) and may also result from taxa replacement, where sensitive taxa in reference land-use sites are substituted by tolerant ones in disturbed sites. In contrast, clearer patterns in assemblage structure as noted during the dry season, is attributable to lower flow conditions that concentrate or reduce pollutants inputs due to reduced surface runoff and connectivity between groundwater and streams enhance their ecological effects, and increase the sensitivity of aquatic communities to land-use-specific stressors (M'Erimba *et al*., 2014; Zhang *et al*., 2012; Masese *et al*., 2023).

The Simpson diversity index indicated stronger taxa dominance in the lower water quality SHA land-use, reflecting communities dominated by a few pollution-tolerant taxa. The dominance of Ephemeroptera in SHA land-use is likely due to the prevalence of taxonomic and functional groups that are more tolerant to sedimentation and organic pollution such as collectors (filterers and gatherers) and predators (Masese and Raburu, 2017; Owade *et al*., 2025). Indeed, even among the EPT orders, some families such as Baetidae, Caenidae and Hydropsychidae, are tolerant to organic pollution and can proliferate in impacted sites (Masese *et al*., 2023). Dominance of Diptera during the dry season, particularly tolerant groups like Chironomidae, reflects their ability to persist under conditions of high turbidity and reduced DO conditions commonly observed in agricultural streams during dry periods (Yegon *et al*., 2021). These patterns are likely influenced by intensive agricultural activities in poorly managed areas, where runoff and habitat disturbance simplify stream habitats and favor tolerant species (Masese *et al*., 2014a, Sitati *et al*., 2021a). Similarly, the Shannon-Wiener diversity index revealed comparable diversity in the NF, SHT, and TTP land-uses during the dry season, with notably lower values in the SHA land-use, reinforcing the notion of stress-induced shifts in community structure.

5.3 Size-spectrum patterns

Macroinvertebrate biomass distribution as a function of body size was more multimodal during the dry season, especially in the NF land-use. This distribution pattern likely reflects the expression of different trophic levels during this season and is compatible with the high diversity recorded for the less disturbed land-use sites. The multimodal distribution appears to support *Sheldon's biomass spectrum*, with a slope equal to zero (Sheldon *et al*., 1972), thereby validating site comparisons using biomass-size spectra derived in this study (also see Sprules and Birth, 2015). The SHA land-use were dominated by small-sized individuals, mostly collector-gatherer taxa in the Chironomidae, Simuliidae, and Oligochaeta groups while, NF and TTP land-uses recorded larger sized individuals- the large-bodied shredders such as freshwater *Potamonautes* (crabs) and *Tipula* (crane flies). The high turbidity levels in the SHA land-use likely precluded grazers/scrapers in favor of collector-gatherers, while larger macroinvertebrates are often more vulnerable to environmental stress due to their greater energy requirements and broader habitat needs (Woodward *et al.*, 2012; Pomeranz *et al.*, 2019) precluding them from the degraded land-use sites.

The decoupling between ecosystem capacity or production (estimated by midpoint height) and trophic transfer efficiency (estimated by slope), found in this study, suggests that they represent different community processes as anticipated from simulation and empirical studies (Kerr and Dickie, 2001). The abundance and biomass size-spectra slopes were all greater than the theoretical equilibrium value of -2.0 and -1.0, respectively, indicating deviation from steady state (Sprules and Barth, 2015; Guiet *et al.*, 2016; Edward *et al.*, 2017). There were quantitative differences between land-use slopes. For example, SHA land-use consistently showed steeper slopes suggesting perturbation-dependent effects on the size structure of the macroinvertebrates and potential low trophic transfer efficiency compared with the more pristine NF land-use, along with the TTP and SHT land-uses which were subject to moderate levels of perturbation had shallower slopes indicating dominance of larger sized macroinvertebrates reflecting possible higher trophic transfer efficiency (Benejam *et al.*, 2018; Murry and Farrell, 2014). However, the absence of statistically significant variation in slopes across land-uses or seasons suggests that size-spectrum slopes may not be strongly responsive to environmental variability in these streams. This lack of statistical responsiveness could imply that other factors, beyond those captured by slope, are influencing macroinvertebrate assemblage structure and trophic transfer efficiency in the Sondu-Miriu River basin. Possible explanations include the limited spatial scale of sampling (Blanchard *et al*., 2009), life-history trait differences due to the fish-based foundation of size-spectrum theory (Rice and Gislason, 1996), and characteristics of river ecosystems such as rapid material throughput, multiple refugia or allochthonous food subsidies that may stabilize the size-spectrum (Guiet *et al*., 2016).

