MACROPLASTICS IN THE ENVIRONMENT: ARE THEY SUITABLE HABITATS FOR MACROINVERTEBRATES IN RIVERINE SYSTEMS?

A thesis submitted in fulfilment of the requirements for the degree of

MASTER OF SCIENCE

Of

RHODES UNIVERSITY

By

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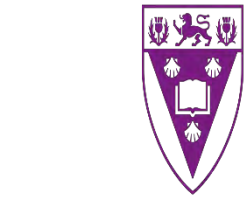

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June 2023

Supervisors:

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ABSTRACT

Emerging pollutants, such as plastics are threat to freshwater ecosystems, and may negatively impact riverine systems. They can modify riverine habitats and affect aquatic organism distribution and composition. Knowledge of how macroplastics alter riverine habitat heterogeneity, and their effects on macroinvertebrate assemblage structure is sparse, especially in Africa. This study examines the effect of hydraulic biotopes on the colonisation, establishment and succession patterns of macroinvertebrates on macroplastic and natural substrates based on the taxonomic and trait-based approach. Four experimental sites from minimally impacted upper reaches of the Buffalo, Kat, Kowie, and Swartkops Rivers in the Eastern Cape of South Africa were selected for the deployment of plastic substrates. Plastics materials, including polyethylene terephthalate (PET) bottles and natural substrate composed of stone and vegetation, were used to formulate three substrate groups: Group 1: 100% natural substrates (NS), Group 2: 50% natural substrates and 50% plastic material (NP), and Group 3: 100% plastic materials (PD). These substrates were placed in litter bags of equal dimension (25 cm by 35 cm, with 2.5 cm mesh) and deployed randomly in three hydraulic biotopes (pools, riffles, runs) over a period of 180 days (October 2021 to April 2022). A total of 216 substrate bags, 54 bags per substrate were deployed per site in the four experimental sites. Twelve bags from each substrate group were retrieved at an interval of 30 days beginning on day 30 after deployment, and analysed for the establishment of macroinvertebrate communities.

Based on composite hydraulic biotope data, Simpson index was significantly higher ($P < 0.05$) for macroinvertebrate assemblage structure on the 50% and 100% macroplastic substrate groups compared to natural substrates. With the exception of Tabanidae, Glossosomatidae, and Psephenidae, all macroinvertebrate taxa recorded showed non-significant positive correlations with all three substrate groups. However, Tabanidae, Glossosomatidae, and Psephenidae showed significant positive correlation with the 100% natural substrates, 50% plastic substrates and 100% plastic substrates, respectively. The parsimony analysis reveal that, within 30 days, all substrate groups underwent similar succession, with high abundance of pioneer taxa which increased on days 60 and 90, and then decreased from days 120 to 180.

For the the pool biotope, Shannon and Simpson indices were significantly higher ($P < 0.05$) for the macroinvertabrates collected over the natural substates compared with those collected on the

macroplastic substrate groups. However, in the riffle and run biotopes, all diversity indices were similar for all substrate groups and no statistically significant difference was observed. Statistically significant higher values for taxonomic richness, diversity, and evenness were found on day 30 to 90 for the riffle biotopes, and day 30 to 60 for the run biotopes. The run biotope presented temporal statistical significant variability in taxonomic composition with different macroinvertebrate communities recorded on days 30 and 60 compared with days 90 to 180. However, in pools and riffles, no temporal variation was observed in the taxonomic composition of macroinvertebrates on all three substrate groups.

The trait-based fuzzy correspondence analysis revealed differential spatial-temporal distribution of macroinvertebrate traits on all three substrate group. The early colonisers i.e. day 30 – 60, were dominated by group of taxa characterised by medium (>10 – 20 mm) and large (20 > 40) body size, flat body, collector-gatherers, free-living, and predators. The late colonisers, collected mainly on day 150 and 180 were dominated by taxa with a preference for high flow velocity (0.3 - 0.6 m/s), permanent attachment, and filter-feeding mode. Traits such as oval and flat body shape, medium body size (>10 - 20 mm), skating and clinging/climbing mobility, temporal attachment, shredders, predators, prey, and plastron and spiracle respiration showed positive correlation with the 100% macroplastic substrates. Filter feeding, crawling, permanent attachment, a preference for fast velocity (0.3-0.6 m/s), and coarse particle organic matter were positively correlated with the 50% macroplastic substrates.

Overall, the results provided critical insights on the impact of macroplastics on the assemblage structure of biological communities by acting as suitable habitats in stream ecosystems. The study elucidated the role of traits of aquatic organisms in mediating the colonisation of plastics substrates, providing insights into the impact of plastics proliferation on riverine ecosystem functioning. Furthermore, the finding provides a baseline insight into the influence of hydraulic biotopes on the colonisation and establishment of macroinvertebrates on macroplastic acting as artificial riverine habitat.

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ABBREVIATIONS AND ACRONYMS

ANOSIM	Analysis of similarity
ANOVA	Analysis of variance
BCMM	Buffalo City Metropolitan Municipality
BHNR	Basic human need reserve
CA	Correspondence analysis
CES	Coastal and Environmental Services
COIA	Co-inertia analysis
DWA	Department of Water Affairs
DWAF	Department of Water Affairs and Forestry
DWS	Department of Water and Sanitation
EIA	Environmental Impact assessment
ER	Ecological Reserve
FCA	Fuzzy correspondence analysis
GA	General authorisation
GSM	Gravel, sand and mud
IWR	Institute for Water Research
MANOVA	Multivariate analysis of variance
MC	Management Class
NP	Natural + Plastic substrate
NS	natural substrate
NWA	National Water Act
NWMS	National Waste Management Strategy
NWRS	National Water Resource Strategy
PCB	polychlorinated biphenyls
PD	Plastic-Dominated substrate
PERMANOVA	Permutational multivariate analysis of variance
PET	Polyethylene terephthalate
POP	Persistent organic pollutants
PRC	Principal response curve

PS	Polystyrene
PVC	Polyvinyl chloride
RDM	Resource-Directed Measures
REC	Recommended ecological categories
RHP	River Health Programme
RQO	Resource Quality Objectives
RSA	Republic of South Africa
SDC	Source Directed Controls
SIMPER	Similarity percentage
UNEP	United Nations Environmental Program
WMA	Water Management Area
WSA	Water Service Act

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to my supervisors, Prof. Oghenekaro Nelson Odume and Dr. Chika Nnadozie, for making my MSc a truly enriching learning experience. They constantly challenged me and provided valuable guidance throughout my studies. I deeply appreciate your valuable advice, guidance, patience, and unwavering support. THANK YOU SO MUCH.

For funding support, I wish to appreciate the African Water Resources Mobility Network (AWaRMN), which has received funding from the Intra-Africa Academic Mobility Scheme of the European Union for funding my MSc degree and the Rhodes University African Cluster Centre for providing funding for the research work. Special thanks also to Rhodes University and the Institute for Water Research (IWR) for providing me with the platform to learn.

My sincere gratitude to Dr. Frank Akamagwuna for his enthusiasm, invaluable mentorship, and unwavering support. He has generously taken the time to teach me new skills. During my time as your adopted student, you consistently provided constructive feedback and kept an open-door policy whenever I sought help with statistics or the project as a whole.

I would like to express my sincere appreciation to my colleagues and friends, who provided invaluable assistance during my fieldwork, laboratory work, and writing stage. I am grateful to Siyabonga, Enahoro, Miracle, Marie, Harriet, Grace, Aphiwe, Havilah, Esther, Sophia, Amy-Grace, Hope, Vuyowethu, Dr. Olivier Amamuddy, and Dr. Darong Gyang for their support. I consider myself lucky to have come across you during my journey to maintain my sanity.

Thank you to the staff and students of IWR, especially Khaya Mgaba, Juanita McLean, and David Forsyth for the administrative and technical support. I really appreciate you all.

Special thanks to my mentor Dr Unique Keke for always believing in and pushing me to be better.

Many thanks to Helen Holeman for proof reading and editing the manuscript, and to the two examiners for the very helpful feedbacks.

I am deeply grateful to my family, especially my parents, Mr. John Philips and Mrs. Cecilia John, as well as my siblings: Mrs. Agatha Oluwagbemi, Mrs. Anthonia Folorunsho, Alpha, Genesis, and Emmanuella, for their unwavering moral support and patience.

Above all, my deepest appreciation goes to God for His unfailing presence and for giving me the strength to face my MSc hurdles. ALL GLORY BELONGS TO GOD.

Collecting permit and ethics approval number for this project: 2022-5286-6498.

DEDICATION

This thesis is dedicated to my niece Saviona Alpha, nephew Lemuel Oluwagbemi, and to all the orphans out there, may doors be open to you at all times.

CHAPTER 1: GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Biodiversity loss in freshwater ecosystems is among the highest compared to other kinds of systems (Dudgeon et al., 2006; Reid et al., 2019). The loss of biodiversity could be attributed primarily to anthropogenic activities such as flow alteration, agriculture, industrialisation, urbanisation, and population growth, which threatens freshwater ecosystems worldwide (Nel et al., 2017). Commonly reported drivers of freshwater biodiversity loss include habitat degradation and destruction, flow alteration, pollution, and invasive alien species (Dudgeon et al., 2006; Darwall et al., 2009; Reid et al., 2019). The increasing scale of freshwater biodiversity loss have led to the identification of new or previously unrecognised threats that have become obvious (Dudgeon et al., 2006; Reid et al., 2019). One such emerging threat to freshwater systems is the proliferation of plastics in these systems (Rochman et al., 2013). Plastic can impact freshwater biodiversity directly or indirectly. For example, the direct effects of plastics include the entanglement of animals such as invertebrates, fish, and birds, or ingesting plastics of different sizes, which reduces their fitness and increases mortality (Windsor et al., 2019; Blettler & Wantzen, 2019; Blettler & Mitchell, 2021). Indirect effects of various sizes of plastics to aquatic biota and habitat may be associated with the release of chemicals absorbed to plastics exposed to the environment or alteration of the habitat heterogeneity of streams, which may impact on biodiversity (Pazos et al., 2017; Blettler & Wantzen, 2019; Windsor et al., 2019). Despite the potential risk posed by plastics to freshwater organisms and habitat heterogeneity, the subject remain considerably understudied in freshwater systems compared to their marine counterparts.

Much of the work done on freshwater plastic research has been on microplastics, with less than 20% on macroplastics (Blettler et al., 2018; Al-Zawaidah et al., 2021). Microplastics are plastics of 0.0001-5 mm, mesoplastic are >5-25 mm, whereas macroplastics are >25-1000 mm (Blettler et al., 2018; Windsor et al., 2019). Studies associated with macroplastics are highly important in freshwater plastic research for at least three reasons. First, macroplastic contributes significantly greater input in terms of mass than microplastics, and these macroplastics are secondary sources of microplastics, which suggests they potentially increase the concentration of microplastics in the

environment (Holzhauer et al., 2016; Schmidt et al., 2017). Second, field-based studies have reported over 100 species of animals entagled by macroplastics, such as discarded fishing lines, bags, food packs and beverage bottles which are common items found in aquatic environment (Windsor et al., 2019; Blettler & Mitchell, 2021), suggestings freshwater organisms can be impacted by macroplastics and therefore are at risk. Lastly, pioneer studies have highlighted the presence of macroplastics in different compartment of freshwater ecosystems (Morritt et al., 2014; Liro et al., 2020; van Emmerik & Schwarz, 2020). These macroplastics are abundant in riverbed, riverbank, water surface, and are occasionally trapped within materials such as macrophytes and boulders, or buried in riverbed sediments (Liro et al., 2020; van Emmerik & Schwarz, 2020). These riverine components serve as habitats for several freshwater organisms, thus, the presence of macroplastics in rivers may alter riverine physical habitat integrity (Thompson et al., 2009; Al-Zawaidah et al., 2021; Azevedo-Santos et al., 2021; Ribeiro-Brasil et al., 2022). To date, limited studies in this area have yielded invariable results for better understanding macroplastics effects on riverine habitat and biota (Wilson et al., 2021).

The few studies conducted on the effect of macroplastic altering riverine habitats and macroinvertebrate composition have focused on pre-existing macroplastics or have been associated with single river network, and these studies typically examined the effects at a single point in time (Czarnecka et al., 2009; Wilson et al., 2021). These studies reported that, pre-existing macroplastic provided habitats for macroinvertebrates that need hard surface in areas where they are scarce, leading to a diverse and considerably different taxonomic composition from the surrounding natural habitats (Czarnecka et al., 2009). However, there is an absence of research that considers the temporal variability by comparing macroplastic substrates to natural substrates and the value of hydraulic biotopes (pools, riffles, runs, etc.) for understanding and monitoring these effects. This is important because, temporal dynamics in relation to macroplastic such as rugosity, persistence, and potential to accumulate detritus in streams may affect the composition of macroinvertebrates observed at different times (Verdonschot et al., 2012). Temporal and spatial heterogeneity of hydraulic biotopes is also fundamental in shaping riverine macroinvertebrate communities, because different riverine macroinvertebrates prefer different flow environments and substrates, which are characteristics of hydraulic biotopes (Jowett, 2003; Monk et al., 2008; Thirion, 2016). Additionally, some macroinvertebrates may respond to macroplastic substrates as a function of their morphological traits and preference to varying ecological conditions (Taurozzi

et al., 2023). Despite the role of temporal variation, hydraulic biotopes and macroinvertebrate traits in influencing the turnover of taxa within macroinvertebrate communities (Reid & Thoms, 2008; Silva et al., 2014; Wilson et al., 2021), not much consideration has been given to how they influence macroinvertebrate responses to the presence of macroplastic in rivers.

The present study is the first investigation into the impact of macroplastics on riverine habitat heterogeneity and macroinvertebrate response at the hydraulic biotope scale in Africa. The study contributes to knowledge of the role of temporal variation, hydraulic biotopes and macroinvertebrate traits in relation to macroinvertebrates colonisation, establishment and succsssion patterns on macroplastics relative to natural substrates in minimally impacted river sites. The study is critical because hydraulic attributes and flow environments hierarchically structure riverine systems, thereby influencing the distribution of macroinvertebrates. Furthermore, the organism's traits and preference for a particular habitat determine the persistence of that organism in its habitat, because, at a microhabitat scale, a macroplastic substrate may create a platform that will naturally filter organisms based on their combination of traits. Thus, this study examines and compared macroinvertebrate assemblages structures on macroplastics relative to natural substrates. It also explores the influence of hydraulics biotiopes and traits on the colonisation, establishment and succession patterns of macroinvertebrates on macroplastics and natural substrates. The study was conducted in minimially impacted sites in the Buffalo, Kat, Kowie, and Swartkops Rivers of Eastern Cape, South Africa. The choice of minimally impacted sites was to minimise the effect of other pollution on the interactions between macroinvertebrates and macroplastics. The rest of this Chapter is a literature review, beginning with South Africa's water resource management framework, followed by a description of plastic contamination in freshwater habitats, biomonitoring of riverine habitats, the rationale and significance of the study, the aim and objectives of the study. The chapter ends with the thesis structure.

1.2 Water Resource Management in South Africa

South Africa's water resource management is a critical issue owing to the country's arid climate, a growing human population, and increasing demands on water resources (DWA, 2013). The National Department of Water and Sanitation (DWS) is responsible for sustainable water resource management in South Africa through policy enactment and directives for equitable, sustainable

and efficient water resources use (DWA, 2010). The legislative framework steering water management in South Africa is the National Water Act (NWA; Act No. 36 of 1998) and the Water Services Act (WSA; No. 108 of 1997; RSA, 1998). The WSA deals with the government delivery of water-related services, such as the provision of potable drinking water. At the same time, the NWA governs the management, conservation, control, protection, and use of water resources (DWAF, 2013). The NWA provides two legally binding water rights, one for human needs and one for maintaining the ecological functions of aquatic ecosystems (Odume, 2022). The NWA mandates the Minister to develop the National Water Resource Strategy (NWRS) for the effective and coordinated management of water resources in South Africa.

The NWRS is vital in achieving the objectives of the NWA by providing a vision for the sustainable use and protection of water resources in South Africa and it is revised every five years. The NWRS identifies critical challenges such as water scarcity, pollution, and climate change and outlines measures to address these challenges (RSA, 1998; DWA, 2013). The resource directed measures (RDM; measures directed towards protecting the water resource) and the source directed controls (SDC; instruments used to restrict and control water resource use) are the two complementary strategies developed to achieve the objective of balancing use and protection (RSA, 1998; DWA, 2013). The NWRS also highlights the importance of stakeholder participation in water resource management to achieve equitable access to water resources (DWAF, 2006).

1.2.1 Strategies for Water Resource Management in South Africa

Water resource management in South Africa involves a range of strategies to ensure the sustainable use and management of water resources (DWAF, 2013). As stated earlier, two complementary strategies employed in this regard to achieve the foundational values of the Act are the resource directed measures (RDM) and source directed controls (SDC) (RSA, 1998; DWAF, 2013). The RDM and SDC are essential strategies for water resource management in South Africa.

Resource Directed Measures (RDM)

The resource directed measures (RDMs) refer to the implementation of measures aimed at protecting and managing the quantity, quality, biota, geomorphology, and habitats of water resources needed for humans and the aquatic environment while ensuring socio-economic growth and development (DWAF, 2004). The RDM measures are implemented are targeted at the water

resources and they include the classification system and classification of every significant water resource, determining the Reserves, and resource quality objectives (RQOs) as summarised in Table 1.1.

Table 1.1: Resource directed measures instruments and application to water resource in South Africa.

RDM instruments	**Overview**
Classification System	A national system of classifying water resources that considers the need to balance ecological requirements and socio-economic development.
Classification of Water Resource	The classification process of every significant water resource into one of three management class: Class I, II and III. Water resources that are minimally used with the overall ecological conditions barely altered from pre-development belong to Management Class (MC) I. Class II are moderately used, and the overall ecological condition has somewhat deviated from pre-development conditions. Class III is heavily used, and the overall ecological condition has deviated considerably from pre-development levels (Department of Water Affairs, 2010).
Determination of the Reserves	The Reserve evaluates water requirements based on quantity, quality, and reliability of supply to fulfil human needs and maintain aquatic ecosystem function (Hughes, 2005). The Reserve consists of two components: the Basic Human Need Reserve (BHNR) and the Ecological Reserve (ER). The BHNR meets people's basic needs for drinking water, cooking, and sanitation, while ER ensures that the quality and quantity of water are maintained to protect and ensure aquatic ecosystem function (King & Pienaar, 2011; Odume, 2022). These components are essential for ensuring effective water resources management.
Determination of the Resource Quality Objectives (RQOs)	RQOs involve setting standards for water resources quality based on their MCs and recommended ecological categories (REC) (DWS, 2016; DWA, 2013). By translating MC and ecological needs determined in

	the Reserve into measurable objectives, the RQOs ensure that progress is accessed towards attaining the desired future condition of water resources (King & Pienaar, 2011).

Source Directed Control

Source directed controls (SDC) use several tools to enforce limits on water resources by water users at individual and corporate levels. The SDC strategy aims to protect water resources from over-exploitation to maintain the desired level of protection by linking the protection of water resources and the regulation of their use. The DWS has progressively implemented several SDC tools, such as general authorisation (GA) and licensing, water-use limits, environmental impact assessment (EIA), and water quality regulations to prevent pollution of water resources through enforcement or economic incentives. These tools are regulated at varying levels of government, indicating the importance of all levels of government in protecting water resources in South Africa (Odume, 2022). Some SDC instruments used in handling the impact of development on water resources are summarised in Table 1.2.

Table 1.2: Source directed control instruments applied to reduce the impact of development on water resources in South Africa.

Instruments	Summary
EIA	Globally, EIA is a practice aimed at understanding the environmental impact of developments and devising means for mitigating such effects.
Water use licence	Licences are control instruments outside the GA and Schedule 1 uses and those confined to lawful use. The Act permits various conditions to be written into and specified in a licence based on the type, nature, extent of water use, potential risk, and severity. Users issued a licence are required to adhere strictly to the conditions of the licence.
Existing lawful use	Before the enactment of NWA, water lawfully used under the previous Act was still maintained by the users.

Water quality standards	Water quality standards are incorporated into the water use licence as part of the licence conditions, which are legally binding and enforceable by the regulators.
Incentive programmes	Government uses incentive programmes such as the Green Drop Certification programme (DWA, 2013) to encourage compliance by the wastewater sectors to minimise their impact on water resources.
General Authorisation (GA)	The GA is a mechanism recognised as part of the Act that allows certain large groups and sections of society to use water for specific purposes without needing individual licences. A GA user must register their water use and abide strictly by its provision, revised periodically in the Government Gazette.
Water use charges	The water use charges involve two instruments: first, the water resource management charge solely enforces the polluter-pay principle plus the user-pay principle. Second, waste discharge charge systems aim to make polluters pay according to waste load and the potential impact of the waste released in the immediate environment.

The combined implementation of the RDM and SDC instruments ensures that water resources are protected and used equitably, sustainably and efficiently. However, the presence of pollutants of emerging concerns such as plastics, antibiotics, pharmaceuticals and other industrial and agricultural chemicals represent a severe threat to the ecology of many rivers in South Africa. Pollutants of emerging concern, such as plastics, pose a significant threat to the biotic and physical features of riverine systems. For example, plastic debris can accumulate and alter streamflow, causing changes in sediment transport and deposition (Blettler & Mitchell, 2021; Carson et al., 2011; Windsor et al., 2019; Honingh et al., 2020). These changes can affect aquatic organisms, the physical habitat, and the overall structure and function of aquatic ecosystems (Akoumianaki et al., 2008; Green et al., 2015; Katsanevakis et al., 2007). Green et al. (2015) reported a decrease in the abundance of macrofaunal species beneath plastic debris compared to demarcated natural areas with no plastic. These findings highlight the need to understand and mitigate the impact of plastic

pollution in riverine systems. The government has recognised the importance of addressing plastic pollution and has developed policies and strategies to manage its impact. For example, the National Waste Management Strategy (NWMS, 2019) set targets for reducing plastic waste from source and decreasing its effects on aquatic ecosystems. Although this strategy has been developed, very little research effort has gone into understanding the ecological impact of macroplastics in riverine systems in South Africa. Thus, the need for the current study.

1.3 Plastic Pollution in Freshwater Ecosystems

1.3.1 Sources of plastic pollution in freshwater systems

Globally, plastic production has increased significantly in recent decades, with an estimated 410 million tonnes (mt) of plastic produced in 2021 alone (UNEP, 2022). With the current global plastic production trend, plastic production is projected to reach 1100 mt by 2050 (PlasticsEurope, 2020; UNEP, 2022). Although most plastic production occurs in Asia, Europe, and North America (PlasticsEurope, 2020), plastic production is also increasing in Africa, with some countries experiencing increasing plastic demand due to population growth and urbanisation (Lebreton & Andrady, 2019; UNEP, 2022; Chitaka et al., 2022). As plastic production increases in these countries, so does the potential for increased plastic pollution in freshwater ecosystems.

Plastic pollution is a growing environmental problem, and freshwater ecosystems are particularly vulnerable because riverine ecosystems typically flow in valleys and lower-elevation terrain, which makes them the primary destination of many types of pollutants entering watersheds (Azevedo-Santos et al., 2021). Africa remains a significant contributor (7% in 2019) to global plastic production, with five countries (Egypt, Nigeria, South Africa, Algeria, and Morocco) listed in the top 20 countries as major waste-generating countries contributing to marine and freshwater plastic pollution (Jambeck et al., 2015; PlasticsEurope, 2020).

Plastic pollution is a critical environmental issue in Africa and, indeed, the rest of the world, with plastic waste often littering rivers, lakes, and other freshwater bodies, especially in urban areas (Lebreton & Andrady, 2019; Dalu et al., 2019; 2020; Chitaka et al., 2022). Much of this plastic waste is generated from inadequate waste management practices, as many African countries lack the proper infrastructure to manage plastic waste effectively (Dalu et al., 2020). For example, as

of 2019, South Africa was producing 2 mt of plastic from raw materials and recycled plastic, 70% of which was collected; however, 58% (110 kt) of the collected plastic waste leaks into the environment owing to mismanagement (Dearmitt, 2020; Peano et al., 2020). Plastic waste management is a monumental challenge because most mismanaged plastics find their way into the environment, and subsequently to freshwater ecosystems where they are either trapped within these systems or discharged into the nearby coastal area (Faure et al., 2015; Jambeck et al., 2015; Liro et al., 2020; van Emmerik et al., 2022).

Freshwater plastic pollution research is still in its infancy although it has gained increased recognition in the last decade. Despite the growing concern about plastic pollution in freshwater ecosystems, there is still a lack of understanding about the impact of different sizes of plastic on the biodiversity of in-stream habitats and on general riverine ecology. The size ranges of plastic are mega-, macro-, meso-, micro-, and nano-plastics with dimensions of >1 m, >25 mm-1 m, 5-25 mm, 0.001–5 mm, and <100 nm, respectively (Al-Zawaidah et al., 2021; UNEP, 2022), and they affect the riverine biome differently. Akindele et al. (2021) analysed 60 items of African plastic research literature and found a bias towards microplastic studies (80%) relative to macroplastic, although both sizes are equally detrimental to aquatic health and ecosystem functioning. Several studies have highlighted the consequences of plastics on biodiversity at the individual level, including ingestion of plastics and entanglement of organisms (Katsanevakis et al., 2007; Gallitelli et al., 2021; Pisani et al., 2022), adsorbing toxic compounds from the environment (Li et al., 2022; Wang et al., 2017), and transporting invasive species (Rech et al., 2016). While the information at individual-level effects cannot be trivialised, evidence of impact at higher levels of biological organisation (e.g., species assemblages, communities, and populations) is often crucial to policymakers since it can assist in forming policy decisions.

Macroplastics (>25-1000 mm) in the environment are pervasive and often in their original form (e.g., beverage bottles and shopping bags; Blettler et al., 2017). They are found in different riverine compartments, such as the water column and riverbed, hence, macroplastics can potentially impact habitat morphology. For example, van Emmerik and Schwarz (2018) noted that plastics are treated similarly to biotic and abiotic river components, such as tree trunks, boulders, sand, and gravel. While comprehensive studies on the interaction of hydraulic structures, vegetation, fauna, and in-stream wood with riverbeds exist, only a few studies exist on the influence of macroplastics on

freshwater habitat (e.g., Wilson et al., 2021; Gallitelli et al., 2023). Macroplastics are as complex as these components owing to their heterogeneous sizes, shapes, densities, and polymer types (Gabbott et al., 2020; Geyer et al., 2017). These characteristics enable macroplastic interference with natural processes and may adversely affect natural freshwater systems. Literature on river macroplastics research is growing, with most research interest in the quantification, fate, and transport of floating, suspended, and riverbank macroplastics (Al-Zawaidah et al., 2021; van Emmerik & Schwarz, 2020). However, evaluating how plastics act as artificial aquatic habitats and the scale of their ecological impact in rivers is critical. This understanding is fundamental in regions where plastic pollution is most prevalent, particularly in South Africa and the rest of the continent.

1.3.2 Factors that influence ecological risk posed by macroplastics

Macroplastics pose specific ecological risks to aquatic ecosystems. It is essential to comprehend these threats, looking at both the direct effects and the indirect consequences on aquatic creatures, such as altered habitats, biota diversity and composition. The ecological risks posed by macroplastics are influenced by several factors, including persistence, size and shape, chemical composition, and the habitat geomorphology of where the plastic is present.

Macroplastic persistence

The persistence of macroplastics in the environment can lead to long-term exposure of aquatic organisms to plastic pollution. Like natural substrates and energy in riverine systems, plastics are widely transported (Jambeck et al., 2015; Meijer et al., 2021). However, plastic remains persistent compared to natural substrates (such as allochthonous organic matter) that quickly degrade with time and are nutritionally beneficial to biota. This persistence of plastic in the environment is due to its durability and lightweight properties (Barnes et al., 2009; Thompson et al., 2009), a persistence that contributes to its wide distribution. Several studies (Ryan et al., 2014; Schwarz et al., 2019) have shown that plastics can carry fouling organisms both short and long distances to a new habitat, as reported in marine ecosystems (Kiessling et al., 2015; Bravo et al., 2011). Some plastics are trapped or buried in sediment load and may remain exposed to organisms in those zones for an unknown duration (Liro et al., 2020; van Emmerik et al., 2020). The longer

macroplastic persists, with all its detrimental tendencies, the higher the chances of interacting with organisms and associated ecological impacts.

Macroplastic size and shape

The size and shape of macroplastics can affect their interaction with aquatic organisms. The bulk of larger macroplastics can physically obstruct the movement of aquatic organisms, while organisms can ingest smaller macroplastics. Numerous studies (Azevedo-Santos et al., 2021; Blettler & Wantzen, 2019; Pazos et al., 2017) highlight adverse ingestion of plastics by aquatic and terrestrial organisms based on the size of the plastics and the organism involved. Blettler et al. (2019) and Pazos et al. (2017) reported a 100% record of plastics in the intestinal gut of fish species from their studies in South America. They attributed this interaction to the detritivore feeding mode of the species and the abundance of plastic particles within the sampled areas (Blettler et al., 2019; Pazos et al., 2017). Larger macroplastics have been observed to entangle larger organisms such as freshwater turtles and birds (Ryan, 2018).

Smaller freshwater organisms (algae, macroinvertebrates, and small fish) are able to use plastics as new habitats, similar to rock, macrophytes, and river debris dams. Gallitelli et al. (2023) reported macroinvertebrate encrusting within and outside plastic bottles and related biota colonisation. Open bottles can trap drift-encrusting organisms and threaten biosecurity as local species become invasive elsewhere. Some organisms may settle in the inside of plastic bottles for a long time and outgrow the circumference of the opening or become buried along with such plastics (Gallitelli et al., 2023; Kolenda et al., 2021; Liro et al., 2023). The inhospitable condition inside these containers may also lead to the mortality of these organisms (Kolenda et al., 2021).

Macroplastic chemical composition

The chemical composition of macroplastics can affect their toxicity to aquatic organisms. Plastics include several compounds, either naturally or added during manufacturing, which may leak out of the plastic matrix into the surrounding environment. Persistent organic pollutants (POPs) like brominated flame retardants and polychlorinated biphenyls (PCBs) can accumulate in the tissue of organisms and spread through the food web (Koelmans et al., 2014). These plastics-related chemicals have been found in humans, birds, and marine life (Rochman et al., 2013; Talsness et al., 2009). Chemical sorption onto plastics depends on environmental conditions such as

temperature, pH, salinity, chemical characteristics, and polymer type (Teuten et al., 2009), while the duration of macroplastic in the river network affects substance discharge and retention. For example, Endo et al. (2005) found that PCBs in plastic pellets on Tokyo beaches increased with residency time of plastic.

Macroplastic and habitat complexity

The characteristics of the habitat where macroplastics are present can influence the ecological risks they pose, while the nature of the habitat influences the residence time of macroplastics. In regions characterised by high water flow (such as run and riffle), macroplastics are susceptible to fragmentation into microplastics due to the mechanical abrasion caused by plastic collision with river structures (e.g., rocks, plants), water discharge, and transported particles (Liro et al., 2020; van Emmerik et al., 2022; Mellink et al., 2022). This fragmentation can result in a more influential ecological impact due to the increased level of microplastics in the environment. By contrast, plastics are quickly buried within regions of slow or still flows due to sediment accretion and biofouling, which adds to the density of the plastic (Liro et al., 2020; van Emmerik et al., 2022; Mellink et al., 2022). Such burial may increase plastics' retention time, increase the chances of biota interacting with plastic with its harmful effects, and facilitate biological degradation (Andrady et al., 2022; Menzel et al., 2021; Schwarz et al., 2019). Gallitelli et al. (2023) hypothesised that more organisms would readily colonise plastic bottles in river pools and that polyethylene terephthalate (PET) bottle deterioration enhances colonisation (Gallitelli et al., 2023). Habitat alteration by macroplastics can lead to changes in the physical structure of the habitat and changes in water quality and hydraulic zones that naturally structure macroinvertebrate assemblages.

1.3.3 Macroplastics as potential hydraulic biotope disturbance

Ecological theories guide investigations of organism-environment interactions. Rivers and streams are the result of a spatial and temporal equilibrium geomorphological processes (Leopold, 1962; Arthington, 2012), and the hydrology comprising water flow and riverbed properties is crucial to freshwater ecosystems (Silva et al., 2014; Hynes, 1968). The abiotic dynamics of hydraulic biotopes affect food and resource availability, substrate type, and organism adaptation (Biggs et al., 2005; Reid & Thoms, 2008), creating specific hydraulic biotopes that favour certain

macroinvertebrate species (Thirion, 2016). For example, pools with non-perceptible water currents, allow fine sediments and detrital matter to settle, enabling biota with the right morphological and functional traits to use the resources and habitats in pools (Shearer et al., 2015).

Bed roughness influence hydrualic biotopes structuring the biotic communities (Wadeson & Rowntree, 1998). Microorganisms, algae, and macroinvertebrates colonising riverbed macroplastics may change the hydraulic biotope; for example, biofilm development on macroplastics may increase their surface roughness, transforming the surrounding habitat's hydraulic roughness and drag coefficient (Downes et al., 2000; Schmocker & Hager, 2013). Furthermore, macroplastic complexity, accumulation and fragmentation may change the hydraulic biotope physically and chemically. For instance, Honingh et al. (2020) found that sizeable macroplastic accumulation modifies water velocity and flow direction, affecting sedimentation patterns and aquatic life dispersion.

Riverine macroplastics may affect established and colonising species in hydraulic biotopes. Macroplastic may influence hydraulic biotopes in the same manner as debris dams by creating diverse habitat types through retention of allochthonous and autochthonous substances, reduction of local flow velocity, and modification of channel morphology (Zhou et al., 2019). For example, macroplastic in pools can smoothen substrates like sediments and detrital debris, disturbing local species and enabling invasion by non-native species (Zettler et al., 2013). Physical changes presumably impact space competition. In the intertidal shores of Ireland, Green et al. (2015) found that biodegradable and conventional macroplastics depleted primary productivity and organic matter, reducing soft-bottom invertebrate abundances. In Italy, primarily pool-dwelling organisms colonised 95 out of 100 beverage bottles sampled in the river (Gallitelli et al., 2023). Plastics may also boost taxonomic richness (Shabani et al., 2019; Carson et al., 2013); in riffle and run, macroplastics may offer a surface for colonising species (Wilson et al., 2021; Gallitelli et al., 2023) but may not be beneficial because, once in the environment, macroplastic breaks down into microplastic with a high tendency to infiltrate the food webs (Valentine et al., 2022). Invasive species may outcompete native species for space and resources and alter ecosystem structure and function when exploiting macroplastic substrates (Zettler et al., 2013; Green et al., 2015). Like filter feeders, invertebrates need substrate stability in runs and riffles to feed on floating debris

(Jowett, 2003; Olivier et al., 2009; Shearer, 2015). Plastic substrates may readily be washed downstream, which might harm inhabiting organisms (Liro et al., 2020; van Emmerik et al., 2022).

Hydraulic biotopes may also impact riverine plastic colonisation. The importance of substrate heterogeneity and hydraulic biotopes on the distribution of biological diversity and habitat quality has been highlighted by several studies (Reid & Thoms, 2008; Zhou et al., 2019). Other studies have also stressed the combined examination of both components – substrate complexity and hydraulic conditions – in monitoring river habitat quality to represent natural patterns and provide a greater understanding of biotic responses (Silva et al., 2014; White et al., 2017). To develop management strategies that minimise negative impacts on freshwater ecosystems while maintaining ecological integrity, monitoring and assessing habitat quality in freshwater systems follows various approaches (Bonada et al., 2006). One approach used in ecosystem monitoring and assessing riverine habitat quality is biomonitoring.

1.4 Biological Monitoring of Riverine Systems

Biological monitoring, or "biomonitoring″ is based on the understanding that resident biota (plants, algae, animals, and microorganisms) can be used to determine the health of an ecosystem (Rosenberg & Resh, 1993; Bonada et al., 2006; Sumudumali & Jayawardana, 2021). Biomonitoring of ecosystem health (water quality, quantity, and available habitat) determines how changes at sub-organismal (genes, cells, and histopathology), organismal, population, community, and ecosystem levels differ from their natural state owing to anthropogenic stressors (Bonada et al., 2006). Biomonitoring is used to search for and identify bioindicators whose presence or absence, abundance and diversity, and behaviour indicate environmental conditions (Bonada et al.,2006). Biomonitoring of river health uses phytoplankton, macrophytes, fish, and macroinvertebrates as bioindicators (Li et al., 2010; Sibanye-Stillwater, 2019; Wang et al., 2021) because of their abundance, diversity, ubiquitous distribution, and ease of collection and identification at the family level, compared to species-level identification, which may not be feasible for specific taxa. In addition, these bioindicators reflect environmental conditions over time; they can detect differential pollution responses, even if no primary pollutants are released (Bonada et al., 2006; Sibanye-Stillwater, 2019; Wang et al., 2021). Furthermore, they are easily seen with the naked eye, spend most or part of their entire life cycles in water, and live on/in a

variety of substrates (e.g., vegetation, stones, gravel, sand, and mud; Dickens & Graham, 2002; Bonada et al., 2006), and, with the intensification of plastic pollution in urban rivers, macroplastics may also provide a substratum for macroinvertebrate dwelling (Wilson et al., 2021; Gallitelli et al., 2023).

Macroplastics as habitats for macroinvertebrates

Macroplastic can potentially alter riverine habitat structure and complexity that essentially determine community diversity and composition (Clifford et al., 2006; Verdonschot et al., 2012; Natsumeda & Iguchi, 2019). Macroplastics act as novel habitats for macroinvertebrates, similar to natural substrata (rocks, macrophytes, wood trunks, leaf litter, etc.) in rivers, particularly because macroinvertebrates readily colonise introduced surfaces as long as they are non-toxic (e.g., artificial substrate samplers; Booth et al., 2013). While the surge in freshwater macroplastic pollution research increases with a focus on source, fate, floatation, temporal storage and sinkage of macroplastics in rivers (Liro et al., 2020; van Emmerik et al., 2022), little is known about the colonisation, establishment, and succession pattern of macroinvertebrates macroplastic artificial habitat in rivers (Wilson et al., 2021; Gallitelli et al., 2023). The limited studies so far have recorded macroplastic effects on physical freshwater habitats to include a decrease in suitable habitats (Czarnecka et al., 2009), taxa-specific density alteration, biological diversity loss, community structure changes (Green et al., 2015), and as a trap for macroinvertebrates (Gallitelli et al., 2023). While this interaction of macroinvertebrates and macroplastics persists, several factors may impact how macroplastics provide habitat for macroinvertebrates. Such factors include, but are not limited to, macroplastic size and exposure duration, colonisation patterns, hydraulic conditions, etc.

The size of macroplastics can reach several metres. Regardless of size, organisms colonise macroplastics and this have severe ecological impacts (Thiel et al., 2018). In marine ecosystems, several studies (Carson et al., 2013; Shabani et al., 2019; Cesarini et al., 2022) revealed a positive correlation between marine litter size and the taxonomic richness of the encrusted community. They reported higher taxonomic richness on larger surfaces due to the provision of sufficient surface area for diverse macrofauna. The researchers noted that discarded items such as fishing gear, for example, were more likely to be inhabited by a diverse biota before being disposed of (Carson et al., 2013; Shabani et al., 2019; Cesarini et al., 2022). In the freshwater environment,

macroplastics are trapped or form part of this substrate in headwaters and foothill streams, where organic and inorganic substrates such as wood and boulders are abundant (Liro et al., 2022; 2023). Macroplastics of varying sizes can also trap sediment, debris, and leaf litter, creating a rich microhabitat (providing shelter, refuge, and food) for macroinvertebrates, similar to debris dams (Zhou et al., 2019). Sediment, debris, and fouling accumulation may contribute to macroplastic persistence and river flow resistance. This may extend macroplastic time in rivers and foster macroinvertebrate colonisation of macroplastics.

Available food sources, available microhabitats, and the dispersal of local assemblages by active or passive drifting facilitate macroinvertebrate colonisation of newly created habitats (Mackay, 1992; Winking et al., 2014; de Donnová et al., 2022). Plastics are hydrophobic, so they are especially prone to biofouling which can lead to the colonisation of microbes and, subsequently, macro-organisms, as seen in the case of the plastisphere in the marine environment (Amaral-Zettler et al., 2020, 2021). Although macroinvertebrates readily colonise clean surfaces without a biofilm, the initial colonist assemblage composition determines the fouling community's subsequent succession (Bravo et al., 2011). For organisms to survive on macroplastics for an extended time, inhabitant macroinvertebrates must overcome specific food acquisition and attachment challenges (Bravo et al., 2011; Kiessling et al., 2015), and because spatial and temporal hydraulic conditions change at small intervals, macroinvertebrate colonisation patterns might be continually reset on non-stable macroplastic substrates. Plastic instability under high flows is also a concern for biodiversity protection. It is, therefore, crucial to evaluate how hydraulic biotopes influence macroinvertebrate colonisation of macroplastics in riverine systems.

Monitoring and assessing the intensifying plastic pollution in freshwater ecosystems is necessary. Macroinvertebrate-based biomonitoring approaches and tools (e.g., taxonomy- and trait-based) have been developed and applied in many countries, including South Africa. Thus, macroinvertebrate taxonomy- and trait-based analyses at the family taxonomic level were employed to evaluate macroinvertebrate response to macroplastic novel habitat quality from different hydraulic biotopes.

Taxonomy-based approach

Taxonomy-based monitoring identifies macroinvertebrate taxa in aquatic systems to measure ecosystem health. This approach is based on the premise that certain macroinvertebrates are more

sensitive to habitat changes in freshwater systems than others so that macroinvertebrate community composition can indicate physical habitat integrity (Odume et al., 2015; González-Trujillo & Alonso-Moreno, 2020). In light of this, biotic indices that include quantitative taxa diversity measures (e.g., taxa richness, evenness, and diversity) and qualitative data on the sensitivity of individual taxa are used to monitor rivers (Rezende et al., 2014).

Taxonomy-based biomonitoring, however, has some drawbacks. First, the variability of taxa identities across different biogeographic regions limits the extent of taxonomic studies (Statzner & Bêche, 2010). Second, using taxonomic identities does not enhance comprehension of the underlying causal mechanisms responsible for the impacts (Ippolito et al., 2012; Collins & Fahrig, 2020). Finally, several studies have linked macroinvertebrate composition with environmental factors. However, the explained variance in these associations is often low, ranging from 5% to 30% (Harris & Heathwaite, 2012). With these limitations, ecologists have been exploring alternative approaches to biomonitoring. One such approach that has gained momentum recently is using macroinvertebrate traits.

Traits, colonisation, and establishment of macroinvertebrates on macroplastics

Traits refer to an organism's behavioural, morphological, physiological features, or life history that are indicative of its biology (McGill et al., 2006; Poff et al., 2006; Odume, 2020). The habitat template model of Southwood (1977, 1988) establishes a relationship between an organism's trait and its external environment. The theory posits that the habitat serves as a template for developing characteristic life-history strategies through evolution (Southwood, 1977, 1988). Identifying trait features that strongly impact ecosystem processes and contribute most to functional diversity is the basis of how traits are connected to ecosystem functioning (Piliere et al., 2015). This linkage may help provide a framework for understanding how organism composition and diversity respond to ecosystem functioning and ecosystem service delivery (Astegiano et al., 2015). For instance, macroinvertebrates suited to high flow rates have streamlined bodies and strong attachment structures. In contrast, those adapted to low flow have flattened bodies and weak attachment structures (Truchy et al., 2015). Traits may help explain the success and persistence of a particular organism in its habitat and have been successfully used to study the vulnerability and resilience of organisms to several natural and anthropogenic disturbances in freshwater systems (Milesi et al., 2016a; Frainer et al., 2018; Akamagwuna et al., 2019; Paz et al., 2022; Taurozzi et al., 2023).

Traits may also help evaluate environmental and biodiversity threats posed by plastic pollution (Al-Zawaidah et al., 2021).

Organisms such as macroinvertebrates with suitable traits may be selectively filtered from the local species pool to coexist on macroplastics by habitat structure at the microhabitat scale. Consequently, habitat structure and macroinvertebrate functional composition may be closely correlated (Poff, 1997; Heino, 2005). Typically, the complexity of a habitat determines how efficiently macroinvertebrates can move around within the habitat and utilise its resources (food, shelter, or refuge) which is evident in the behaviours, feeding strategies, and food types that macroinvertebrates use in a structure that the trait-based approach may reveal.

Macroinvertebrates with a particular trait or a combination of trait attributes are likely to persist in novel macroplastic habitats. While some macroinvertebrates freely roam diverse environments for food, others adopt various attachment techniques to adhere to surfaces. Macroinvertebrate assemblages that live in macroplastic environments probably have a number of specific behaviours, such as attachment and filter feeding (Gallitelli et al., 2023). Filter feeders cling to solid objects like wood, pebbles, and macrophytes to feed on transported debris. Given their little or no nutritional value, macroplastics may also act as rigid substrates for macroinvertebrates, so enabling the attached macroinvertebrates to rely on food from moving water. However, attachment problems might occur when unstable macroplastics are remobilized or change locations due to hydraulic circumstances (Bravo et al., 2011), or when transport particles scour the macroplastic during high discharge (Cooper & Corcoran, 2010; Andrady et al., 2022). Macroplastic fragmentation may also alter the microchemical environment necessary for a creature to respire (Katsanevakis et al., 2007; Akoumianaki et al., 2008; De Souza Machado et al., 2018). As a result, it is assumed that macroinvertebrates with specialised respiratory equipment, such as teguments and breathing tubes, may benefit more from macroplastics than trait attributes such as respiratory gills that are sensitive to depleted oxygen levels.

Despite the potential effects of macroplastic pollution on riverine habitats and the predictive role of traits in underpinning the mechanistic response of organisms to stressors, no research has used traits to comprehend the mechanism mediating macroinvertebrate communities on macroplastics in the Afrotropical region. Thus, a multidimensional trait-based method was utilised to analyse macroplastic macroinvertebrate communities and identify indicator species, traits, and ecological

preferences influencing macroinvertebrate-macroplastic interactions in the selected Afrotropical rivers.