Lack of spatio-temporal variation in the slope metric may also imply that although agriculture is having an impact on the ecological condition of streams and rivers in the region, their impact is still within tolerable limits for most taxa, and sampling across a gradient of other land-use types, such as urban land-use, may uncover a stronger impact on the size-distribution of macroinvertebrates. Indeed, the %EPT metric recorded consistently high values (>50%), considered a threshold for highly impacted streams. Similarly, the diversity indices varied minimally, suggesting that the level of impact among the land-uses was comparable to some degree. These findings should be confirmed (or falsified) by similar studies in other tropical headwater streams and in larger basins impacted by different types and levels of impacts. The adoption of size-spectrum models that incorporates life history-traits of the benthic macroinvertebrates will likely capture more of the factors that affect size-distribution and trophic transfers in river systems (Hartvig *et al*., 2011).

In contrast to the slopes, the midpoint heights significantly varied with land-use and seasons and hence appeared more sensitive to changes in stream conditions. Prior research suggests that size-spectrum heights reflects system productivity (Kerr and Dickie, 2001;

Daan *et al*., 2005) contributed by species richness, biomass and primary carbon sources, attributes that are sensitive to environmental perturbations (Guiet *et al*., 2016). The largest midpoint heights were recorded at land-uses with the highest taxon richness during the dry season (SHT and NF), while in the wet season, increased nutrient (e.g. SRP) and organic matter concentrations likely contributed to higher productivity, particularly at SHA and SHT land-uses, suggesting the utility of spectrum heights in discriminating trophic conditions as found for the Laurentian Great Lakes (Evans *et al*., 2022). The responsiveness of midpoints heights to environmental variability in the streams is a significant finding as few studies have demonstrated this for freshwater systems (Evans *et al*., 2022). Instead, other theories, such as the metabolic theory of ecology (MTE, Brown *et al*., 2004), the dynamic energy budget theory (DEB, Kooijman, 2010) and the riverine ecosystem synthesis model (RES, Thorp *et al*., 2006), among others, have been advanced to explain assemblage structure and functioning of riverine ecosystems. More studies testing the responsiveness of size-spectrum metrics to environmental changes in tropical streams are required to add to the theories accounting for ecosystem functioning and monitoring of these systems.

5.4 Abundance-Biomass Comparison (ABC) curves

The W-statistic values derived from the ABC plots quantitatively supported the separation of the biomass from the abundance curves with the biomass curve consistently being above the abundance one at all sites and seasons. Across all the four land-uses and seasons, the W-statistic remained positive, indicating undisturbed stream conditions also depicted by the more elevated biomass curves. Higher positive W-statistic values in TTP and NF land-uses, indicated minimal environmental stress at these sites, while slightly lower positive values in SHA and SHT land-use suggested marginally less optimal conditions but still within the undisturbed classification. Similar patterns of higher W-statistic from ABC curves for less impacted sites and slightly reduced values under moderate pressures have been reported for e.g. wetlands (Wijeyaratne and Bellanthudawa, 2018), and rivers (Clarke and Warwick, 2001; Mise *et al*., 2018). The seasonal decline in W-statistics observed in NF and TTP land-use in the dry season concurs with the findings of the physico-chemical variables as aligned to reduced flow and dilution during low-water periods, which can lead to subtle increases in stress or altered habitat structure. Comparable seasonal variability in W-statistics, with changes linked to hydrological conditions and nutrient dynamics, has been documented in other aquatic systems (Villanueva and Ibarra, 2016; Yan *et al*., 2022).