1.5 Rationale and Significance of the Study

Plastic proliferation and its negative ecological effect pose a threat to biological assemblage structure. Macroplastic proliferation in rivers can shape in-stream hydro-morphological characteristics (Honingh et al., 2020; van Emmerik et al., 2022), necessary for conserving biological diversity and the functioning of riverine habitats. Numerous studies (Windsor et al., 2019; Wilson et al., 2021; Gallitelli et al., 2023) demonstrated that the characteristics of plastics (size, shape, density, form, etc.) determine their different interactions with the physical, chemical, and biological environment in different ways. In riverine habitats, macroplastics add to substrata roughness, provide rigid substrates for organisms in depositional zones and trap detritus and sediment particles in high-flow zones. As a result, hydraulic variation and habitat complexity may be impacted.

As a consequence of macroplastic proliferation, macroinvertebrate assemblage structure and composition may vary according to substrate-hydraulic conditions, exposure duration, and macroplastic characteristics (e.g., Wilson et al., 2021; Gallitelli et al., 2023). In a Gallitelli et al. (2023) study, most macroinvertebrates colonising PET bottles were pool dwellers and were tolerant of low water quality. The authors also noted a correlation between macroinvertebrate abundance and degradation level, possibly resulting from duration of exposure to macroplastic (Gallitelli et al., 2023). Depending on the composition of the initial colonists, some macroinvertebrates may be able to colonise the macroplastic, while others may find it difficult. Additionally, different macroinvertebrates may colonise macroplastics at different times of exposure, possibly owing to the gradual development of biofilms on macroplastics, which contribute to macroplastic surface rugosity and provide food and habitats for different macroinvertebrates with different preferences for surface rugosity or food resources. It is imperative to pinpoint the factors underlying these responses to understand better how macroplastics could impact riverine habitats and macroinvertebrates.

Factors such as topography and hydraulic biotopes of riverine ecosystems can influence the nature and magnitude of transport and storage of macroplastics across riverine ecosystems. Hydraulic

biotopes can control the level of interaction (i.e., colonisation and establishment of biota community) of macroinvertebrates with their habitat. It can also impact the exposure duration of organisms to their habitat and the stability of that habitat (i.e., the duration for which the habitat, such as macroplastic, might remain stable and available to macroinvertebrates). For example, because of the infrequent changes in hydraulic conditions within hydraulic biotopes, established macroplastic macroinvertebrate communities will continually reset to the initial stage or be covered by sedimentation during higher discharge or sediment deposition (van Emmerik et al., 2022; Liro et al., 2020). However, information on the colonisation of macroplastics at the hydraulic biotope scale, which is characterised by varying attributes (flow, substrates, etc) and plays a vital role in the distribution of substrates, biological assemblage, and energy within riverine systems, remains a constraint.

In addition to improving our knowledge of plastic proliferation and the suitability of macroplastics as habitats for macroinvertebrates, and understanding how hydraulic biotopes influence this interaction, it is vital to understand how macroinvertebrate traits respond to macroplastic proliferation. Macroinvertebrate traits are essential in revealing the adaptation of macroinvertebrates to the environment (Zhu et al., 2020). For example, because macroinvertebrates have physical habitat preferences, their traits determine how they adapt to their environment (Taurozzi et al., 2023; Poff et al., 2006). The traits also determine how macroinvertebrates evolve to adapt to the environment by reflecting their survival strategies under natural selection pressure over time. These strategies may include the ability of macroinvertebrates with specific traits to establish on macroplastics and withstand local and external disturbance, or their ability to recover after a disturbance in their local habitat.

An investigation of the macroplastic impact on riverine habitat heterogeneity and macroinvertebrate response at the hydraulic biotope scale in Africa is presented here for the first time. The study contributes to knowledge of the role of temporal variation, hydraulic biotopes, and macroinvertebrate traits in relation to macroinvertebrate colonisation, establishment, and succession patterns on macroplastics relative to natural substrates in minimally impacted river sites. The study is critical because hydraulic attributes and flow environments hierarchically structure riverine systems, influencing macroinvertebrate distribution. Furthermore, the organism's traits and preference for a particular habitat determine its persistence. This is because,

at a microhabitat scale, a macroplastic substrate may create a platform that naturally filters organisms based on their combination of traits.

1.6 Aim and Objectives of the Study

1.6.1 Aim

This study aims to investigate the effect of hydraulic biotopes on macroinvertebrate colonisation, establishment and succession patterns on macroplastic and natural substrates based on the taxonomic and trait-based approach in minimally impacted sites in the Eastern Cape of South Africa.

1.6.2 Objectives

1. To examine and compare macroinvertebrate assemblage structure on macroplastic and natural substrates in four selected, minimally impacted sites.
2. To assess the influence of hydraulic biotopes on the taxonomic assemblage structure of macroinvertebrates on macroplastics and natural substrates in minimally impacted upper reaches.
3. To analyse the distribution of macroinvertebrate traits and ecological preferences on macroplastics and natural substrates in minimally impacted headwater reaches.

1.7 Thesis Structure

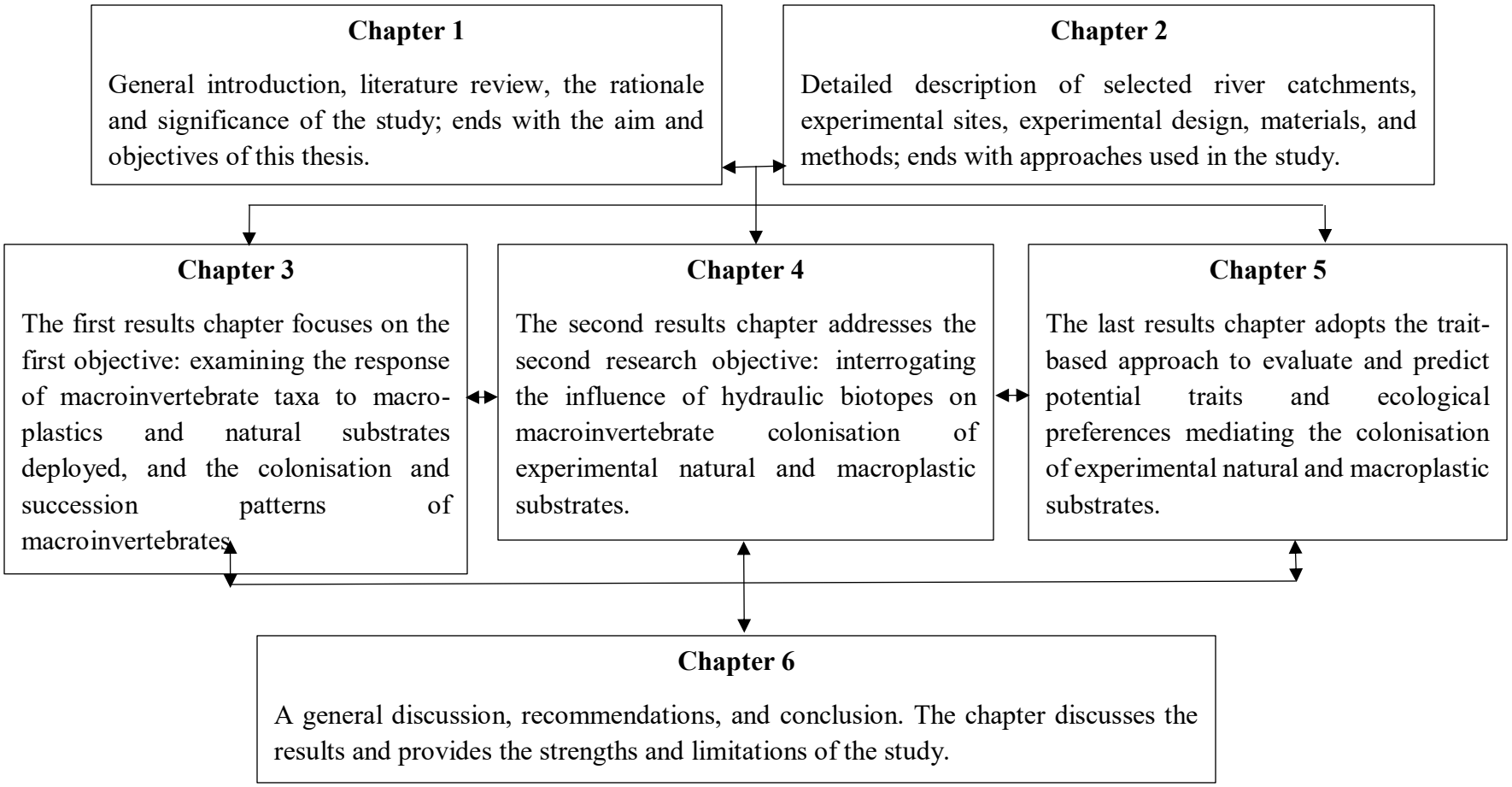

Figure 1: Graphical presentation of the thesis structure showing the connection between chapters and a brief overview of each chapter.

CHAPTER 2: GENERAL MATERIALS AND METHODS

2.1 Introduction

This chapter summarises the river catchments and experimental sites. The geology, soil, topography, vegetation, climate, and ecological importance of each river catchment are given, together with each experimental site physical, chemical, and habitat descriptions. This chapter also summarises the study materials and procedures.

2.2 Study Area and Site Descriptions

The four river catchments studied are within the Eastern Cape Province of South Africa (Figure 2.1). The river catchments include the Kowie, Buffalo, Kat, and Swartkops. All river catchments fall within Mzimvubu to Keiskamma Water Management Area (WMA) based on the proposed reconfiguration from nine to six WMAs in the third National Water Resources Strategy (NWRS-3, 2022).

In each river, one sampling site was selected in the headwaters. The sites represent near-pristine water quality conditions with minimal anthropogenic disturbances. Anthropical activities such as urbanisation, informal settlements, and industrialisation lead to the accumulation of emerging contaminants, such as plastics, in river systems, which can alter the natural condition of river quality (Corenblit et al., 2007; Gurnell et al., 2016). The initial visual assessment of the river catchments placed the sites in the Kat and Swartkops rivers as slightly impacted by anthropogenic activities (citrus and livestock farming, and sand mining, respectively) more than the Kowie and Buffalo rivers. However, all sites were considered suitable for the experiment as they were the least impacted points closest to the source. Near-natural sites were selected to minimise the effect of pollution and other disturbances on the colonisation of macroinvertebrates on macroplastic and natural substrates. The availability of diverse hydraulic biotopes (pool, riffle, and run) within the site was also considered in site selection.

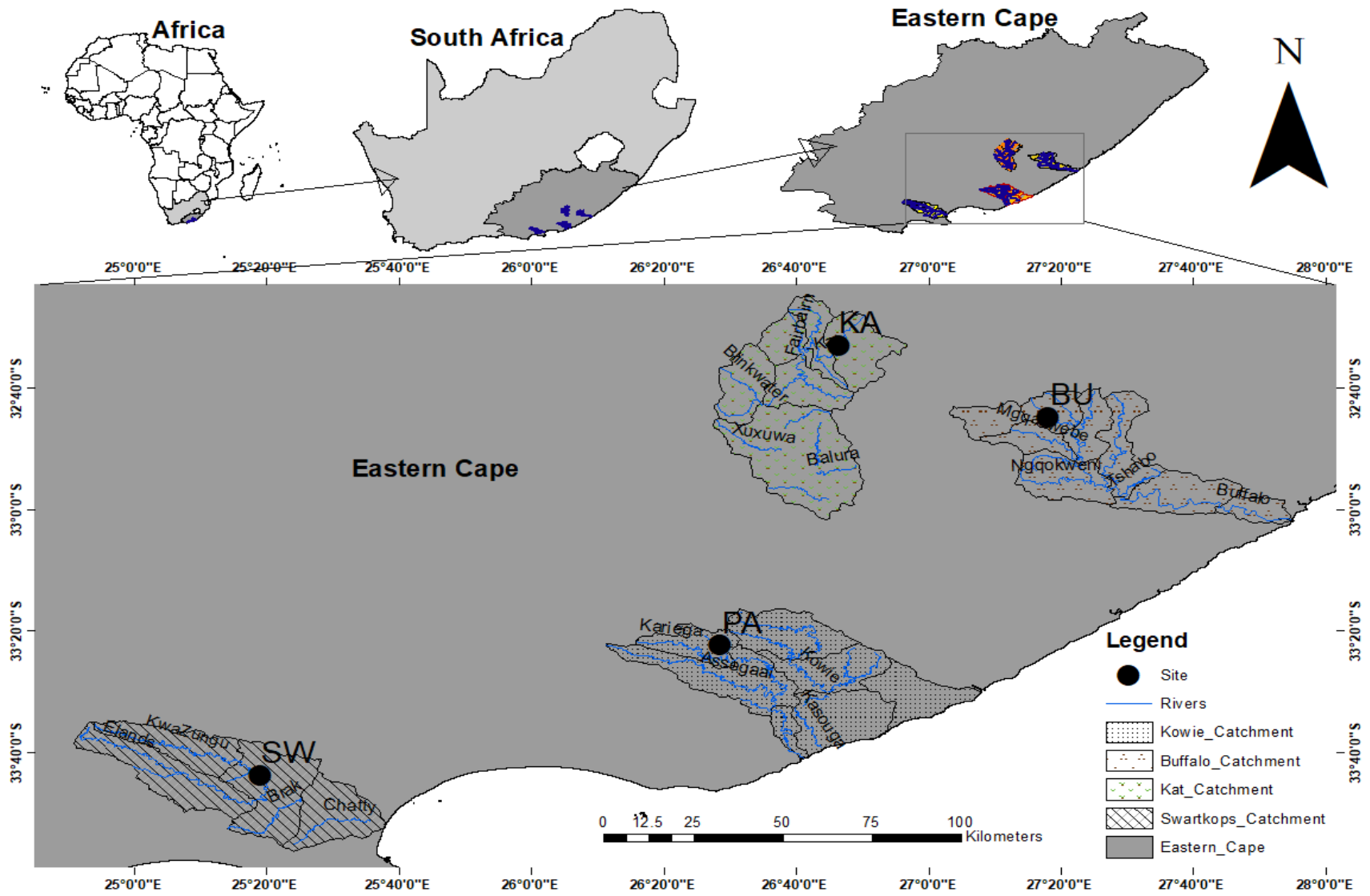

Figure 2.1: Map of the study catchment areas within the Eastern Cape Province of South Africa, demarcated on the map of Africa, South Africa and the Eastern Cape. Rivers: Site PA (Kowie River), Site BU (Buffalo River), Site KA (Kat River), and Site SW (Swartkops River).

2.2.1 The Kowie River Catchment and Experimental Site

The Kowie River location (PA) (33.371006° S, 26.476634° E), lies 353 m above Thomas Baines Nature Reserve (Figure 2.2). The Palmiet River was chosen as the least-impacted headwater tributary of the Kowie River. Kowie River's main tributaries are Palmiet, Lushington, and Bloukrans. Boulders, cobbles, pebbles, gravel, sand, and mud (GSM) characterise the experimental location in-stream. Large fallen trees from bush burning, high rainfall, and wash-off events cause stream bank erosion during floods. Boulders, stones, and marginal and riparian vegetation make up the run. Large rocks and riparian flora border the pool. The pool bed has plant detritus, rocks, marginal or riparian vegetation, and settled sand and mud.

The Kowie River lies in a subtropical climate zone in the Makana Municipality, Eastern Cape Province of South Africa, with a catchment area of approximately 800 km^2 (Figure 2.2; Whitfield' et al., 1994). The Kowie River has its source in the hills of Grahamstown and runs for approximately 70 km before discharging into the Indian Ocean at Port Alfred (Rivers-Moore et al., 2010; Sinchembe & Ellery, 2010). In most of its catchment, the river meanders through the Bokkeveld shales. Water supply to the river is through groundwater influx and direct rainfall, with an average annual rainfall of 650 mm (Whitfield et al., 1994; Dalu et al., 2016), mainly summer-fed (November – March) (Mangadze et al., 2019).

The geology of the Kowie River belongs to the Cape and Karoo and Cenozoic deposits (Smetherham et al., 2019). The Cape Supergroup comprises Bokkeveld Groups and Witterberg Groups (Palmer et al., 2004). The soil composition varies considerably due to the geology and topography, with Karoo systems comprising a range of well-structured to a mix of coarsed and unstructured shallow and nutrient-poor soils. The Cape supergroups yield coarse textured rocks and shallow rocky soil, greatly influencing water quality (Smetherham et al., 2019).

The vegetation comprises five biomes: the thicket, Nama-Karoo (Southern Karoo Riviere), grasslands (Bedford Dry grassland), forest (Southern Mistbelt Forest), and fynbos (Suurberg Quartzite Fynbos, Suurberg Shale Fynbos) (Palmer et al., 2004). The essential vegetative unit is the Albany Thicket, comprising Great Fish Thicket, Kowie Thicket, Albany Broken Veld, Great Fish Noorsveld, and Eastern Cape Thornveld (Palmer et al., 2004).

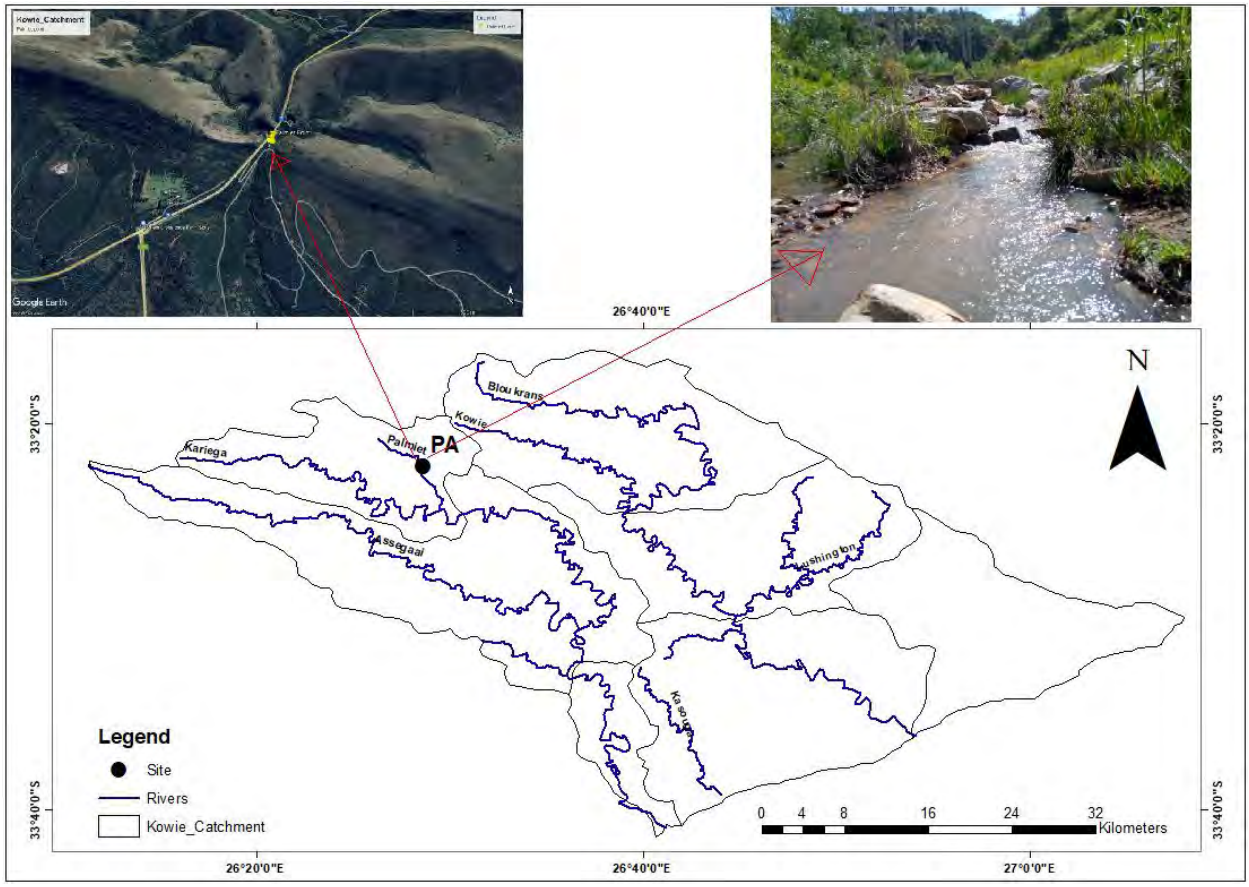

Figure 2.2: The map of Kowie River Catchment with Google Earth land cover map (top left) and site photograph (top right)

The leading cause of anthropogenic disturbance in the upper reaches of the Kowie River and its tributaries include suburban and informal settlements and agricultural activities. The middle reach occasionally receives partially treated sewage. Wastewater effluent predominates the primary water quality deterioration in the middle reach of the river (Wutor et al., 2009; Gininda, 2016). The lower reaches of the Palmiet tributary drain a protected area (Bloukrans Nature Reserve) privately owned by the community. As the river passes through the nature reserve, it forms a pool preserved for recreational and religious ceremonies by local communities.

2.2.2 The Buffalo River Catchment and Experimental Site

The Buffalo River is in the Buffalo City Metropolitan Municipality (BCMM) in Eastern Cape Province, South Africa (Figure 2.3). The river is about 126 kilometres long and covers a catchment area of 1287 km^2 (The National River Health Programme RHP, 2004). The river originates from

the seeps and sponges of the Amathole Mountains at an altitude of 1200 m. It is joined by the Mgqakwebe River upstream of King William's Town (Qonce) and the Ngqokweni River at Zwelitsha as a major western tributary. From the north, another important tributary (Yellowwoods River) meets the Buffalo River, where they feed into the Laing Dam (Mandela, 2016). The river flows 40 km from the Laing Dam into the Bridle Drift Dam, which forms a confluence with the Tshabo tributary at Mdantsane and continues for another 20 km before discharging into the Indian Ocean at the estuary harbour of East London (Mandela, 2016).

The Buffalo River site, BU (32.743906° S, 27.302445° E), was selected in the upper reach at an altitude of 530 m between the Maden and Rooikrans Dams at the foot of the Amathole mountains. The land cover in this area makes it a forested area. The presence of the Maden Dam upstream of the site did not considerably affect the river's flow owing to the fast flow observed throughout the study period. Several hydrological factors affect river plastic transport, including water level, flow velocity, and discharge (van Emmerik et al., 2020; Honingh et al., 2020). However, in the Buffalo River catchment, no management strategies are in place to control the simulation of seasonal flow except for overflow from Maden Dam and a crack in the Rooikrans Dam (Mandela, 2016).

The in-stream characteristic of the Buffalo experimental site includes large fallen trees, boulders, cobbles, and pebbles with significant organic matter in the form of plant debris due to erosion during flood events. The run zone consists of boulders, cobbles, and stones, with fallen wood and riparian vegetation. In contrast, the pool bed comprises settled sediments with riparian aquatic macrophytes on bedrock surfaces. The riffle zone is of intermediate depth with a smooth water flow; the bed property includes bedrock with boulders and cobbles and very little trapped debris between cobbles.

The upper catchment is rural, but the river drains a predominantly urban and industrialised area through King William’s Town (Qonce), Zwelitsha, Mdantsane, and East London (RHP, 2006). The major tributaries of the Buffalo River include the Mgqakwebe River, Ngqokweni River, Yellowwoods River, Tshabo River, and Mncotsho River, which joins the river in various towns from the upper-reach to mid-reach. Natural vegetation is scarce owing to industrialisation and urbanisation in the Buffalo catchment, except for the forests in the upper parts, which are also vulnerable to fire outbreaks.