These W-statistic patterns are consistent with the water quality results, which indicated comparable physico-chemical variables across sites. However, ABC curves of macroinvertebrate assemblages may require higher environmental perturbation levels to be sensitive to land-use effects and be useful for monitoring stream status.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

The results of physico-chemical analysis showed a clear influence of catchment land-use activities on stream water quality. Consequently, 1) the smallholder agriculture (SHA) land-use exhibited greater perturbation compared to the natural forest (NF) and tea and tree plantation (TTP) land-uses, with smallholder tea (SHT) showing an intermediate level of perturbation. 2) These conclusions are supported by macroinvertebrate assemblage structure, especially the %EPT and community structural indices. 3) Further impacts of land-use change on the ecological condition of the studied streams were captured by the community size-spectrum metrics, especially the midpoint heights, that reflect ecological processes governing food-web capacity or community production. 4) Results show uniformity in community spectral slope, indicating they are less useful indicators of disturbances in the streams. However, midpoint height, an estimator of food-web capacity or ecosystem productivity, varied between land-uses and appeared to be more elevated in less impacted sites. Although the study was conducted within a limited spatial and temporal scope, and therefore potentially captured a snapshot of the ecosystem condition, the findings are quite promising in terms of the utility of size-spectrum metrics being able to detect and respond to low levels of disturbance within a rural river setting. Future long-term studies along a gradient of low to high disturbance (e.g., urban land-use) and stream sizes may uncover the utility of macroinvertebrate size-spectrum metrics as indicators of ecological conditions in streams and rivers.

More comprehensive data, including gradient of perturbation (e.g., urban, mining, etc.) and large spatial scales spanning different hydrologic and climatic conditions will also be required to evaluate the stability and utility of the community spectra and ABC curves as proxies for perturbation. Most community size-spectra studies have concentrated on fishing effects at larger spatial scales. Differences caused by river ecosystem dynamics including allochthonous food subsidies, benthos-pelagic coupling of communities, different life-history traits, among other attributes, may require different formulation of community size-spectrum models to cater for species interactions and the analysis of spectral patterns at variable gradients of perturbations and variable spatio-temporal scales. These differences may also require the application of ABC curves at different levels of land-uses including urban types to determine the critical threshold perturbations for their application. Nonetheless, the results of this study provide a pioneering attempt to generate simple quantitative frameworks for monitoring and managing data-scarce tropical river basins based on macroinvertebrate assemblages.

6.2 RECOMMENDATIONS

Following the results of this study, the following recommendations are provided:

1. Given the observed spatial and temporal differences in water physico-chemical conditions and macroinvertebrate assemblage structure, future monitoring programs in headwater streams should be designed to capture both seasonal and site-specific variability at longer scales. Regular assessment during both wet and dry seasons is essential to identify short-term fluctuations and long-term trends.

2. Management interventions for headwater streams should focus on land-use practices that degrade water quality, particularly in smallholder agriculture areas (SHA) that have been found to have greater effects on water quality variables and assemblage structure. The management interventions may include promotion of riparian buffer zones (especially around SHA sites) and reducing nutrient and sediment inputs through effective watershed management.

3. Given that water physico-chemical conditions were found to influence the distribution of macroinvertebrates; ataxic community size class distributions can serve as indicators of ecological condition in headwater streams. Conservation strategies should therefore prioritize maintaining optimal physico-chemical conditions that sustain a balanced distribution of community size classes. This can be achieved through measures such as reducing agricultural runoff, regulating pesticide use, and preserving natural flow regimes. By safeguarding these conditions, managers can help maintain a functional and resilient aquatic food web, as reflected in the structure of ataxic size classes.

4. The sensitivity of midpoint heights of the size-spectra to land-use differences highlights its potential as a diagnostic indicator of disturbance from land-use change, reflecting shifts in community structure and resource allocation. This makes it useful in tropical stream monitoring, where resources can be scarce. It is recommended that more studies of midpoint height variability be replicated in different basins to validate the stability of their responsiveness to riparian use effects on stream quality. The results of these studies will support the use of midpoint heights as proxies for disturbance in streams.