The Buffalo River's primary raw water users are the Buffalo City Metropolitan Municipality (BCMM) comprising King William's Town, East London, Bhisho, Breidbach, Berlin, Zwelitsha, Mdantsane, public works, textile, and informal and rural dwellers. Subsistence farming occurs in the middle reaches, with less than 1% intensive irrigation of fresh produce and crops such as lucerne (Mandela, 2016). The lower reaches consist of the Umtiza Coastal Nature Reserve (~ 560 ha of natural forest), coastal forest, and the East London harbour. Generally, dryland cultivation is practised in approximately 8% of the catchment associated with the four dams, which delineates the importance of the river to the entire catchment (Mandela, 2016).

The river catchment has a warm and temperate climate, with mean annual temperatures of 21°C and 18 °C in the coastal and inland zones, respectively (Mandela, 2016). The catchment receives mainly summer rainfall, with a mean annual rainfall of about 736 mm, with 40% of the run-off influenced by the upper reaches (Mandela, 2016).

The Buffalo River Catchment geology consists of rocks of the Balfour Formation and Middleton Formation (Adelaide Subgroup, Beaufort Group). The soil content comprises primarily minimally developed soils, apparent on hard and weathering rocks (Calcisols, Leptosols, Durisols, and Regosols). Over 78% of the catchment is overlaid by sedimentary rocks of the Lower Beaufort Series (Adelaide Subgroup) of the Karoo Systems (Mandela, 2016), with dolerite outcrops located at Evelyn Valley, below Rooikrans Dam, and on the northern and southern rim of the middle and lower catchment (Mandela, 2016). Such underdeveloped soil content may contribute to sediment accumulation within the river course and, thus, the likely burial of plastics.

Four vegetation types – Fynbos, Yellowwood, grassland, and Acacia karoo – dominate and are widely distributed in the Buffalo River catchment (Zuma, 2010). Seven per cent of the catchment area is covered by indigenous forest, with an additional 4% surrounded by pine and blue gum plantations (Mandela, 2016).

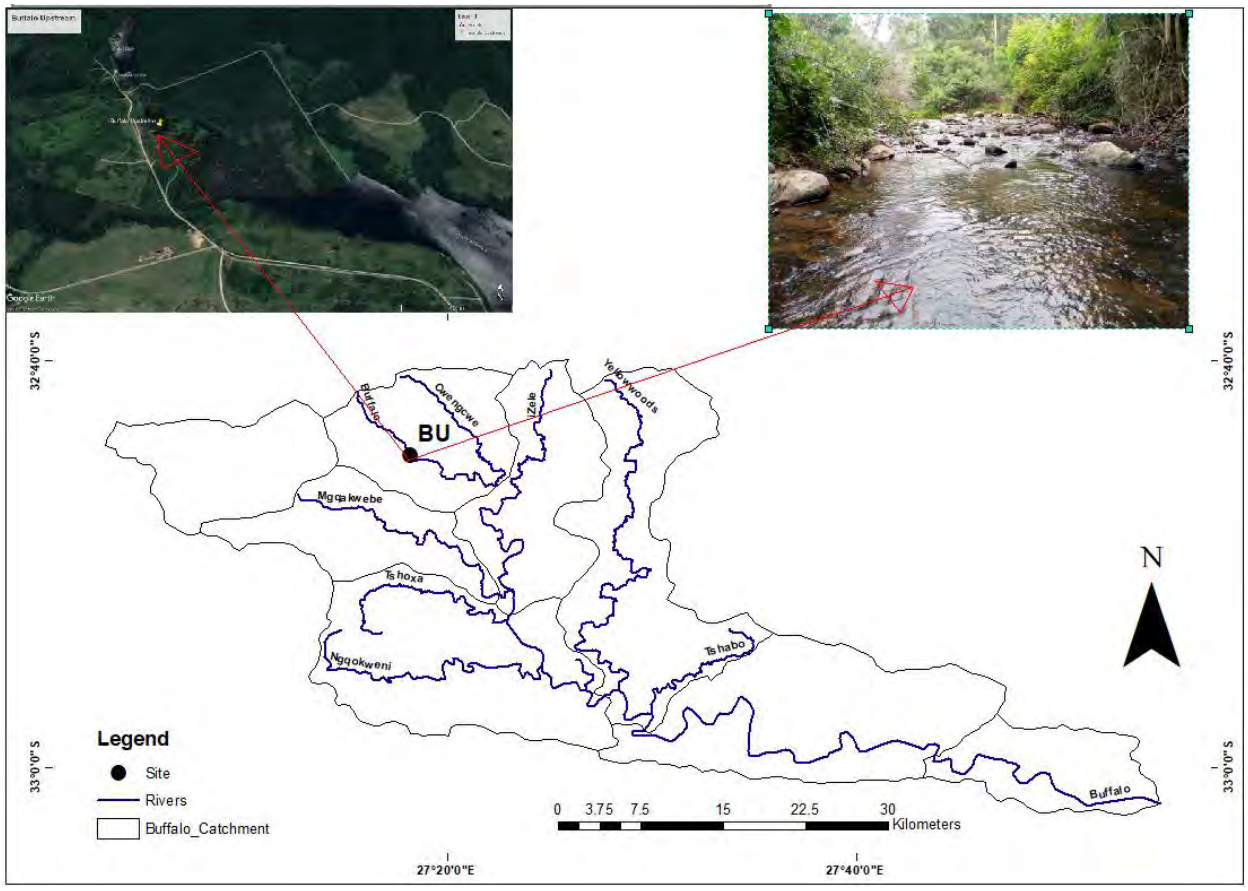

Figure 2.3: Map of the Buffalo River catchment with Google Earth land cover map (top left} and picture of the Buffalo site within the upper reaches (top right).

2.2.3 The Kat River Catchment and Experimental Site

The Kat River catchment area lies in Nkonkobe Municipality in Amathole District, Eastern Cape, with the river source from the Hogsback Mountains in the northeast and the Katberg Mountains in the northwest through Seymour and Fort Beaufort (Figure 2.4). The catchment covers an area of approximately 1,700 km^2, and the Kat River is a major tributary of the Great Fish River. Balfour, Fairbairn, and Blinkwater streams are the main tributaries of the Kat River, which flows south for about 90 km before discharging directly into the Great Fish River and then to the Indian Ocean at its estuary, northeast of Seafield.

The socio-ecological importance of the Kat River to the immediate municipality includes water for agriculture (irrigation and livestock farming) and spiritual, domestic, and recreation sites. However, non-native invasive plant species and arable farming have been reported to impact its

integrity (Mgaba 2018). Cultivation may increase the susceptibility of the surrounding soil to get into the headwater streams, leading to sediment accretion, reduction in the level of transportation of matter downstream, and habitat loss in the river. Additionally, it is likely to bring about the burial of plastics in such systems, thus increasing the plastic's duration in such a river.

The Kat River site KA (32.546921° S, 26.774686° E), is at an altitude of 770 m. The site is situated in the headwaters, upstream of the Katriver Dam; the most extensive bulk water infrastructure in the Kat River catchment, located northwest of the catchment, is in Seymour. The in-stream characteristics include marginal plants, mainly of the *Cyperaceae* spp., and riparian vegetation of trees, shrubs, and grasses, making the site a forested zone. The riffles are smooth-running waters except for run-off events with small pebbles and GSM characteristics. The runs comprise boulders, cobbles, pebbles, and GSM with high flow and marginal vegetation.

The climate of the river catchment is described as sub-humid in the north to semi-arid in the south. The catchment's mean maximum and minimum annual rainfall ranges between 1200 mm and 400 mm (Schoombe et al., 1997). The maximum mean daily temperature varies from 30 ºC in February to 21 ºC in July, while the minimum mean daily temperature varies from 17 ºC in January to 8 ºC in July (Motteux & McMaster, 2002; Mgaba, 2018).

The river catchment area geology belongs to the Beaufort series of the Karoo, at the Molteno, Red Bed, and Cave Sandstones Stages, with shale, mudstones, sandstone, and limestone as dominant rocks (Lerotholi, 2005). The minimum and maximum altitudes in this catchment area range from 600 m to 1600 m from the confluence point with the Fish River to the source of the Kat River, respectively (Geological Map of Southern Africa, 1970). The dominant vegetation includes the Valley Bushveld and River Thicket type, with pockets of Afromontane Forest and grassland vegetation evident at high altitudes (Mgaba, 2018).

Various land use characterises the catchment, ranging from the export of citrus farm produce and commercial rangeland stock farming to small-scale vegetable and crop production as well as stock farming. Commercial farmers are mainly located in the middle Kat and lower Kat, whereas smallholders and emerging farmers primarily practice agriculture in the upper catchment.

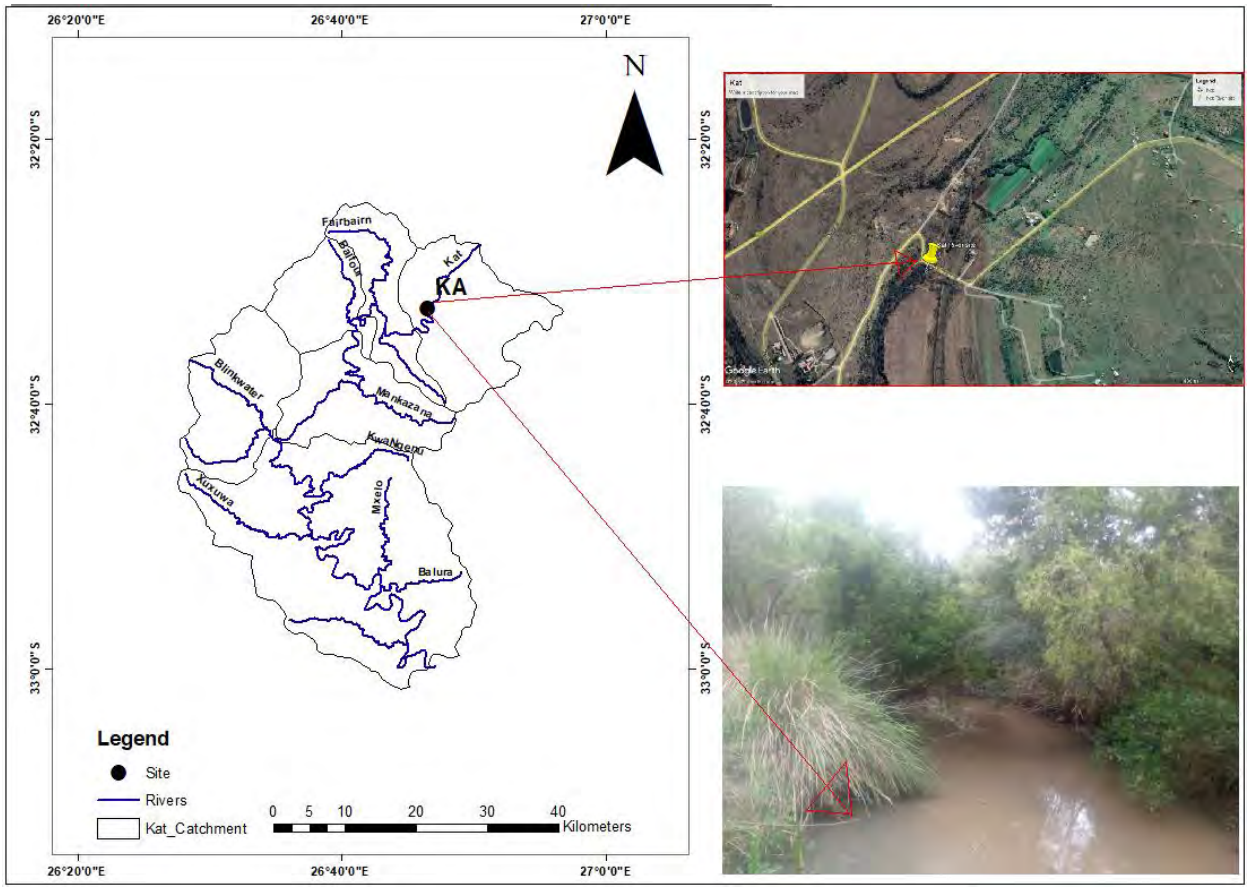

Figure 2.4: Kat River catchment boundary with Google Earth land cover map (top right) and site photograph (bottom right).

2.2.4 The Swartkops River Catchment and Site

The Swartkops River originates in the Groot Winterhoek Mountains in the Eastern Cape, South Africa (Figure 2.5). The Swartkops River emerges from the confluence of the KwaZunga River to the north and the Elands River to the southwest, just above Uitenhage, in an area called Kruisrivier (Odume et al., 2012). The river is a 155 km river system with a catchment area of about 1555 km² (Haigh, 2002). Two small tributaries (the Brak and Chatty Rivers) join the river in the middle and lower reaches, north of Port Elizabeth, before emptying into the Indian Ocean at Algoa Bay in Gqeberha (Port Elizabeth) (Odume et al., 2012).

Among its ecological benefits, the river supports an estuary that provides critical breeding areas for water birds and fish (Odume et al., 2012). Despite this, the river suffers varying degrees of human-induced impacts, including industrial and domestic effluent discharges, impoundments,

deforestation, and agricultural land use within its catchment (DWAF, 2003; Odume et al., 2012). The upper catchment is protected by a nature reserve (Groendal Nature Reserve); however, sand mining occurs just below the nature reserve. This process will likely impact the water quality and habitat due to rock blasting and the destruction of the surrounding floral communities. The middle reaches comprise mainly agricultural and industrial activities, and informal and formal residents and industries make up the lower regions from Uitenhage town further downstream to the river mouth (Odume et al., 2012).

The Swartkops River site, SW (33.728962° S, 25.317314º E), was selected just below the Groendal Nature Reserve at an altitude of 70 m (Figure 2.5). Within the Swartkops River, this site represents the best available condition owing to less interaction with anthropogenic activities. The heterogeneity of this site's biotopes could be considered good, with the runs and riffles characterised by fast-running waters, stones, pebbles and medium-sized boulders, and riparian vegetation of shrubs and grasses. The pools consist of mud-covered cobbles, and the marginal vegetation comprises floating water hyacinths and submerged *Cyperaceae* spp. The site flows through a privately owned sand mining group with heavy machinery transporting broken rocks for crushing through the water.

The climate of the Swartkops River catchment is warm and temperate, with a mean daily temperature of about 6 °C in July and 27 °C in January (Nelson Mandela Bay Municipality, 2008). The catchment is an all-year-round rainfall area with a minimum monthly average of 60 mm to the highest rainfall events in June and October of about 760 mm (Nelson Mandela Bay Municipality, 2008; Odume et al., 2012).

Geologically, the Swartkops River catchment consists of an upper base of cretaceous shale and mudstones. The base is overlain by marine sedimentary deposits in the high regions by alluvial deposits on the flood plain (Odume et al., 2015). The upper catchment is of Table Mountain Group quartzite, with weakly consolidated cretaceous shales of marine origin found in the lower reaches (Odume et al., 2015).

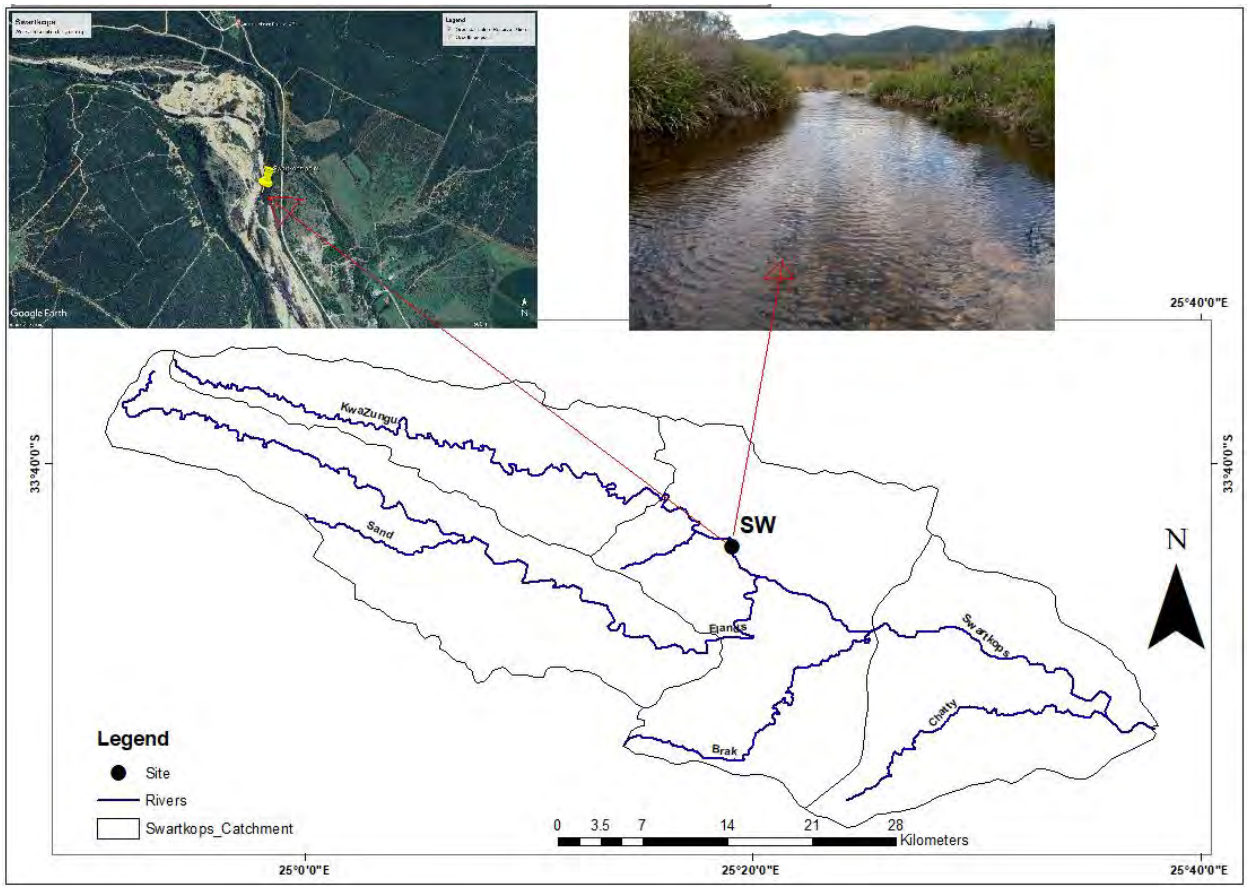

Figure 2.5: The Swartkops River Catchment map with Google Earth land cover map (top left) and picture of the site (top right).

The soils of the Swartkops River Catchment are mainly alluvial, derived from the Uitenhage group (Haigh, 2002). Sand, loam, and clay of consolidated sand dunes and lime-rich sandy clay are easily erodible and are the significant soil texture present, making the catchment suitable for agriculture (DWAF, 1996).

The natural vegetation in the Swartkops River catchment is dominated by the Bushveld or Succulent thicket (Kleynhans et al., 2005). However, extensive encroachment of exotic or invader vegetation such as *Acacia* spp has severely impacted this vegetation. (black wattle and Port Jackson willow) and *Eucalyptus* spp. (gum trees) (DWAF, 2006, Odume et al., 2012).

2.3 Field Work

2.3.1 Experimental design

An *in-situ* field experiment was conducted in the four experimental sites described in Section 2.2. Polyethylene Terephthalate (PET) bottles were deployed at each site for 180 days, from October 2021 to April 2022 (Figure 2.6a). The PET is referred to as riverbed plastic owing to its higher density than water, which makes it sink into the riverbed (Dhaka et al., 2022). Before deployment, the plastics were washed with hydrochloric acid and deionized water using the Institute for Water Research (IWR) standard acid wash protocol. The washed plastic materials were cut into 7 x 7 cm sized pieces and placed into plastic litter bags (dimension: 25 cm in width by 30 cm in length, and mesh size of 25 mm; Figure 2.6B). The sizes ensured easy colonisation of the plastic substrate by providing sufficient surface area without the risk of being trapped, guaranteeing free movement of macroinvertebrates (in and out) of the plastic litter bags.

Three substrate compositions were formulated. The first substrate group comprised 100% natural substrate (NS), the second substrate group a combination of natural substrates and plastic substrate (NP) in equal proportions (50:50%), and the third comprised 100% plastic materials – the plastic-dominated (PD) substrate (Figure 2.6 C-E). These formulations ensured a plastic gradient from natural (0%) to 50% to 100% plastic materials in the litter bags. The natural substrates (NS) were composed of stones, debris, and vegetation, with no plastic materials added (Figure 2.6E). Substrate materials in these litter bags mimic the natural condition of the river systems, thus colonisation is hypothesised to reflect those naturally occurring within the system without plastic materials. The bags containing natural substrates and plastic materials were composed of a mixture of natural substrates and PET bottles in equal proportions (50%:50% of natural and PET bottles) (Figure 2.6D). The combination explains the role of habitat patches made of natural and plastic substrates. Recent studies show that macroplastics submerged in water columns are sometimes trapped by large cobbles and aquatic vegetation such as water hyacinth (van Emmerik et al., 2019) and sometimes buried by sediment accretion (Liu et al., 2021). This combination is intended to simulate a condition that assumes macroplastics are trapped in large stones, vegetation and boulders. The 100% macroplastic substrates (PD) contain 100 g of weighed PET bottles with no

natural substrates introduced experimentally (Figure 2.6E). The PD substrate logically imitates a situation where plastic clogging takes over a habitat (van Emmerik & Schwarz, 2020).

A total of 54 bags (NS: 18, PD: 18 and NP: 18) were deployed per site during the experimental period. For each substrate category, six bags per hydraulic habitat – riffles, runs and pools – were deployed in a randomised manner (Figure 2.7) because the in-stream conditions and biotic assemblage structures within each habitat tend to differ (Wadeson & Rowntree 1998). The deployed materials spanned different seasons to capture seasonality (spring, summer, and autumn; from October 2021 to April 2022). Across the four rivers, a total of 216 bags were thus deployed as described.

Deployed plastic litter bags were anchored using steel bars of 20 cm drilled into the riverbed, attached using twine, and supported by a large stone until retrieval to avoid loss of bags (Figure 2.6E). The plastic materials (PET bottles) used in this study were obtained from the Makhanda recycling centre and were returned afterwards for proper disposal.

Figure 2.6: Experimental materials and substrate used; Pictures A (woven plastic litterbag), B (cut PET bottles), C (natural substrate NS), D (natural+plastic NP substrate), and E (plastic-dominated substrate with a sample picture of steel bar).