5. Conservation measures should prioritize protecting less disturbed sites, such as natural forest (NF) and tree and tea plantation (TTP) areas, as reference conditions, while implementing targeted rehabilitation in more impacted smallholder agriculture zones as found in this study.

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APPENDICES

Appendix I: Site-level slopes and midpoint heights of normalized abundance size-spectra (NASS) and normalized biomass size-spectra (NBSS) across 24 stream sites under four land-uses: natural forest (NF), smallholder agriculture (SHA), smallholder tea (SHT), and tea and tree plantation (TTP) during wet and dry seasons. R^2 = coefficient of determination.

Site ID	Land-use	Season	NASS slopes	NASS Midpoint heights	NASS R^2	NBSS slopes	NBSS Midpoint heights	NBSS R^2
1	NF	Dry	-1.57	-0.69	0.95***	-0.57	5.3	0.73***
2	NF	Dry	-1.43	-1.1	0.97***	-0.41	4.86	0.77***
3	NF	Dry	-1.43	-0.33	0.94***	-0.43	5.57	0.57**
4	NF	Dry	-1.47	-0.68	0.97***	-0.48	4.99	0.71**
5	NF	Dry	-1.41	-0.15	0.94***	-0.42	5.79	0.60**
9	NF	Dry	-1.28	0.51	0.87***	-0.32	5.91	0.28
13	SHA	Dry	-1.39	-2.16	0.96***	-0.4	4.24	0.67***
14	SHA	Dry	-1.28	-1.08	0.95***	-0.26	3.86	0.46*
15	SHA	Dry	-1.5	-0.25	0.95***	-0.49	5.18	0.68**
16	SHA	Dry	-1.7	-1.35	0.96***	-0.62	4.33	0.77***
17	SHA	Dry	-1.63	-1.04	0.96***	-0.65	4.38	0.83***
18	SHA	Dry	-1.4	-0.88	0.93***	-0.39	4.47	0.48*
6	SHT	Dry	-1.2	0.01	0.95***	-0.22	5.45	0.37*
7	SHT	Dry	-1.3	0.64	0.95***	-0.28	5.6	0.51*
11	SHT	Dry	-1.37	-0.42	0.86***	-0.41	4.96	0.34
12	SHT	Dry	-1.58	-0.62	0.95***	-0.58	5.34	0.78***
20	SHT	Dry	-1.57	-1.02	0.96***	-0.6	4.36	0.80***
21	SHT	Dry	-1.36	1.55	0.92***	-0.4	6	0.51*
8	TTP	Dry	-1.54	-0.56	0.92***	-0.53	5.41	0.60**
10	TTP	Dry	-1.55	-0.26	0.91***	-0.5	5.32	0.59**
19	TTP	Dry	-1.5	-1.5	0.97***	-0.49	4.95	0.79***
22	TTP	Dry	-1.43	-1.76	0.97***	-0.42	4.56	0.68***