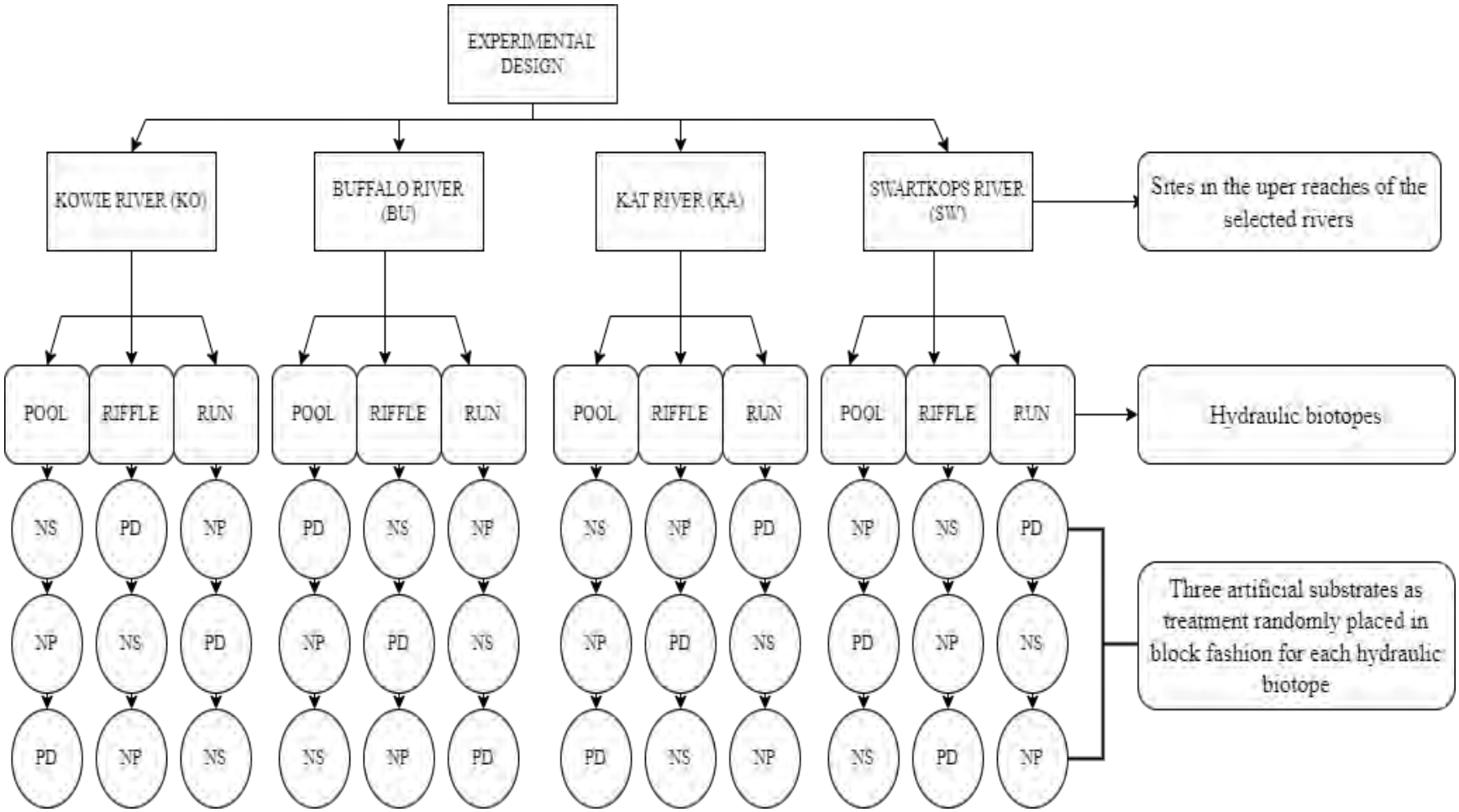

Figure 2.7: Summary of the experimental design setup employed in this study. The set-up is graphically represented reflecting all four selected sites within the upper reaches of the rivers.

2.3.2 Sample Collection and Retrieval

After deployment, the bags were left for at least 30 days for colonisation. The first set of bags (12 per substrate group) representing all substrates across hydraulic biotopes was retrieved from the four rivers on day 30 and days 60, 90, 120, 150, and 180. The plastic litterbags were retrieved using a hand net (dimension 300 x 300 mm frame and 250 µm mesh size), held directly downstream of the plastic litterbags at each site. The hand net was used to scoop the plastic litter bag from the riverbed. The process was swiftly and carefully done to ensure colonised macroinvertebrates were not dislodged and washed downstream.

After the plastic litter bags were retrieved, each was emptied into a round white tray, and the substrates were thoroughly washed with river water to dislodge macroinvertebrates. The substrates were carefully screened, and attached macroinvertebrates were removed by hand using a soft brush. The retrieved plastic substrates and polyethene nettings were inserted into a zip-lock plastic bag and transferred to the laboratory for proper disposal.

The collected macroinvertebrates were preserved in 70% ethanol using labelled 350 ml polyethylene honey jars, placed in an ice cooler box, and transported to the laboratory for sorting, identification, and abundance count.

2.3.3 Water sampling and physicochemical measurement

Water physicochemical variables were measured in all hydraulic biotopes during deployment (October 2021) and retrieval over the experimental period (November 2021–April 2022). The experiment spanned three seasons (spring, 2021; summer to autumn, 2022). For each sampling event, the physicochemical variables measured *in situ* included the width (m) of the hydraulic biotopes using a tape measure, and water depth (m) using a depth stick. The flow velocity (m/s) was measured using a Marsh-McBirney Flo-Mate^{TM} portable flow meter (Model 2000). The streamflow (m^3/s) was calculated as the product of the unit's width, average depth, and average flow velocity. Dissolved oxygen (DO) in mg/L, electrical conductivity (EC) in µS/cm, pH, and temperature were measured using the Hannah multiparameter probe model H198. Turbidity was measured using the portable turbidity Orbeco-Helliage 966 meter.

Water samples for nutrient concentration were collected in 250 ml acid-washed bottles and transported in ice coolers to the water quality laboratory of the Institute for Water Research, Rhodes University. The water samples were preserved following UNEP/WHO (1996)

standards (Bartram et al., 1996) in a refrigerator at a temperature of 4 °C until they were analysed. Samples were preserved for not more than 24 hours before analysis for nitrite-nitrogen (NO_2-N), nitrate-nitrogen (NO_3-N), ammonium-nitrogen (NH_4-N), total inorganic nitrogen (TIN), and orthophosphate-phosphorus (PO_4-P).

2.4 Laboratory Analysis

2.4.1 Macroinvertebrate identification

Macroinvertebrate specimens were sorted and identified at the family level using the keys described by Gerber and Gabriel (2002). Family level identification was applied because this is the resolution rountinely used in biomonitoring in South Africa and also due to inadequate taxonomic key and expertise on species level information on Afrotropical macroinvertebrates. (Mueller et al., 2013). The identified families were recorded separately for each substrate category per hydraulic biotope for each river site. For example, at the substrate scale for each sampling event, overall replicates amounted to 12 (3 hydraulic biotopes x 4 rivers), and four replicates at the hydraulic biotope scale for individual substrate group analysis.

2.4.2 Trait Analysis and Selection

The study employed nine traits categories, classified into 36 attributes (see Chapter 5, Section 5.2.1). The traits include behavioural (mobility), morphology and physiology (body size, body shape, and respiration), and ecological preference (attachment strategies, substrate preference, velocity preferment, feeding preference, and food material). A detailed explanation of traits and ecological preferences used in this study is provided in Chapter 5 (Section 5.2.1).

2.4.3 Nutrient analysis

For laboratory chemical analysis of nutrients, the preserved water samples were analysed for nutrients (nitrate-nitrogen, nitrite-nitrogen, ammonium-nitrogen, and orthophosphate-phosphorus) in the laboratory using the Palintest Photometer (Model 7500) according to the manufacturer's instructions. Before analysis, the water samples were allowed to equilibrate to room temperature. The total inorganic nitrogen (TIN) concentration was calculated by adding the concentrations of nitrate-nitrogen, nitrite-nitrogen, and ammonium-nitrogen, according to Palmer et al. (2007).

2.4.4 Statistical Analysis

The data were analysed using appropriate statistical techniques, fully described in Chapters 3, 4 and 5.

CHAPTER 3: SPATIO-TEMPORAL ASSEMBLAGE STRUCTURE OF MACROINVERTEBRATES ON MACROPLASTIC SUBSTRATES IN MINIMALLY IMPACTED SITES IN SELECTED RIVERS, EASTERN CAPE, SOUTH AFRICA

3.1 Introduction

The proliferation of plastic in the aquatic environment has tremendous ecological impacts, including altering habitat and adversely affecting biodiversity. The fact that plastics persist in the environment and can travel widely compared to natural substrates threatens global biosecurity (Thompson et al., 2009). While plastics pose a significant threat to freshwater ecosystems – particularly near urban areas – their presence is understudied, especially in African cities. Africa contributed approximately 8% of the global plastic production in 2019, with the global plastic production rate projected to be about 1100 mt by 2050 (PlasticsEurope, 2021; UNEP, 2022). Africa is an essential contributor to the global plastic waste and mismanagement problem, with approximately 21% of the estimated 80 mt mismanaged globally in 2015 (Lebreton & Andrady, 2019). Jambeck et al. (2015), in a global list of 192 countries generating plastic waste, ranked South Africa among the top fifteen contributors, a substantial waste generation country.

Upon reaching freshwater systems, the generated waste, mostly of plastics, for example, can be trapped by riverbanks, macrophytes, rocks, or buried in sediment (Faure et al., 2015; Weber & Opp, 2020; van Emmerik et al., 2022). These freshwater ecosystems are often situated in valleys and low-elevation terrains, making them suitable plastic conduits and reservoirs. The accumulation of plastic wastes, particularly of bigger sizes, the so-called macroplastics, can modify the physical habitats and create artificial microhabitats, and thus affect the biotic communities and ecological processes.

Freshwater ecosystems serve as plastic reservoirs for most plastics that leak into the environment (Liro et al., 2020; van Emmerik et al., 2022). The characteristics of the plastic, such as size, shape, density, and transport regime within the rivers, facilitate plastic retention. For example, a high flow regime can alter the buoyancy of macroplastics, affecting whether or not they sink to the riverbed. Macroplastics that sink to the riverbed, or even floating ones, can provide habitats for organisms (Czarnecka et al., 2009; Wilson et al., 2021, Gallitelli et al., 2023). The more macroplastics are retained in affected rivers, the more they impact and interact

with aquatic organisms such as macroinvertebrates. They may also displace natural microhabitats, potentially impacting habitat heterogeneity, quality, stability, and diversity.

Macroplastics have different physical and chemical properties from natural substrates, such as surface texture and water permeability. They are also prone to fragmentation and degradation (physical and biological) into secondary microplastics (Liro et al., 2020; Vaid et al., 2021). The fragmentation of macroplastics by macroinvertebrates through biting, chewing, and digesting is gaining recognition (Zhang et al., 2021; So et al., 2022; Valentine et al., 2022), and the filling of macroinvertebrate guts by these plastic materials presents a critical ecological risk. Also, studies on interactions (e.g., colonisation) between macroplastics and biota remain overlooked, yet a potential threat to freshwater biota and riverine habitats. In this regard, understanding the ecological risk posed by macroplastics from a habitat quality perspective and its effect on biological colonisation and ecological processes is critical in freshwater ecology.

In South Africa and, indeed, the rest of the continent, not much research work has been done on the modifying effects of macroplastics on the habitats and biological assemblages of riverine systems. Such studies are critical to understanding better how to address the potential risk posed by macroplastics. The few published studies in the freshwater system relate mainly to microplastics (e.g., Naidoo et al., 2015; Bouwman et al., 2018). Habitat heterogeneity is an important driver of biological diversity in freshwater systems and the presence of macroplastic may modify this heterogeneity. Therefore, the objective of this chapter is to examine and compare macroinvertebrate assemblage structure on macroplastic and natural substrates in minimally impacted sites within the upper reaches of the Kowie, Buffalo, Kat, and Swartkops rivers. This objective thus addresses objective one of this thesis stated in Chapter 1, Section 1.6.2.

3.2 Materials and Methods

Detailed descriptions of the study area, sampling design, and laboratory analysis are presented in Chapter 2. Four experimental sites in four river catchments between October 2021 and April 2022 were used (Chapter 2; Section 2.2). Polyethylene terephthalate (PET) plastic materials and natural substrates were classified into three substrate groups: 100% natural substrate (NS = group 1), 50% natural + 50 % plastic substrate (NP = group 2), and 100% plastic-dominated substrate (PD = group 3) (Chapter 2; sub-section 2.3.1). Three-substrate groups representing gradients of the macroplastic substrates were deployed into the stream hydraulic biotopes, including Pools, Riffles, and Runs. Physical attributes, including stream depth, width, and flow

velocity, were measured at each site per hydraulic biotope. Concurrently, environmental variables, including dissolved oxygen (DO), electrical conductivity (EC), pH, ammonium-nitrogen (NH_4-N), nitrite-nitrogen (NO_2-N), nitrate-nitrogen (NO_3-N), and orthophosphate-phosphorus (PO_4-P), were measured.

A total 54 bags (NS: 18; PD: 18 and NP :18) were deployed per site during the experimental period. For each category of substrate, six bags were deployed per hydraulic habitat: riffles, runs and pools (Figure 2.7). The bags were deployed within each hydraulic habitat in a randomised manner to span across seasons (spring, summer, and autumn, October 2021 to April 2022). Across the four rivers, a total of 216 bags were deployed as described. Owing to substrate loss during the experiment, retrieved substrates (NS: 48; NP: 52 and PD :49) within all sites were used as replicates for statistical analyses.

3.3 Data Analyses

3.3.1 Water physicochemical parameters across experimental sites

A multivariate analysis of variance (MANOVA) identified differences in environmental variables across sites. The four selected upper reaches were minimally impacted sites and were used as replicates across the site rather than pseudo-replication. Thus, the MANOVA of physico-chemical variables was used to reveal distinct replicates at the site level. The MANOVA is a parametric test used to test simultaneously the differences in the means of two or more samples using multiple dependent variables. When the MANOVA was significantly different, a one-way analysis of variance (ANOVA) determined the differences in individual environmental variables between the experimental sites. Where necessary, Tukey's post-hoc test distinguished between sites. Shapiro-Wilk's and Levene's tests were used to ascertain the data normality distribution and homogeneity of variance. The data were log (x + 1) transformed and normalised before MANOVA and ANOVA tests. The MANOVA and ANOVA tests were analysed using the Statistica software package version 13.

3.3.2. Spatio-temporal distribution pattern of macroinvertebrates assemblage structure on macroplastic and natural substrate

Taxonomic metrics

To assess the spatial and temporal taxonomic response of the macroinvertebrate community utilising macroplastic and natural substrates, five metrics representing various taxonomic

measures were selected. These metrics included richness in terms of the number of taxa and four diversity indices (Margalef's richness, Pielou's evenness, Shannon diversity, and Simpson's dominance; Table 3.1). The Kruskal-Wallis non-parametric test and clustered chart were used to test for significant differences and visualise the standard deviation values of the metrics between the substrate groups. Before the Kruskal-Wallis test, Shapiro-Wilks and Levene's tests ascertained the normality and homogeneity of the macroinvertebrate summary metrics dataset, respectively. Diversity indices and Kruskal-Wallis tests were computed using Vegan package 2.4.3 within R statistical environment version 4.2.0 (Oksanen, 2013; R Core Team, 2022).

Table 3.1: Macroinvertebrate taxonomic-based metrics applied to the macroinvertebrate taxa collected in all substrate groups from all hydraulic biotopes in the four selected rivers (October 2021 to April 2022).

Measures	Metrics	Summary
Number of Taxa	Absolute number of macroinvertebrate taxa found spatio-temporally in a substrate group	Gives an account of the number of taxa encountered in a population.
Diversity	Margalef's richness index	Accounts for both the number of taxa and individuals in a population
	Shannon diversity index	An abundance-based information index that assumes all taxa are represented in the sample with potential maximum value increasing as the number of taxa increases (Morris et al., 2014).
	Simpson's diversity index	A dominance-based diversity index that estimates the probability of finding a second individual in a sample from the same taxa as the first. This index is influenced by common taxa rather than taxa richness (Morris et al., 2014)
	Pielou's evenness index	An index that measures the equitability of taxa diversity based on the Shannon-Weiner diversity index. It indicates

		whether a few taxa dominate and whether a relatively equal number of individuals belong to each taxon. Low values indicate that a few taxa dominate, and high values imply that most taxa prevail (Morris et al., 2014).

Taxa preference for a particular substrate group

Pearson's point-biserial correlation was used to determine the preferences of macroinvertebrate taxa for specific substrates. Pearson's point-biserial correlation coefficient also determines the relation between the taxa and the substrate groups (NS, NP, PD) (de Cáceres et al., 2010). The purpose of the analysis is to determine the influence of the substrate groups on the macroinvertebrate assemblage structure and to identify community types based on the preferred substrates. Pearson's point-biserial analysis uses the Monte Carlo permutation test with 999 permutations at a 0.05 significance level to evaluate the association. Pearson's point-biserial correlation coefficient was analysed using the Indicspecies package version 2.0.2 within the R version 4.2.2 statistical environment (de Cáceres, 2013; R Core Team, 2023).

3.3.3. Macroinvertebrate community response to substrate groups

The principal response curve (PRC) analysis was used to elucidate whether the macroinvertebrate community structures on macroplastic substrates differed from those of the natural substrates over time. The analysis also identifies macroinvertebrates responsible for such departure, and their relative contribution. The PRC is a multivariate method explicitly developed for analysing experimental community response to treatments over time by focusing on the taxonomic composition of the treatment (in this case, NP and PD) and those of the control (NS) collected simultaneously (van den Brink & ter Braak, 2009). The PRC model allows for variance partitioning into parts, including differences in time (between sampling date), and treatment (substrate). The PRC model uses the first axis of a Principal Coordinate analysis using Bray-Curtis similarity to generate a correlation coefficient point (substrate) and taxon scores. The treatment (substrate) scores are interpreted as the principal response of the community to a substrate. The taxa score allows the determination of a taxa-specific response to a substrate group. A positive taxon weight indicates an increase in spatial and temporal abundance of the taxon on the natural substrate relative to macroplastic substrates. A negative

taxon score reveals less influence of treatment (NP and PD) on taxon abundance (van den Brink & ter Braak, 1999), while a taxon score close to zero indicates no specific response pattern to either natural or macroplastic substrates. The direction of taxon weight determines the direction of the taxon's response to the treatments. The PRC test analysis uses an ANOVA-like permutation test to assess the significance of the PRC model using the "anova.cca" function of the vegan R package (Oksanen, 2013). The PRC was conducted using the R statistical environment version 4.2.0 (R Core Team, 2022).

The analysis of similarity (ANOSIM) is a non-parametric permutation test of (dis)similarity based on a rank similarity matrix between defined sample groups. Similarity percentage (SIMPER) indicates the percentage contributions of taxa to the (dis)similarity among different gradients of plastic substrate. One-way ANOSIM was used to compare the similarities of replicate samples within a substrate group and other substrates using the Bray-Curtis similarity index. The ANOSIM analysis produces a global *R* test statistic as the result of the similarities of replicates within a substrate compared to those from other substrates. The global *R*-value ranges between 0 and 1. A value close to or equal to 1 indicates that replicates within substrates were more similar than those from other substrates. A value close to 0 indicates differences between substrates with dissimilarities between and within substrate replicate samples almost equal (Clarke & Warwick, 1994). Both analyses were conducted using the vegan package version 2.4.3 within the R statistical software version 4.2.0 (Oksanen, 2013; R Core Team, 2022).

3.3.4. Colonisation pattern of macroinvertebrate assemblage structure on substrate groups

Succession reveals the pattern by which species inhabiting different substrate groups are structured periodically (Wenzel & Luque, 2008). Under the same continuous process and environmental conditions facilitating colonisation and interaction of species with their habitat, spatio-temporal patterns exist in species assemblages. Ordinarily, statistical techniques predict how species assemble from a community pool based on components of variance. For instance, popular multivariate methods, such as Discriminant Analysis (DA), treat data as direct variance by comparing the DA result of various plots in Period 1 to those of Period 2, and Period 3. Lambshead & Paterson (1986) demonstrated the use of the cladistic parsimony technique to comprehend ecological pattern. This technique has been successfully deployed in ecological succession and changes in community structure (Wenzel & Luque, 2008, Rachlin et al., 2012).

Unlike conventional multivariate techniques, the cladistic parsimony technique provides special power with respect to successional ecology by revealing the strict hierarchical order of species colonisation, so identifying the most likely order in which different species colonised and interacted with their habitat over time, and providing insights into mechanisms driving ecological succession, such as competition, facilitation, and environmental filtering.

The cladistic parsimony tree was used to summarise the ecological succession of macroinvertebrates on the substrate groups. Cladistic parsimony analysis is non-parametric and multivariate, enabling a strict hierarchy with data from experiments. This analysis helps identify the precise role that individual taxa played in determining successional patterns, regardless of temporal dominance, offering insight that complements variance-based statistical analysis (Luque et al., 2007; Wenzel & Luque, 2008). Parsimony analysis graphically exhibits taxa fostering diversity, rooted at time zero or, tendering to either various duration, or with branches indicating marginal assemblages of taxa over time. Additionally, taxa that uniquely support a specific axis are marked (solid circle) and identified differently from those that are not. These marks depict their occurrence, decrease (>0) or increase (>1) to diversity in a particular period. The presence/absence dataset constructed an artificial row of zeros representing an ideal cladogram root when there would be no taxa (Luque et al., 2007) for all substrate groups used for the analysis. To create solution trees, the matrices were submitted to the ordinary phylogenetic programme, WinClada (Nixon, 1999) running Nona (Goloboff, 1993).

3.4 Results

3.4.1. Water physicochemical variables

The means, standard deviations, and ranges of the environmental variables analysed during the experiment are presented in Table 3.2. The global multivariate MANOVA revealed a significant difference between sites (Table 3.3). Besides mean dissolved oxygen DO, one-way ANOVA indicated significant difference between sites in all physicochemical variables. Tukey's post-hoc test revealed site SW's (Swartkops River) mean temperature to be significantly higher than those of the other sites. The mean EC values of sites BU and KA were significantly lower than those of sites PA and SW. The mean nutrient (NO_2-N, NO_3-N, NH_4-N, TIN, and PO_4-P) and turbidity values across all sites were statistically different from site KA (Table 3.2).

Table 3.2: Mean ± standard deviation and range (in parenthesis) of the analysed environmental variables in all experimental sites during the study period (October 2021– April 2022). P values are indicated by ANOVA. The same superscript letter between sites per variables indicates insignificant difference. Different superscript letters per variable across sites indicate significant differences revealed by Tukey's HSD post-hoc test.

Variable	**Rivers**				
	Kowie (PA)	**Buffalo (BU)**	**Kat (KA)**	**Swartkops (SW)**	**p-value**
Temperature (°C)	19.15 ± 5.03 (16.6 - 24.7)^{a}	17.97 ± 8.15 (15 - 24.9)^{a}	20.77 ± 4.32 (12.9 - 28.1)^{a}	25.94 ± 2.57 (23.1 - 29.9)^{b}	**0.000**
Dissolve Oxygen (mg/L)	8.18 ± 2.06 (5.37 - 9.45)	7.17 ± 3.10 (7.98 - 9.1)	8.23 ± 0.68 (7.37 - 9.4)	7.71 ± 0.99 (4.81 - 10)	0.34
Electrical Conductivity (µS/cm)	224.4 ± 53.56 (213.5 - 265)^{a}	52.88 ± 23.34 (54.5 - 74.4)^{b}	112.9 ± 28.58 (68.7 - 151.1)^{c}	260.4 ± 8.35 (241 - 281)^{a}	**0.000**
Turbidity (NTU)	10.63 ± 7.35 (3.13 - 24.5)^{a}	4.68 ± 3.28 (1.65 - 11.91)^{a}	118.3 ± 231.1 (8.4 - 665)^{b}	3.88 ± 1.9 (1.6 - 9.3)^{a}	**0.003**
Nitrite-Nitrogen NO_2-N (mg/L)	0.018 ± 0.02 (0.02 - 0.08)^{a}	0.015 ± 0.02 (0.002 - 0.09)^{a}	0.73 ± 1.78 (0.003 - 6.11)^{b}	0.013 ± 0.01 (0 - 0.05)^{a}	**0.02**
Nitrate-Nitrogen NO_3-N (mg/L)	1.22 ± 1.7 (0 - 6.25)^{a,b}	0.46 ± 0.4 (0.04 - 0.86)^{a}	3.13 ± 4.52 (0.14 - 15.15)^{b}	0.67 ± 0.50 (0.04 - 2.31)^{a}	**0.002**
Ammonium NH_4 (mg/L)	0.02 ± 0.03 (0 - 0.1)^{a}	0.02 ± 0.2 (0 - 0.11)^{a}	0.55 ± 1.38 (0 - 4.9)^{b}	0.03 ± 0.06 (0 - 0.3)^{a}	**0.03**
Total Inorganic Nitrogen (mg/L)	1.27 ± 1.69 (0.03 - 6.2)^{a,b}	0.50 ± 0.42 (0.04 - 1.43)^{a}	4.42 ± 7.51 (0.28 - 24.96)^{b}	0.72 ± 0.50 (0.14 - 2.34)^{a}	**0.005**
Orthophosphate PO_4-P (mg/L)	0.1 ± 0.07 (0.01 - 0.33)^{a}	0.09 ± 0.06 (0.03 - 0.2)^{a}	2.27 ± 5.20 (0 - 15.5)^{c}	0.37±0.28 (0 - 6.12)^{b}	**0.03**

Table 3.3: Global MANOVA results indicating a significant difference between sites based on the environmental variables between sites from October 2021 to April 2022.

	Multivariate Tests of Significance					
Effect	Test	Value	F	Effect (df)	Error (df)	p
Intercept	Wilks	0.032509	238.0893	9	72.0000	0.00
Rivers	Wilks	0.031601	17.6835	27	210.9194	0.00

3.4.2. Macroinvertebrate assemblage structure on substrate groups

The total number of macroinvertebrates identified during the experimental period was 17,314 individuals from 48 families belonging to 12 orders (Table 3.4). Generally, the order Odonata had eight families represented: seven families each for Hemiptera, Trichoptera, Coleoptera, and Diptera orders. Order Ephemeroptera had five Families, Gastropod two families, Annelida, Crustacea, Plecopteran, Lepidoptera, Zygoptera, and Turbellaria each represented one family throughout the experiment. The predominant taxa were the Chironomidae, Hydropsychidae, and Baetidae (Table 3.4). The predominance of the family Chironomidae was recorded in 100% natural substrate group NS and least in 50% plastic substrate group NP.

In the NS substrate, the families Chironomidae, Hydropsychidae, Baetidae, and Simuliidae dominated the assemblage structure, contributing 37.6%, 21.4%, 12%, and 7.3%, respectively (Table 3.4). The families that contributed least to the relative abundance were Chlorocyphidae (0.01%), Planaria (0.01%), Hydrophilidae (0.01%), Leptoceridae (0.01%), Helodidae (0.01%), Belostomatidae (0.01%), Corixidae (0.01%), and Gerridae (0.01%).

The NP substrate macroinvertebrates assemblage was dominated by families Hydropsychidae (29.1%), Chironomidae (24.5%), Baetidae (13.5%), and Simuliidae (12.6%) (Table 3.4). Pisuliidae, Naucoridae, and Corduliidae, had the lowest relative abundance, with each contributing 0.01%.

The relative abundance of macroinvertebrates in the 100% plastic substrate PD was dominated by families Chironomidae, Hydropsychidae, Baetidae, and Simuliidae, contributing 31.2%, 23.1%, 16.3%, and 11.8%, respectively (Table 3.4). The lowest contributors to relative abundance were the families Perlidae (0.02%), Coenagrionidae (0.02%), Pleidae (0.02%), Nepidae (0.02%), and Pisuliidae (0.02).

Throughout the study, NS substrate recorded the highest number of taxa (40), followed by NP with 35 taxa, and the least (34) by PD, but not significant (Table 3.4). Taxa Chlorosyphidae, Pyralidae, Belostomatidae, Helodidae, Hydrophilidae, Ephydridae, Tipulidae, and Physidae were only recorded in NS (Table 3.4). Synlestidae and Corduliidae were solely found in NP, while only PD substrate recorded Nepidae, Veliidae, and Hydraenidae taxa throughout the study.

Table 3.4: Percent of relative abundance of substrate per taxon (left) and taxon per substrate group (right), and number of taxa on each substratum (NS, NP, PD) irrespective of hydraulic biotopes throughout the experimental period (October 2021–April 2022)

Taxa/Substrate		Substrate per Taxon			Taxon per Substrate		
Order	Family	NS	NP	PD	NS	NP	PD
ANNELIDA	Oligochaeta	0.44	0.36	0.20	2.09	1.45	0.91
CRUSTACEA	Potamonautidae	0.44	0.28	0.29	0.92	0.48	0.57
PLECOPTERA	Perlidae	0.46	0.46	0.08	0.11	0.09	0.02
EPHEMEROPTERA	Baetidae	0.27	0.35	0.37	12.63	13.53	16.32
	Caenidae	0.34	0.38	0.28	3.19	2.99	2.56
	Leptophlebiidae	0.33	0.30	0.36	3.02	2.27	3.13
	Tricorythidae	0.24	0.59	0.17	3.32	6.81	2.31
	Heptageniidae	0.50	0.25	0.25	0.08	0.03	0.04
ODONATA	Aeshnidae	0.32	0.30	0.38	0.55	0.44	0.63
	Chlorosyphidae	1.00			0.02		
	Synlestidae		1.00			0.06	
	Coenagrionidae	0.82	0.12	0.06	0.26	0.03	0.02
	Corduliidae		1.00			0.02	
	Gomphidae	0.27	0.37	0.37	0.21	0.23	0.27
	Lestidae	0.42	0.24	0.34	2.09	0.97	1.61
	Libellulidae	0.48	0.29	0.23	1.77	0.89	0.82
LEPIDOPTERA	Pyralidae (Crambidae)	1.00			0.02		
HEMIPTERA	Belostomatidae	1.00			0.02		
	Pleidae	0.75		0.25	0.06		0.02
	Corixidae	0.25	0.75		0.02	0.05	
	Gerridae	0.33	0.67		0.02	0.03	
	Nepidae			1.00			0.02
	Naucoridae	0.38	0.04	0.58	0.17	0.02	0.25
	Veliidae			1.00			0.05
TRICHOPTERA	Ecnomidae	0.75	0.25		0.17	0.05	
	Glossosomatidae		0.93	0.07		0.20	0.02

	Hydropsychidae	0.26	0.43	0.30	21.48	29.12	23.11
	Philopotamidae	0.13	0.37	0.50	0.51	1.21	1.86
	Sericostomatidae	0.21	0.50	0.29	0.06	0.11	0.07
	Leptoceridae	0.33		0.67	0.02		0.04
	Pisuliidae		0.50	0.50		0.02	0.02
COLEOPTERA	Dytiscidae	0.36	0.18	0.45	0.08	0.03	0.09
	Elmidae	0.43	0.40	0.17	0.25	0.19	0.09
	Gyrinidae	0.36	0.28	0.37	0.60	0.39	0.59
	Helodidae	1.00			0.02		
	Psephenidae		0.14	0.86		0.05	0.32
	Hydraenidae			1.00			0.04
	Hydrophilidae	1.00			0.02		
DIPTERA	Athericidae	0.12	0.42	0.46	0.21	0.61	0.75
	Chironomidae	0.38	0.30	0.33	37.65	24.50	31.25
	Ceratopogonidae	0.85	0.08	0.08	0.42	0.03	0.04
	Ephydridae	1.00			0.04		
	Tabanidae	0.61	0.32	0.07	0.47	0.20	0.05
	Simuliidae	0.21	0.43	0.36	7.30	12.58	11.87
	Tipulidae	1.00			0.04		
GASTROPODA	Ancylidae	0.13	0.25	0.63	0.06	0.09	0.27
	Physidae	1.00			0.04		
TURBELLARIA	Planaria	0.06	0.94		0.02	0.23	
Total Abundance		5299	6421	5594			
Total Number of Taxa		40	35	34			

Temporally, days 120 and 180 recorded the highest and lowest number of taxa in NS, respectively (Figure 3.1). The Kruskal-Wallis test indicated a significant difference ($p < 0.05$, Kruskal Wallis chi square values (KW-H) = 23.99) between sampling days of NS substrate

group with a higher number of taxa recorded on days 30, 60, and 90 than days 120, 150, and 180. In NP, a significantly (KW-H = 11.59, $p = 0.04$) high number of taxa was recorded on days 30 and 60, and the lowest on days 150 and 180. For PD, although not significant ($p > 0.05$, KW-H = 6.02), sampling days 30 and 120 had more macroinvertebrate taxa than other sampling days (Figure 3.1).

Generally, more macroinvertebrate taxa were recorded between substrates on days 60 and 90 in NS than the other substrates (Figure 3.1). On days 30 and 180, NP and PD substrates shared equal taxa greater than the NS substrate. The PD substrate recorded more macroinvertebrate taxa than NS and NP substrates on day 120 (Figure 3.1).

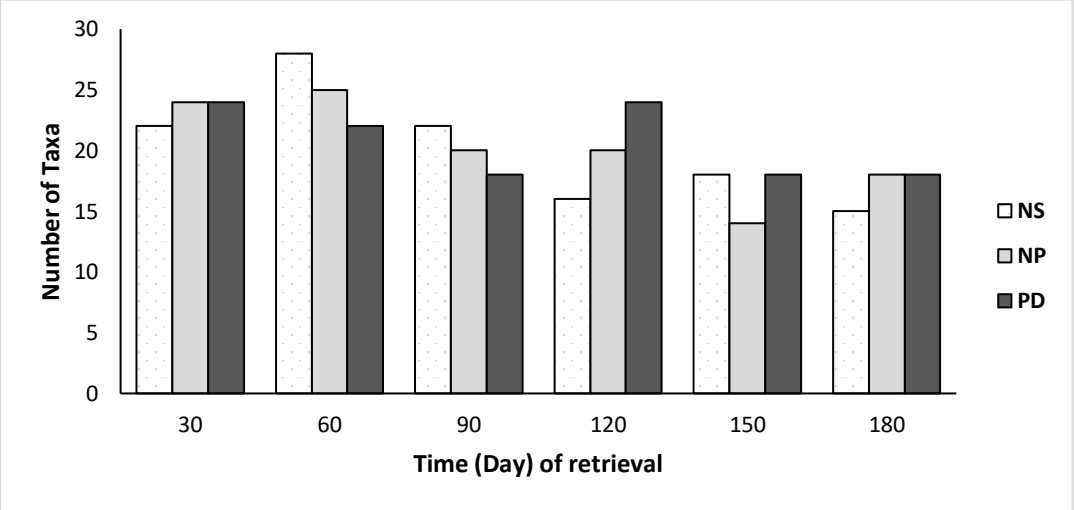

Figure 3.1: Number of taxa of macroinvertebrates for the natural substrate (NS), natural + plastic substrate (NP), and plastic-dominated substrate (PD) during the study (October 2021 to April 2022).

Clustered charts were used to graphically represent selected macroinvertebrate metrics between defined substrate groups spatio-temporally (Figure 3.2 A-D). The Kruskal-Wallis test result revealed that, of all summary metrics, only the Simpson's index indicated significant differences (Kruskal-Wallis H = 41.1, $p < 0.05$) between the substrate groups (Figure 3.2). The NS substrate macroinvertebrate assemblages had the lowest Simpson's index value compared to NP and PD macroinvertebrate assemblages. Temporally, except equitability, all other summary measures indicated significant variation over time. All substrate macroinvertebrate assemblages within days 30, 60, 90 were significantly (with all KW-H values having a $P < 0.05$) higher in diversity, richness, and dominance than the later days. At no time of sampling were there significant differences in richness, diversity and dominance between the three substrates (NS, NP and PD; all KW-H values for the main effect of the group had $P > 0.05$).

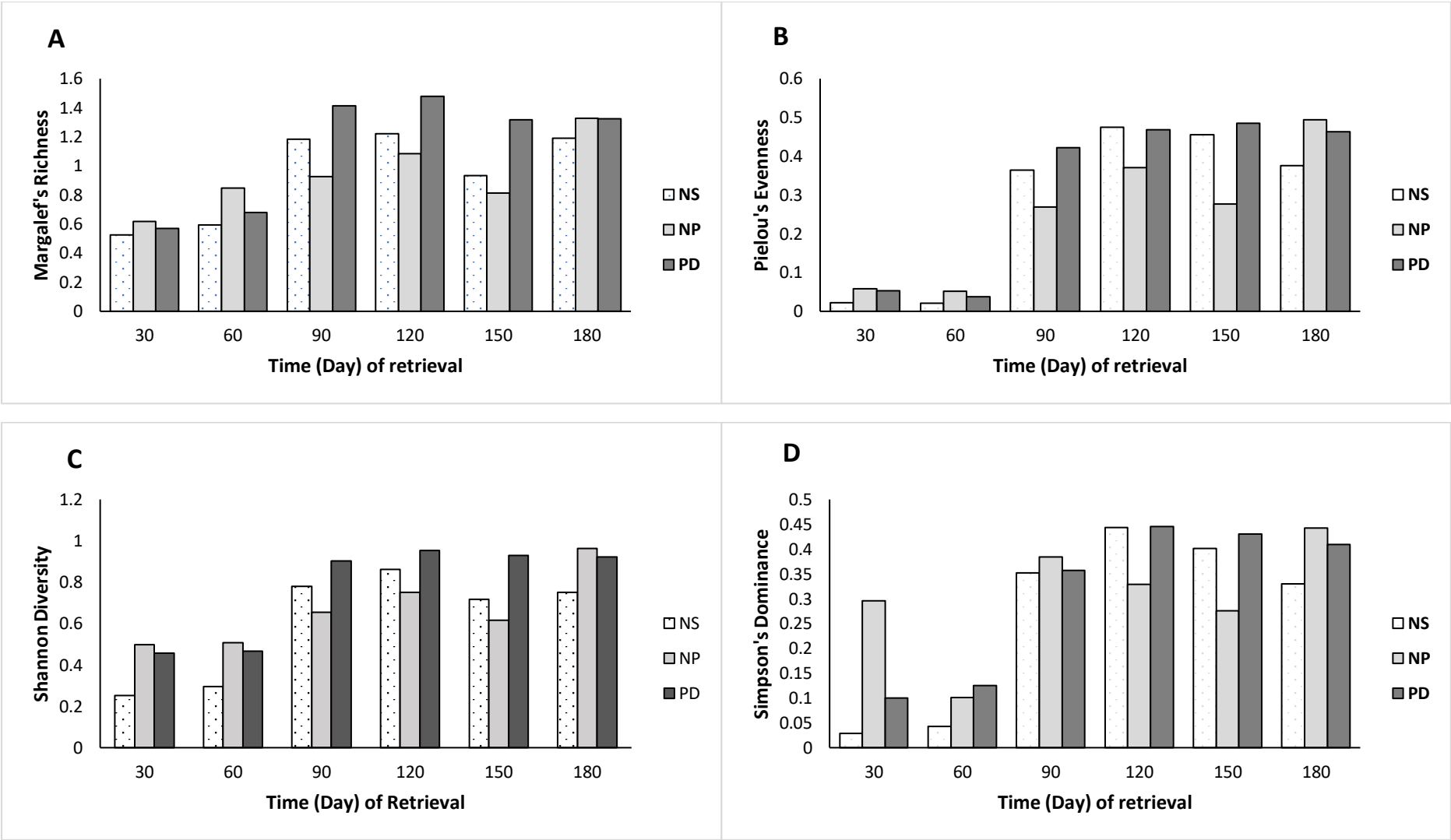

Figure 3.2: Standard deviation of taxonomic metrics (Margalef's richness, Pielou's Evenness, Shannon Diversity, and Simpson's Diversity; A-D, respectively) of substrate groups macroinvertebrate assemblage structure per period throughout the experiment (October 2021–April 2022).

3.4.3 Macroinvertebrate preferences for substrate groups

The Pearson's point-biserial correlation was used to assess preferences of macroinvertebrate taxa to the different substrate groups, with a view to identifying community types based on the substrate groups. A total of six community types were identified (Table 3.5). The first community type showed a preference for the NS substrates, with a total of 17 families in this community. Of these families, only the family Tabanidae had a significant preference ($p < 0.05$) for the NS substrate (Table 3.5). The second community type preferred the NP substrate and consisted of eight families, of which the glossosomatids had a significant association. The third community consisted of six families that preferred the PD substrate. Only the family Psephenidae was significantly correlated with the PD substrate (Table 3.5).

The fourth community type showed a preference for the NS and NP substrates, with a total of five families (Table 3.5). Of the five families, none displayed significant correlation with the substrates. The fifth community type consisted of six families and was associated with the NS and PD substrates. Of the six families, none was significantly correlated with the substrates. The sixth community type was associated with the NP and PD substrates. This community was composed of six families, none of which showed significant correlation with the substrates.

Table 3.5: Preferences of macroinvertebrate taxa to different substrate groups: natural substrates (NS); a mixture of natural and plastic (NP), and plastic dominated (PD) in the Bloukrans, Buffalo, Kat, and Swartkops river systems during the experimental period (October 2021–April 2022). Taxa preferences are indicated by the numbers 1 and 0 under the substrate as a family preferring a particular substrate or not preferring a specific substrate, respectively. Index (substrate preference index) indicates families preferring the NS (1), NP (2), PD (3), both NS and NP (4), both NS and NP (5), and both NP and PD (6). A significant association ($p < 0.05$) is indicated by a boldface value under the *p*-value column.

Families	**NS**	**NP**	**PD**	**Index**	**Point-Biserial Co-efficient**	***p*-value**
Potamonautidae	1	0	0	1	0.126	0.274
Heptageniidae	1	0	0	1	0.074	0.723
Coenagrionidae	1	0	0	1	0.13	0.252
Libellulidae	1	0	0	1	0.172	0.081
Belostomatidae	1	0	0	1	0.118	0.316
Pleidae	1	0	0	1	0.094	0.573
Pyralidae	1	0	0	1	0.118	0.319

Ecnomidae	1	0	0	1	0.106	0.339
Helodidae	1	0	0	1	0.118	0.327
Chironomidae	1	0	0	1	0.089	0.55
Hydrophilidae	1	0	0	1	0.118	0.328
Ceratopogonidae	1	0	0	1	0.113	0.569
Ephydridae	1	0	0	1	0.168	0.101
Tabanidae	1	0	0	1	0.181	**0.049**
Tipulidae	1	0	0	1	0.168	0.091
Chlorosyphidae	1	0	0	1	0.118	0.312
Physidae	1	0	0	1	0.118	0.333
Tricorythidae	0	1	0	2	0.114	0.4
Synlestidae	0	1	0	2	0.187	0.113
Corduliidae	0	1	0	2	0.114	1
Corixidae	0	1	0	2	0.14	0.326
Glossosomatidae	0	1	0	2	0.198	**0.015**
Hydropsychidae	0	1	0	2	0.071	0.716
Sericostomatidae	0	1	0	2	0.048	0.921
Planaria	0	1	0	2	0.117	0.542
Aeshnidae	0	0	1	3	0.056	0.763
Nepidae	0	0	1	3	0.117	0.654
Veliidae	0	0	1	3	0.204	0.075
Psephenidae	0	0	1	3	0.155	**0.046**
Hydraenidae	0	0	1	3	0.166	0.214
Ancylidae	0	0	1	3	0.151	0.144
Oligochaeta	1	1	0	4	0.084	0.683
Perlidae	1	1	0	4	0.122	0.333
Caenidae	1	1	0	4	0.058	0.774
Gerridae	1	1	0	4	0.1	0.674
Elmidae	1	1	0	4	0.127	0.256
Leptophlebiidae	1	0	1	5	0.05	0.842
Lestidae	1	0	1	5	0.099	0.48
Naucoridae	1	0	1	5	0.126	0.293
Dytiscidae	1	0	1	5	0.082	0.586

Leptoceridae	1	0	1	5	0.079	0.76
Gyrinidae	1	0	1	5	0.048	0.866
Baetidae	0	1	1	6	0.08	0.621
Gomphidae	0	1	1	6	0.04	0.866
Philopotamidae	0	1	1	6	0.095	0.54
Pisuliidae	0	1	1	6	0.082	1
Athericidae	0	1	1	6	0.136	0.234
Simuliidae	0	1	1	6	0.083	0.621

3.4.4. Macroinvertebrates community response to substrate groups

The principal response curve (PRC) analysis displayed marked fluctuating deviations between the macroplastic and natural substrate macroinvertebrate communities (Figure 3.4). The greatest deviation of macroinvertebrate community from the natural substrate by macroinvertebrates in the macroplastic substrates was observed on day 150 (Figure 3.4). The results suggest that in the early days of the experiment, from day 0 – 60 not much difference could be detected in the assemblage structures of the substrate groups. The PRC analysis indicates that the substrate groups' effect accounted for 4.45% of the total variance. The variance attributed to sampling time differences accounted for only 10.05%. However, the remaining 85.50% was due to variability between replicates over time. Based on the Monte Carlo permutation test, the PRC test result was not statistically significant (F = 0.56, pr(>F) = 0.95).

The PRC taxa scores indicated that the abundance of the family Lestidae was substantially reduced in macroplastic substrates (NP and PD), but the Lestidae abundance in NS increased over time. However, the abundance of Chironomidae, Simuliidae, Tricorythidae, and Hydropsychidae increased in the NP and PD substrates compared to NS treatment at the time of deviation. In Figure 3.4, the remaining taxa had weights close to zero and were determined as non-responsive to the PRC.

The analysis of similarity (ANOSIM) revealed that macroplastic and natural substrate macroinvertebrate communities were not statistically distinct between the substrate groups (Global R = -0.019; $p > 5\%$). Macroinvertebrate communities of natural + plastic – NP and natural substrat – NS substrates were distinct (Global R = 0.114; $p < 5\%$; Table 3.6) only on day 150. However, macroinvertebrate community compositions significantly differed across

retrieval days (Global R = 0.075; p < 1%; Table 3.6). The macroinvertebrate community similarities within substrate groups were 42.51% for NS, 39.09% for PD and 34.10% for NP. The families Chironomidae, Baetidae, and Hydropsychidae were mainly responsible for the observed similarities in all substrate groups. SIMPER analysis revealed macroinvertebrate assemblage dissimilarities between the substrate groups, with the highest dissimilarity of 63.04% observed for NP and PD substrates, 61.66% between the NS and NP substrates, and 59.04% for the NS and PD substrates (Figure 3.4). With the exception of tricorythids, the SIMPER result also reported the main contributors to the dissimilarities between the substrate groups to be mainly Chironomidae, Baetidae, Hydropsychidae, Simuliidae, Caenidae, Leptophlebiidae, and Lestidae across all substrate dissimilarity pairs (Figure 3.4).