Appendix 1 continued

23	TTP	Dry	-1.5	-0.81	0.96***	-0.5	4.64	0.77***
24	TTP	Dry	-1.42	-0.71	0.94***	-0.43	4.2	0.60**
1	NF	Wet	-1.34	-1.19	0.95***	-0.34	4.73	0.51**
2	NF	Wet	-1.49	-1.07	0.94***	-0.47	3.97	0.67**
3	NF	Wet	-1.33	-1.73	0.95***	-0.36	3.89	0.58**
4	NF	Wet	-1.5	-2.04	0.98***	-0.46	3.94	0.80***
5	NF	Wet	-1.44	-1.81	0.98***	-0.44	3.63	0.82***
9	NF	Wet	-1.13	0.39	0.85***	-0.12	5.31	0.06
13	SHA	Wet	-1.5	-0.9	0.95***	-0.52	4.94	0.70***
14	SHA	Wet	-1.09	-2.03	0.85***	-0.11	3.06	0.05
15	SHA	Wet	-1.6	-1.61	0.97***	-0.61	3.7	0.81***
16	SHA	Wet	-1.57	-0.87	0.97***	-0.57	5.08	0.80***
17	SHA	Wet	-1.49	-0.89	0.94***	-0.53	4.52	0.70**
18	SHA	Wet	-1.61	-1.08	0.97***	-0.63	3.84	0.82***
6	SHT	Wet	-1.47	-0.54	0.96***	-0.47	4.86	0.69**
7	SHT	Wet	-1.33	0.08	0.91***	-0.32	4.44	0.34
11	SHT	Wet	-1.31	-0.02	0.89***	-0.3	4.4	0.31
12	SHT	Wet	-1.45	-1.01	0.95***	-0.43	4.42	0.68**
20	SHT	Wet	-1.47	-1.98	0.97***	-0.46	3.92	0.76***
21	SHT	Wet	-1.5	1.23	0.90***	-0.54	4.61	0.58*
8	TTP	Wet	-1.54	-1.66	0.97***	-0.51	4.45	0.78***
10	TTP	Wet	-1.53	-1.76	0.97***	-0.54	4.15	0.82***
19	TTP	Wet	-1.29	-0.83	0.92***	-0.3	5.07	0.39*
22	TTP	Wet	-1.26	-1.8	0.94***	-0.26	4.21	0.40*
23	TTP	Wet	-1.45	-2.29	0.97***	-0.44	4.17	0.76***
24	TTP	Wet	-1.37	-1.47	0.94***	-0.32	3.71	0.45*

Appendix II: List of Identified Aquatic Macroinvertebrate Taxa (Order, Family, and Genus)

Order	Family	Genus
Amphipoda	Paramelitidae	*Paramelita*
Annelida	Oligochaetidae	*Oligochaetes*
Arachnida	Araneae	*Araneae*
Bivalvia	Sphaeriidae (Pisidiidae)	*Pisidium*
Coleoptera	Carabidae	*Trechus*
	Chrysomelidae	*Altica*
	Curculionidae	*Bagous*
		Stenopelmus
	Dryopidae	*Rapnus*
	Dytiscidae	*Copelatus*
		Hydroporus
		Laccophilus
		Rhantus
		Yola sp.
	Elmidae	*Ctenelmis*
		Elmis
		Haplelmis
		Leielmis
		Leptelmis
		Microdinodes sp.
		Potamocares
		Potamodytes
		Pseudancyronyx
		Stenelmis
	Gyrinidae	*Aulonogyrus sp.*
		Dineutus sp.
		Gyrinus
		Orectogyrus
	Haliplidae	*Haliplus*
	Hydraenidae	*Aulacochthebius*
		Hydraena
		Prosthetops
	Hydrochidae	*Hydrochus*
	Hydrophilidae	*Acidocerus*
		Amphiops
		Berosus
		Hydrophilus
	Lampyridae	*Lampyris*
	Limnichidae	*Byrrhinus*
	Melyridae	*Astylus*
	Noteridae	*Hydrocanthus*
	Hydrophilidae	*Helochares*
	Ptilodactylidae	*Ptilodactyla*

Appendix II continue		
	Scirtidae	*Elodes*
	Scarabaeidae	*Rhyssemus*
		Spercheus
	Staphylinidae	*Paederina*
		Stenus
	Helophoridae	*Helophorus*
	Hydrophilidae	*Anacaena*
Decapoda	Potamonautidae	*Potamonautes brincki*
		Potamonautes clarus
Diptera	Anthomyiidae	*Anthomyia*
	Athericidae	*Suragina*
	Blephariceridae	*Elporia*
	Ceratopogonidae	*Ceratopogon*
		Culicoides
	Chaoboridae	*Chaoborus*
	Chironomidae	*Chironomus*
		Tanypus
	Dixidae	*Dixa*
		Dixella
	Dolichopodidae	*Hydrophorus*
	Empididae	*Clinocera*
	Ephydridae	*Brachydeutera*
		Ochthera
	Limoniidae	*Antocha*
		Gonomyia
		Limnophila
	Muscidae	*Musca*
	Psychodidae	*Clogmia sp.*
	Scathophagidae	*Scathophaga*
	Simuliidae	*Paracnephia sp.*
		Simulium
	Stratiomyidae	*Hermetia*
		Stratiomys
	Syrphidae	*Eristalis*
	Tabanidae	*Chrysops sp.*
		Tabanus
	Tipulidae	*Tipula spp.*
Ephemeroptera	Baetidae	*Acanthiops*
		Acentrella
		Afrobaetodes
		Centroptiloides
		Cheleocloeon sp.
		Cloeon
	Caenidae	*Afrocaenis caenis*