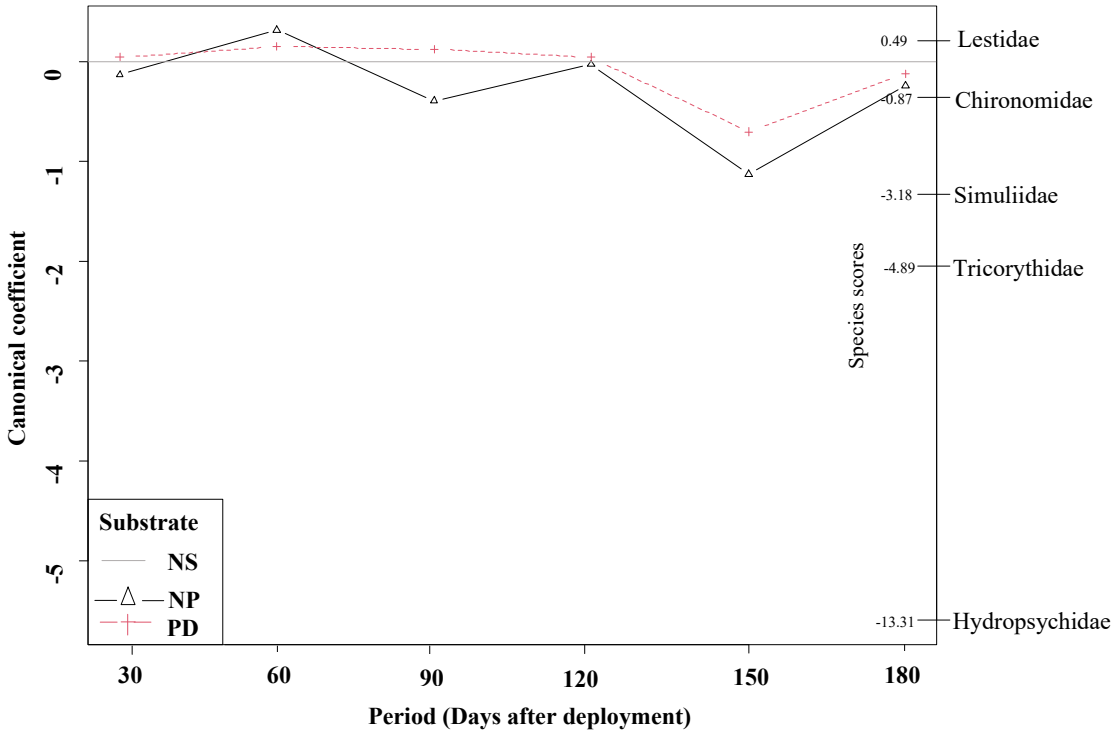

Figure 3.3: Principal response curve plot with taxa weights indicating the macroinvertebrate community response to the plastic substrates from the four selected river systems. Taxa whose weights are between 0.4 and -0.4 were omitted for clarity. Substrate groups are natural substrates (NS); natural + plastic substrates (NP); and plastic-dominated substrates (PD).

Table 3.6: Results of ANOSIM permutational test of substrate groups, day of retrieval, and interaction of substrate group per retrieval day showing global R statistics, pair-wise R statistics and significance level based on the macroinvertebrate taxa abundance and composition during the period of experiment (October 2021–April 2022). Pairs with significance level (p<5%) are bolded. Substrate groups are natural substrates (NS); natural + plastic substrates (NP); and plastic-dominated substrates (PD). Retrieval days are days 30, 60, 90, 120, 150, and 180.

Substrate pairs	R statistics	Significance level %	Substrate per retrieval day	R statistics	Significance level %
Global test	-0.019	90.9	Global test	-0.015	63.01
NS, NP	0	49.8	NS30, NP30	-0.053	92.3
NS, PD	-0.025	89.4	NS30, PD30	-0.013	54.1
NP, PD	-0.035	96.7	NS60, NP60	-0.064	95.1
Retrieval day pairs	R statistics	Significance level %	NS60, PD60	-0.015	51.7
Global test	0.075	**0.1**	NS90, NP90	-0.003	43.5
30, 60	0.117	**0.2**	NS90, PD90	-0.018	64.7
30, 90	0.122	**0.1**	NS120, NP120	0.013	33.1
30, 120	0.066	**2.2**	NS120, PD120	-0.023	62.1
30, 150	0.17	**0.1**	NS150, NP150	0.114	**2.8**
30, 180	0.188	**0.1**	NS150, PD150	-0.061	94.7
60, 90	-0.003	53.4	NS180, NP180	-0.005	44.8
60, 120	0.097	**0.2**	NS180, PD180	-0.021	56.3
60, 150	0.137	**0.2**	NP30, PD30	-0.052	88.5
60, 180	0.13	**0.1**	NP60, PD60	-0.024	62
90, 120	0.036	10.6	NP90, PD90	-0.063	92.2
90, 150	0.052	5.8	NP120, PD120	-0.057	87
90, 180	0.015	27.5	NP150, PD150	0.043	17.6
120, 150	0.012	29.8	NP180, PD180	-0.057	91.7
120, 180	0.077	**1.8**			
150, 180	0.009	36.1			

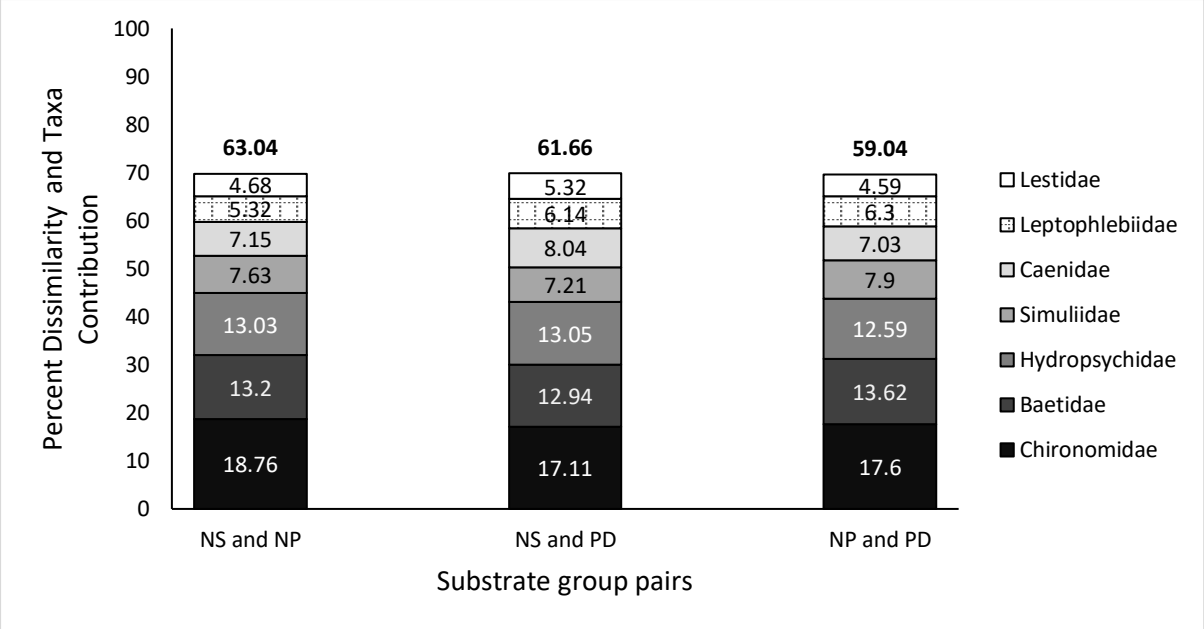

Figure 3.4: SIMPER analysis result showing percent average dissimilarity (bold numbers on top of bars) between substrate groups and macroinvertebrate taxa contributing to dissimilarity during the study period (October 2021–April 2022). Substrate groups are natural substrates (NS); natural + plastic substrates (NP); and plastic-dominated substrates (PD).

Responses of the taxa distinguishing substrate groups indicated by the PRC analysis

Lestidae

Lestids occurred in natural and macroplastic substrate groups throughout the experiment. Except for day 180, Lestid abundance was similar in NP and PD substrate (Figure 3.5). Although no significant differences (ANOVA = $p > 0.05$) between the natural and macroplastic substrate was observed, their abundance was highest on days 30, 90, and 120 on 100% natural substrate.

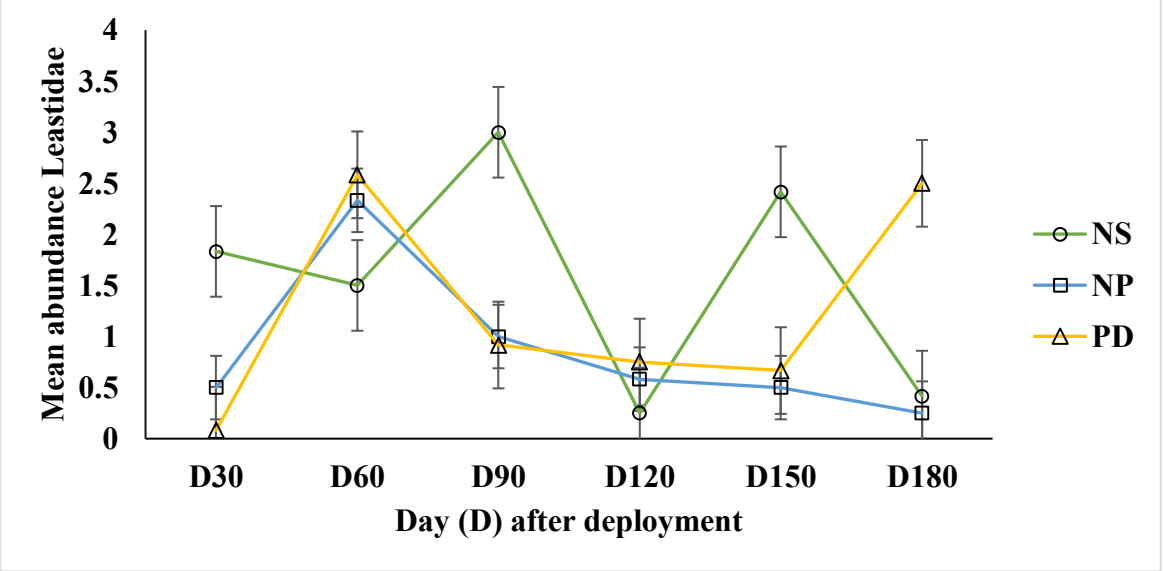

Figure 3.5: Mean abundance ± standard error of Lestidae in natural (NS) and macroplastic (NP, PD) substrates on each retrieval day, showing that the abundance of these taxon was mostly higher in the natural substrate than in the macroplastic substrates during the experimental period (October 2021–April 2022). Substrate groups are natural substrates (NS); natural + plastic substrates (NP); and plastic-dominated substrates (PD).

Chironomidae, Simuliidae, Tricorythidae, and Hydropsychidae

The Chironomids were more abundant in NS substrates compared to NP and PD substrate (Figure 3.6). Highest number of Chironomidae was recorded on NS and PD substrates on day 60. On day 60, one-way ANOVA, followed by Tukey's HSD revealed Chironomid abundance on NP substrates to be significantly lower than in NS and PD. All other sampling days had a similar trend in Chironomid abundance on all substrates (Figure 3.6).

The abundance of Simuliidae was consistently higher on the NP and PD substrates, and lowest in the NS substrates (Figure 3.6). Although no Simuliidae were recorded in NS substrate on day 30, on day 60 and 180, Simuliidae abundance was slightly insignificantly higher in PD substrate than in NS and NP, while the highest abundance of Simuliidae was recorded in NP substrates on day 90 with no significant differences to NS and PD (Figure 3.6).

Tricorythidae were significantly less abundant in NS substrates on days 30, 60, and 90 relative to macroplastic substrates ($p < 0.05$). Throughout the experiment, the abundance of Tricorythidae was similar between the NP and PD substrates, except for days 120 and 150 (Figure 3.6). From day 120 to 180, only an individual Tricorythid was recorded in NS substrate on day 150.

On retrieval day 150, the abundance of Hydropsychidae was insignificantly higher in NP and PD substrates than in NS substrate (Figure 3.6). Aside days 90 and 150, Hydropsychidae abundance was similar in both natural and macroplastic substrates.

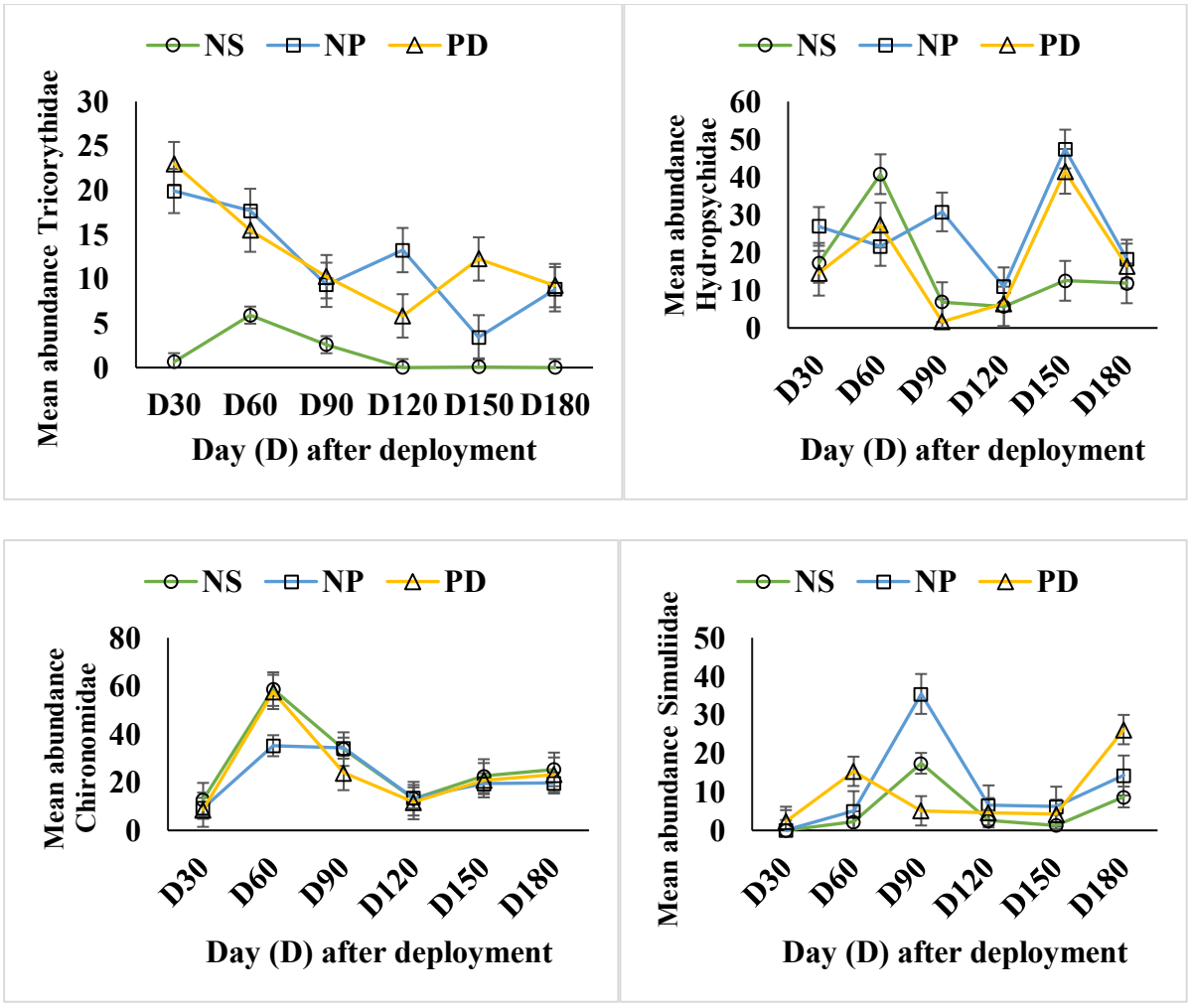

Figure 3.5: Mean abundance ± standard error of Lestidae in natural (NS) and macroplastic (NP, PD) substrates on each retrieval day, showing that the abundance of these taxon was mostly higher in the natural substrate than in the macroplastic substrates during the experimental period (October 2021–April 2022). Substrate groups are natural substrates (NS); natural + plastic substrates (NP); and plastic-dominated substrates (PD).

3.4.5. Macroinvertebrate colonisation patterns on substrate groups

The parsimony analysis was used to examine the colonisation pattern of macroinvertebrates on the different substrate groups. The analysis starts from a hypothetical time zero (NS0, NP0, PD0) to the subsequent substrate's retrieval days (Figure 3.7). Chronologically, sampling days for each substrate group were arranged to capture the changes in succession and colonisation patterns over time. The parsimony plots revealed taxa contributing substantially to increasing

(unique) or decreasing diversity within a root (continuous to other time) or terminal branch (particular to a period; Figure 3.7).

In the NS tree, taxa 10 (Coenagrionidae), 15 (Belostomatidae), 21 (Naucoridae), 29 (Leptoceridae, and 33 (Helodidae) were unique to day 30 as early colonists. Taxa 6 (Tricorythidae) and 42 (Simuliidae) were unique from Day 60 onwards as intermediate to stable colonists (Figure 3.7A). Taxa 16 (Pleidae), 17 (Corixidae), and 46 (Planaria) were unique late colonisers of NS substrate on Day 180.

The NP substrate unique early colonists found on Day 30 were taxa 10 (Coenagrionidae), 11 (Corduliidae), 22 (Naucoridae), and 30 (Pisuliidae) (Figure 3.7B). The intermediate to stable colonists from Day 60 onwards were taxa 2 (Perlidae), 6 (Tricorythidae), 38 (Athericidae), 41 (Tabanidae), and 42 (Simuliidae) with taxa 41 (Tabanidae) and 2 (Perlidae) lost or decreased on Days 150 and 180, respectively. Taxon 8 (Heptageniidae) was the only unique late colonist on day 150, with none on day 180 (Figure 3.7B).

The unique early colonists of the PD substrates were taxa 23 (Glossosomatidae), 29 (Leptoceridae), and 30 (Pisuliidae) (Figure 3.7C). From day 60 onward, taxa 0 (Oligochaeta), 38 (Athericidae), 42 (Simuliidae), and 44 (Ancylidae) were recorded as stable colonists throughout, except for taxon 38 (Athericidae) that became absent on day 120. Unique late colonists include taxa 2 (Perlidae) and 28 (Sericostomatidae) found on day 150 with none recorded on day 180 (Figure 3.7C).

Generally, generalist taxa with high representation in all substrate groups throughout the experiment showed no succession trend. Taxa 3 (Baetidae), 24 (Hydropsychidae), and 35 (Chironomidae) are steady colonists (Figures 3.7A-C). From Day 60 onward, only taxon 42 (Simuliidae) was unique to both macroplastic and natural substrates, indicating increased establishment on all substrates. Thus, simuliids were intermediate and stable coloniser of all substrate groups in this experiment (Figures 3.7A-C). On Day 30, taxa 10 (Coenagrionidae), 29 (Leptoceridae), and 30 (Pisuliidae) were uniquely documented on NS, NP, and PD and are considered early colonists in all substrate categories (Figure 3.7).

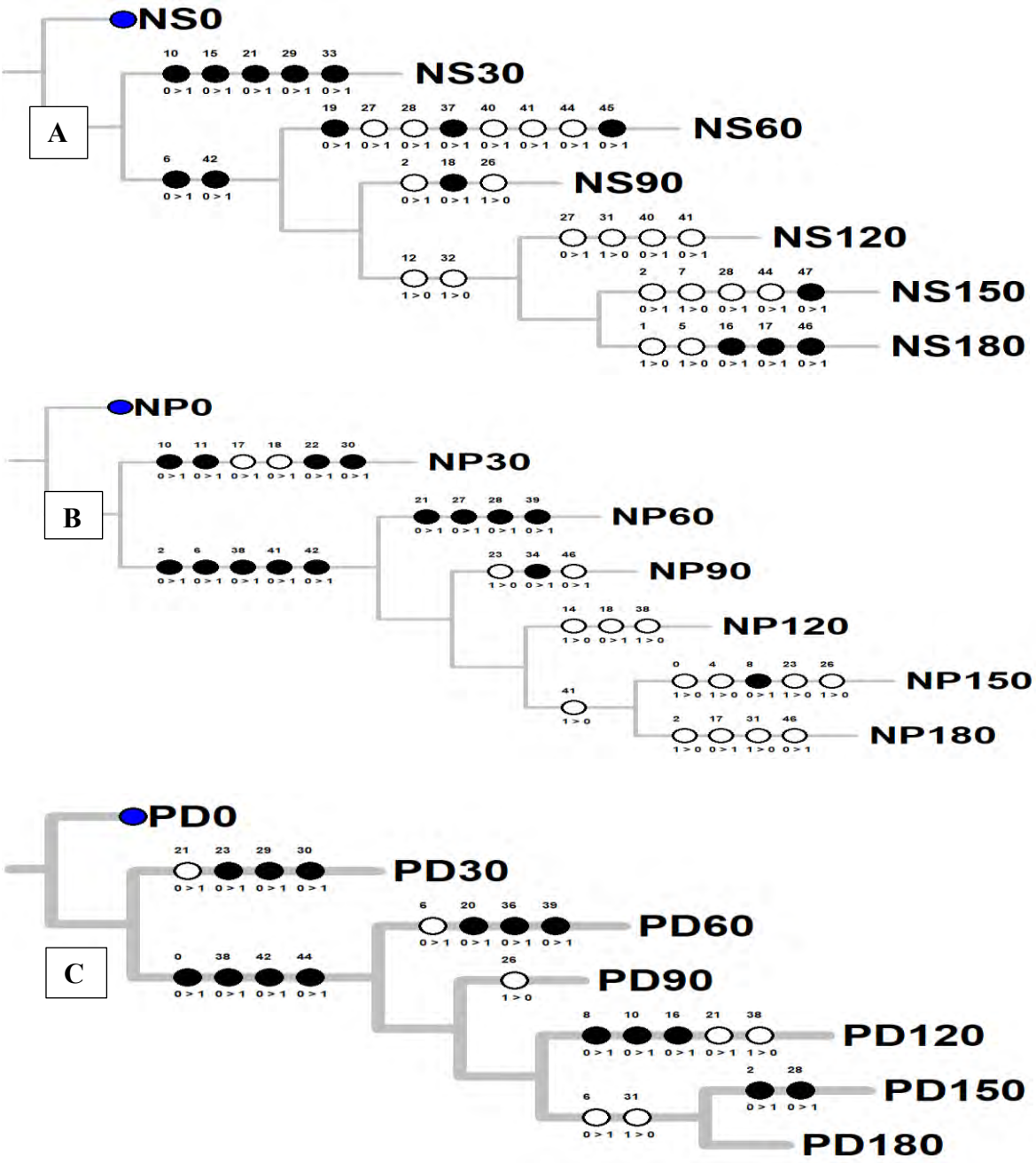

Figure 3.7: Consensus tree derived from parsimony analysis of presence/absence data matrix of NS (A), NP (B), and PD (C) substrates showing colonisation pattern over time. Closed black circles represent taxa unique to a substrate at a root branch leading to other periods or terminal branch of a particular time. Open circles represent the taxa not unique to a substrate or axis. Code numbers above the circles represent the taxon identified below the trees. Code "0 > 1" below any circle depicts the presence of that taxa, while "1 > 0" represent its absence. Taxon numeric codes: 0 = Oligochaeta; 1 = Potamonautidae; 2 = Perlidae; 3 = Baetidae; 4 = Caenidae; 5 = Leptophlebiidae; 6 = Tricorythidae; 7 = Aeshnidae; 8 = Heptageniidae; 9 = Synlestidae; 10 = Coenagrionidae; 11 = Corduliidae; 12 = Gomphidae; 13 = Lestidae; 14 = Libellulidae; 15 = Belostomatidae; 16 = Pleidae; 17 = Corixidae; 18 = Gerridae; 19 Pyralidae; 20 = Nepidae; 21 = Naucoridae; 22 = Ecnomidae; 23 = Glossosomatidae;

24 = Hydropsychidae; 25 = Veliidae; 26 = Philopotamidae; 27 = Dytiscidae; 28 = Sericostomatidae; 29 = Leptoceridae; 30 = Pisuliidae; 31 = Elmidae; 32 = Gyrinidae; 33 = Helodidae; 34 = Psephenidae; 35 = Chironomidae; 36 = Hydraenidae; 37 = Hydrophilidae; 38 = Athericidae; 39 = Ceratopogonidae; 40 = Ephydridae; 41 = Tabanidae; 42 = Simuliidae; 43 = Tipulidae; 44 = Ancylidae; 45 = Chlorosyphidae; 46 = Planaria; 47 = Physidae.