Appendix II: Continue

	Dicercomyzidae	*Dicercomyzon*
	Ephemeridae	*Afromera*
	Heptageniidae	*Afronurus*
	Leptophlebiidae	*Aprionyx*
	Oligoneuriidae	*Elassoneuria*
		Oligoneuriopsis
	Polymitarcidae	*Ephoron*
	Prosopistomatidae	*Prosopistoma*
		Ephemerellina
		Lithogloea
	Tricorythidae	*Tricorythus*
Hemiptera	Aphelocheiridae	*Aphelocheirus*
	Belostomatidae	*Appasus*
	Corixidae	*Micronecta*
		Sigara
	Gerridae	*Eurymetra*
		Gerris
		Limnogonus
	Hebridae	*Hebrus*
	Hydrometridae	*Hydrometra*
	Mesoveliidae	*Mesovelia*
	Naucoridae	*Laccocoris*
		Neomacrocoris
	Nepidae	*Laccotrephes*
		Ranatra
	Notonectidae	*Anisops*
		Enithares
	Pentatomidae	*Banasa*
	Pleidae	*Paraplea*
		Plea
	Saldidae	*Saldula*
	Veliidae	*Microvelia*
		Rhagovelia
		Xiphovelia
Hirudinea	Glossiphoniidae	*Helobdella*
		Marsupiobdella
Isopoda	Ligiidae	*Ligia*
Lepidoptera	Crambidae	*Acentria*
		Elophila
		Nymphulinae
		Paraponyx
		Synclita
Megaloptera	Corydalidae	*Chauliodinae*
		Chloroniella peringueyi
		Corydalinae
Mollusca	Ampullariidae	*Lanistes*
		Pila occidentalis

Appendix II: Continue

	Bulinidae	*Bulinus*
	Lymnaeidae	*Lymnaea*
	Physidae	*Stenophysa*
	Planorbidae	*Ancylinae*
		Biomphalaria
	Thiaridae	*Melanoides*
	Viviparidae	*Bellamya capillata*
Nematomorpha	Gordiidae	*Chordodes*
Odonata	Aeshnidae	*Aeshna*
		Anax
	Calopterygidae	*Phaon*
		Platycypha
	Coenagrionidae	*Pseudagrion*
	Gomphidae	*Ceratogomphus*
		Ictinogomphus
		Notogomphus
		Onychogomphus
	Libellulidae	*Bradinopyga*
		Crocothemis
		Notiothemis
		Orthetrum
		Trithemis
		Zygonyx
	Macromiidae	*Phyllomacromia*
	Platycnemididae	*Allocnemis*
	Synlestidae	*Chlorolestes*
Plecoptera	Perlidae	*Neoperla*
Porifera	Spongillidae	*Spongilla*
Trichoptera	Barbarochthonidae	*Barbarochthon*
	Calamoceratidae	*Anisocentropus*
		Heteroplectron
	Dipseudopsidae	*Dipseudopsis*
	Ecnomidae	*Ecnomus*
	Glossosomatidae	*Agapetus*
	Hydropsychidae	*Aethaloptera*
		Leptonema
	Hydroptilidae	*Oxyethira*
	Hydrosalpingidae	*Hydrosalpinx sericea*
	Lepidostomatidae	*Lepidostoma*
	Leptoceridae	*Adicella*
		Athripsodes
		Oecetis
		Setodes
		Triaenodes
	Philopotamidae	*Chimarra*
		Thylakion

	Phryganeidae	*Phryganea*
	Pisuliidae	*Pisulia*
		Silvatares
	Rhyacophilidae	*Rhyacophila*
	Sericostomatidae	*Rhoizema*
Tricladida	Dugesiidae	*Dugesia*

APPENDIX III: SIMILARITY REPORT

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