3.5 Discussion

3.5.1 Macroinvertebrate assemblage structure on substrate groups

This study provides the first baseline information on macroinvertebrate assemblage structure on macroplastic substrates in relation to natural substrates in four river systems. Plastic has been reported to modify the existing physical habitat by creating new habitat for faunal assemblages due to its complex makeup and durability both in the marine (Katsanevakis et al., 2007; Green et al., 2015; Torn et al., 2022) and freshwater systems (Czarnecka et al., 2009; Wilson et al., 2021, Gallitelli et al., 2023). However, knowledge concerning whether these novel habitats are suitable for macroinvertebrates remains sparse, and hence the present study aims to contribute to filling this knowledge gap.

The results indicated that macroinvertebrate assemblage structures of macroplastic and natural substrates were similar in richness, diversity and evenness. Significantly higher macroinvertebrate Simpson dominance values were recorded on 50% plastic (NP) and 100% plastic (PD) substrates than 100% natural substrates (NS). This result indicates that the dominance of macroinvertebrates assemblage on macroplastic substrates was due to the ability of fewer taxa capable of successfully establishing on NP and PD substrates. These dominant species made up the largest proportion of the total abundance of macroinvertebrates in this experiment. One possible explanation could be taxa-specific interactions (competition) or adaptations to macroplastic habitats that resulted in a few taxa dominating a wider range of taxa. As a result, less competitive and adaptive taxa were excluded or suppressed, consequently reducing diversity (Morris et al., 2014). This is contrary to previous research using a variety of discarded litter, such as plastic and glass, that indicated higher diversity on litter, such as plastic bottles and glass, in comparison to local, natural habitats (Czarnecka et al., 2009; Garcia-Vazquez et al., 2018; Wilson et al., 2021). Wilson et al. (2021) reported a significantly higher diversity of macroinvertebrate assemblages on plastic and fabric than those on natural rocks in

three United Kingdom rivers and attributed the high diversity to the complexity of plastic and fabric surfaces. However, their studies did not estimate the duration of residence of the anthropogenic litter in the three rivers. This may have confounded their results since the plastic and other waste used to compare with local natural habitats may be of different ages and origins or may have travelled a great distance with macroinvertebrates community (Chapman & Clynick, 2006; Kiessling et al., 2015).

Temporally, all substrates had significantly higher diversity, richness, and dominant macroinvertebrates assemblage in the first half (days 30– 90) of the experiment compared to the later days (120–180), indicating taxa diversity of macroinvertebrate species was higher, and individuals were distributed more equally among taxa in the first three retrieval events. Nevertheless, assemblage diversity decreased at subsequent sampling events, suggesting a change in the assemblage structure for both macroplastic and natural substrates. As these metrics decreased significantly in the later days of retrieval, it is possible that external disturbances, such as hydroperiodicity during the second half of the experiment could have influenced these measures. This stochastic disturbance may have altered the stability of all substrate groups, reset macroinvertebrate successions, and favoured fewer species that could colonise rapidly or withstand disturbances within those period (Czarnecka et al., 2009). Dinh and Death (2018) posited that less diverse communities are likely to be recorded in highly dynamic habitats. The macroplastic and natural substrates deployed in this experiment were anchored to maintain their position; however, spate could have potentially moved the substrates within the plastic litter bags and washed away inhabitant macroinvertebrates and available food resources within these substrates.

In Sydney Harbour, Chapman and Clynick (2006) compared three waste materials (tyres, wood, or metal) to a natural sandstone patch reef of identical age and size over 19 months and found similar algae and invertebrates on all sampled days. The summary metric results showed that macroinvertebrate assemblage was never different between macroplastic and natural substrates over time (Figure 3.2). This matches Chapman and Clynick's (2006) trend of macroinvertebrate recruitment on plastics, glass and natural surfaces over time. Although both studies differ in time, substrate, and ecosystem examined, this study suggests that macroinvertebrates colonise macroplastics similarly to natural surfaces within 180 days of deployment if the macroplastics and natural substrates are of similar proportion and exposure duration and deployed in the same hydraulic conditions.

3.5.2 Preferences of macroinvertebrate taxa to substrate groups

Stream substratum structure shapes macroinvertebrate assemblage structures in many freshwater systems (Dallas, 2007). Habitat heterogeneity supports biological diversity more than homogenous habitats does. With regard to macroinvertebrate substrate preferences, six community types were identified (Table 3.5). For each substrate group (NS, NP, PD), one taxon each indicated significant correlation to a particular substrate group. The NS group supported more families of macroinvertebrates than macroplastic substrate groups. This suggests that the natural substrate (NS) supported a diverse range of organisms, indicating its excellent ecological health and potential for high ecosystem resilience compared to macroplastic substrates. Despite the wide variety of taxa supported by the NS substrates, macroinvertebrate groups displayed a great deal of plasticity along the macroplastic substrate gradients as there were less than two taxa showing significant correlations to either natural or macroplastic substrate. This shows that macroinvertebrates find macroplastic substrate as suitable habitat similarly to natural substrates over time.

3.5.3 Community response of macroinvertebrates to natural and macroplastic substrates

Macroplastic and natural substrate had similar macroinvertebrate community compositions. The PRC and ANOSIM results indicated similarity in the macroinvertebrate community composition to be due to the variability between the substrate replicates (associated with time) rather than the variability between the substrate groups or substrate groups within sampling times (Table 3.6; Figure 3.5). A lack of habitat specialisation and niche differentiation among macroinvertebrate communities is evident from the similarity in macroinvertebrate community composition across the three substrate groups. Consequently, macroplastic substrates and natural substrates possibly provides similar ecological conditions, resulting in similar taxonomic adaptations (Wilson et al., 2021). Of the three substrates, SIMPER analysis showed that 50% plastic (NP) substrate macroinvertebrate community was the most dissimilar from natural substrate (NS) macroinvertebrate community (Figure 3.6). In accordance with this finding, PRC and ANOSIM found that macroinvertebrate communities differed significantly between NP and NS substrates for day 150 retrieved samples (Table 3.5; Figure 3.5), suggesting that NP substrates have the potential to influence macroinvertebrates over long periods of time. The NP substrate had higher abundances of dominant macroinvertebrates than NS and PD during the later days of the experiment. The observed similarity of macroinvertebrate community composition between substrate groups was mainly attributed to

the families Hydropsychidae, Chironomidae, Baetidae, Simuliidae, Tricorythidae, and Lestidae (Figure 3.5).

Lestidae abundance in the NS substrate was twice that of NP, with an average abundance in PD substrates. Lestoids are known predators found in vegetation as their preferred habitat (Samways, 2008; Suhling et al., 2015). Their abundance in the NS substrate could be due to deployed vegetation (as part of the NS component) and debris that mimic the naturally occurring substratum, of which foliage is part. A predatory feeding mechanism is advantageous in the natural substrate compared to the macroplastic-related substrates, which offer a more complex surface area for prey to avoid predators such as the Lestoids. The PRC taxa weight of Hydropsychidae, Simuliidae, Tricorythidae, and Chironomidae indicated support rendered by the 50% plastic substrate by their increased abundance compared to the 100% natural substrate towards the latter retrieval days (Figure 3.4). For example, Chironomids had high abundance in NS on days 30 and 60, and reduced relative to NP. A similar trend was seen with the Simuliidae and Tricorythidae, which were absent on day 30 and appeared on day 60 onward with greater abundance on the macroplastic substrates. These taxa also prefer rigid substrates such as stones and plastic substrates used in this study. Substrates such as plastic provided a surface for the attachment of hydropsychids and simuliids, and a deposit point for food sources and shelter against the flow current for the tricorythids (Okano et al., 2012, Adler, 2022).

The result reflected that the most dominant families of macroinvertebrates recorded were generalists that readily occupy any introduced substrate, such as macroplastic. These families are able to colonise and exploit a diverse range of habitats and resources, making them successful in freshwater system (Wallace & Anderson, 1995; Carvalho et al., 2013). The dominance of the macroinvertebrate assemblages by families Chironomidae, Hydropsychidae, Baetidae, and Simuliidae concurred with other experimental studies on artificial substrate (Downes et al., 2000; Molokwu et al., 2014; Leite-Rossi et al., 2015). Chironomidae is referred to as generalist because of their low selectivity for available resources (de Carvalho & Uieda, 2004; Carvalho et al., 2013), and the order Diptera are prominent consumers of biofilms on the surface of substrates such as wood (Bond et al., 2006). The growth of biofilm in the different substrates, among other factors such as accumulation of detrital matter, may have contributed to the dominance of Chironomidae in all the substrate groups as this family comprises a wide range of species with varying feeding modes. With regard to simuliids, it is possible that their dominance is as a result of their ability to anchor themselves firmly to the different substrate groups. Simuliids are filter feeders with apparatus for anchorage onto solid substrates (Shearer

et al., 2015; Adler, 2022). The Hydropsychidae was the dominant Trichopteran in this experiment, probably because the hard surface of both macroplastics and natural substrates provided a suitable surface for this taxon to attach to while filtering their food source, as was the case with submerged wood (Lyon et al., 2009). Family Baetidae is commonly established on different substrates in a wide array of southern African rivers, and some species can survive under varying conditions (Pereira-Da-Conceicoa et al., 2012). This experiment, conducted in four selected rivers, supports the report on their ability to colonise different substrate types. Baetidae and Chironomidae are known generalists in terms of habitat occupation and preferences, while Simuliidae and Hydropsychidae could be good conpetitors of hard substrates, such as PET macroplastics.

3.5.4 Macroinvertebrate colonisation and succession patterns on the substrate groups

Despite succession being a fundamental aspect of ecological theory, comparative studies on the succession pattern of macroinvertebrate colonisation of macroplastic and natural habitat remains sparse. Such comparison is imperative because colonisation and succession provide insight into the relative importance of community assembly mechanisms (e.g., environmental filtering and biotic interactions) at different stages of succession (Chang & Turner, 2019), and across spatial and temporal scales (Walker & Wardle, 2014). Successional trajectories of macroinvertebrates will assist in understanding whether macroinvertebrate community colonisation differs in macroplastic and natural substrates (Prach et al., 2016) and also provide a basis for understanding and informing mitigation and management of plastic pollution (Suding & Hobbs, 2009).

Taxa dispersion and ecological factors like food and microenvironments drive the colonisation of new substrates (Mackay, 1992; Molokwu et al., 2014). The first and most stable colonists in all substrate groups were the families Chironomidae, Hydropsychidae, and Baetidae that dominated throughout the experiment. These taxa were, however, not displayed by the parsimony tree due to their ambiguous distribution devoid of any succession pattern in the study. These taxa have been reported to be among pioneer colonists of submerged substrates in previous experiments (Molokwu et al., 2014; Winking et al., 2014; Milesi et al., 2019). The persistence of these taxa is due to their wide distribution, vagility, eurytopic nature, and feeding habits. For instance, Milesi et al. (2019) noted that early colonists are often common in water columns and riverbeds with a high tendency for drifting and clinging to substrates and are mainly collectors and/or filter feeders. On the other hand, other early colonists that were not

persistent, yet were associated with two or more substrates, were the coenagrionids and naucorids (NS, NP), leptocerids (NS, PD), and pisuliids (NP, PD; Figure 3.7). The free-living characteristics of these taxa may have been the reason for their recruitment in these substrates, especially as passive and active drifting plays a huge role in short-term colonisation (Winking et al., 2014). Additionally, the presence of prey, such as Chironomidae, in new habitats has been reported to facilitate the short-term colonisation of Odonata predators (e.g., Coenagrionidae and Naucoridae) in previous study (de Donnova et al., 2022). The result concurs with studies highlighting coenagrionids as highly mobile and opportunistic colonists of newly created habitats made of plastics and mixed substrates (Booth et al., 2013).

A general trend observed was that, macroinvertebrates colonised both macroplastic and natural substrates within the first 30 days of the experiment (Figure 3.7). High abundance and colonisation by macroinvertebrates were recorded mainly on 100% natural substrate (NS) and 50% plastic (NP) substrates on days 60 and 90, respectively. However, at the later stages of the experiment, while macroinvertebrates still showed substantial colonisation of natural substartes, macroplastic substrates (NP and PD) macroinvertebrate colonists decreased especially on day 180 (Figure 3.7). The colonisation of all substrate groups could be as a result of resident streambed inhabitants crawling into deployed substrates as reported by other experiment of introduced substrates to freshwater systems (Molokwu et al., 2014; Winking et al., 2016). The different late successional trajectory recorded in the macroplastic substrates in comparison to the more natural substrates, is alarming and may be driven by the quality of this macroplastic substrate groups. These differences could be as a result of reduced stability due to flow disturbance, less interstitial spaces available for specialist taxa, and spatial competition by inhabitants. For example, an extreme increase in the abundance of Simuliids was observed at the later stage on PD substrates. This taxon is known to be fiercely competitive for smooth surfaces compared to their counterparts, such as Hydropsychids that are ecosystem engineers and preferentially construct their nets on rough surfaces (Braccia et al., 2014; Molokwu et al., 2014). Conversely, as of day 60, Simuliids (#42) constituted a significant portion of stable colonies across all substrate types, as intermediate and established colonists of macroplastic and natural substrates throughout the study (Figure 3.7). Their preference for attachment substrates, such as trailing vegetation, stones, and refuse (such as plastics) (Greenwood, 1993; Adler, 2022), could be the reason that they thrive. These substrates provided them a platform to attach to while feeding on fine particulate matter captured within the water column.

Colonisation and successional comparison of substrate groups showed that substrates with the greatest macroinvertebrate dominance underestimated various taxa. This is particularly true for substrate dominated by plastic (PD) which is the most unstable, enabling just a few taxa to colonise and establish. The mixed substrate had a similar successional trend as the natural substrate (NS). This supports the univariate and multivariate findings that macroinvertebrates may find macroplastics habitat suitable. The results showed a succession of taxa expressed in ecological terms following the theory that pioneer species move, expand, or contract their realised niche due to competition for space or predation, after a certain period (Antoniadou et al., 2010; Toledo et al., 2020). Understanding the successional trajectories of riverine macroinvertebrates along a gradient of plastic pollution can help predict ecosystem responses to plastic pollution and how such pollution affects habitat selection by macroinvertebrates.

3.6 Conclusion

Macroinvertebrates respond to macroplastic ecological risk from a habitat quality perspective; their effect on biological colonisation in freshwater systems is recent, with no studies in the Afrotropics. The spatio-temporal analysis of macroinvertebrates represents a valuable starting point for ecological and biotic responses to macroplastic pollutant impacts on river habitat quality. The results showed that macroinvertebrates can colonise macroplastic and natural substrates in rivers with substrate characteristics playing a minimal role, especially in the early stages, which showed structural and compositional differences from macroinvertebrate community of the later stages of colonisation on natural and macroplastic substrate. This implies that comparing both macroplastics and natural substrate communities in rivers may require more time, and that other external factors such, as hydraulic conditions, may unconfound these findings. An additional implication for managing and conserving riverine ecosystems is that macroinvertebrates find macroplastic habitats attractive but not necessarily more suitable, and mitigation measures are imperative for curtailing the long-term effects of macroinvertebrate-macroplastic interaction. Hence, this chapter contributes to reducing the knowledge gap about macroinvertebrate community responses to macroplastic pollution in Afrotropical riverine systems.

CHAPTER 4: INFLUENCES OF HYDRAULIC BIOTOPES ON THE TAXONOMIC ASSEMBLAGE STRUCTURE OF MACROINVERTEBRATES ON MACROPLASTICS.

4.1 Introduction

Hydraulic biotopes are important features in riverine systems, capable of structuring macroinvertebrates assemblage structure and composition (Jowett, 2003; Reid & Thoms, 2008; Silva et al., 2014). Given the role played by hydraulic biotopes at the reach scale, they are important variables to consider in ecological studies of rivers. For example, empirical evidence suggests that certain macroinvertebrate taxa often show preferences for specific hydraulic biotopes. Families such as Simuliidae have been shown to prefer the more turbulent hydraulic biotopes, such as runs and riffles (Principe et al., 2007; Thirion, 2016). The abiotic dynamics of different hydraulic biotopes are also important in structuring food and resource availability, the nature of substrate that may be found within the biotope, as well as the trait necessary to adapt by biological organisms (Biggs et al., 2005; Reid & Thoms, 2008). For instance, pools with non-perceptible water currents are usually amenable to fine sediments settling, thereby providing a non-stable substrate for biota (Shearer et al., 2015). Unlike pools, substrate stability in reaches with higher water current (e.g., run and riffle) is advantageous for organisms such as filter-feeders to attach while feeding on drifting particles (Jowett, 2003; Olivier et al., 2009; Shearer et al., 2015). The presence of plastic materials in riverine systems may modify the hydraulic biotope habitat patch distribution. In turn, the hydraulic biotopes may also govern how colonisation takes place on macroplastic materials within riverine systems.

The interactions between hydraulic biotopes and plastic materials in riverine systems is poorly studied. The way in which such interactions may regulate the colonisation, establishment and succession patterns of macroinvertebrates is also not well documented in the literature (Gallitelli et al., 2023). In pool-dominated river systems, plastic materials may rapidly become buried in sediments, allowing burrowers to become the dominant colonisers, whereas in runs, the continuous flow of water current implies that plastic surfaces may be dominated by biofilms, supporting organisms that have a natural preference for high-flow velocity (Thirion, 2016). Thus, hydraulic biotope-macroplastic interaction may result in novel habitats at the reach scale, which may have implications for biodiversity conservation and non-native species taking advantage of such novel habitats. For example, Akoumianaki et al. (2008) reported that non-native species might benefit more from novel plastic habitats than native species,

especially in unstable sediment habitats. However, the instability of plastic substrates under high flows is also a concern for biodiversity protection. Identifying how hydraulic biotopes influence macroinvertebrate colonisation of macroplastics in riverine systems is thus critical for effective management strategies.

Approximately 90% of South Africa's rivers (including the four river systems studied here) are foothills (Nel & Driver, 2015). Thus, in the same way flow patterns in conjunction with natural rocks, wood, and aquatic macrophytes affect habitat heterogeneity, so plastic litter acting simultaneously with the hydraulic biotope may increase habitat heterogeneity (Pilotto et al., 2014). The ecology of macroplastic novel habitat, like any other freshwater system substrate such as wood and leaf litter, requires understanding how hydraulic biotopes facilitate macroinvertebrate colonisation of macroplastics at the microhabitat scale (Roque et al., 2010; Siqueira et al., 2012). Understanding how macroinvertebrates colonise macroplastic substrates under varying hydraulic conditions is crucial to how novel habitats created by macroplastics affect biological diversity at the reach scale. Several studies have found that different substrate and surface flow types result in different assemblage compositions (Duan et al., 2008; Reid & Thoms, 2008). However, Silva et al. (2014) argue for the need to examine both components together, or to consider a range of substrate and hydraulic habitat types to represent natural patterns better. Therefore, the objective of this chapter is to analyse the influences of hydraulic biotopes on the structuring and colonisation patterns of macroinvertebrates in the studied river systems. This objective addresses objective 2 of the study, stated in Chapter 1, Section 1.6.2.

4.2 Materials and methods

Detailed descriptions of the study area and sampling design are provided in Chapter 2. The study was conducted in four selected sites in four river systems (Kowie, Buffalo, Kat, and Swartkops) between October 2021 and April 2022. Macroplastic materials and natural substrates were classified into three substrate groups: group 1, 100% natural substrate (NS); group 2, 50% plastic substrate (NP); and group 3, 100% plastic substrate (PD). The three substrate groups representing a gradient of macroplastic substrates were deployed into three hydraulic biotopes: pool, riffles, and runs.

Macroinvertebrates were collected on six different occasions, on days 30, 60, 90, 120, 150, and 180 from October 2021 to April 2022. Concurrently, physicochemical variables at each site for each hydraulic biotope were recorded. Detailed descriptions of tools, sampling techniques, and study design are outlined in Chapter 2. Six bags of substrates per substrate group were

randomly deployed throughout each hydraulic biotope per river location. Each substrate group received 18 substrate bags. Each experimental site contained 54 bags (6x3x3) throughout the study, giving a total of 216 substrate bags (4 x 6 x 3 x 3). Each substrate group within a hydraulic biotope had four replicates per sampling event. Due to the lack of hydraulic biotopes and substrate loss from high flow current and vandalism, the following replicates for pool (NS = 18, NP = 19, PD = 18); riffle (NS = 14, NP =15, PD = 18); and run (NS = 18, NP = 18, PD = 17) were retrieved and used for analysis.

4.3 Data analysis

4.3.1 Macroinvertebrate assemblage structure of substrate groups in relation to hydraulic biotopes

Macroinvertebrate data were analysed for each dataset at hydraulic biotope level for all substrate groups. Four metrics representing different taxonomic measures were used to assess the structural response of macroinvertebrates to the three substrate groups within the hydraulic biotopes (See Chapter 3, Table 3.1 for a full description of metrics). Cluster charts were used to graphically represent the metrics, and a Kruskal-Wallis test used for statistically significant differences between macroinvertebrate metrics of the substrate groups. Before the Kruskal-Wallis test, macroinvertebrate diversity data was subjected to Shapiro-Wilks and Levene's tests to ascertain normality and homogeneity of variance and normalised using log (x + 1) transformation, respectively. All tests were conducted using Statistica software v13 (Tibco Software, 2018).

Macroinvertebrate preference for substrate group within hydraulic biotopes

To examine whether macroinvertebrates from a particular hydraulic biotope showed a preference for natural or macroplastic substrate, the Pearson's point-biserial correlation was employed. Pearson's point-biserial correlation coefficient enables the determination of the relation between the taxa and substrate groups within the hydraulic biotopes (pool, riffle, run) (de Cáceres, 2013). The purpose of the analysis was to determine the influence of the hydraulic biotope group on the macroinvertebrate assemblage structure and to identify community types based on the preferred substrate group. Pearson's point-biserial correlation analysis uses the Monte Carlo permutation test with 999 permutations at a 0.05 significance level to evaluate the association. The Pearson's point-biserial correlation was conducted using the Indicspecies

package version 1.7.12 within the R version 4.3.0 statistical environment (de Cáceres, 2013; R Core Team, 2022).

4.3.2. Macroinvertebrate community response to substrate groups within hydraulic biotopes

The principal response curve (PRC) analysis was used to determine whether macroinvertebrate communities on macroplastic and natural substrates differed spatio-temporally within individual hydraulic biotope. The analysis found macroinvertebrates responsible for such divergence and their proportionate contribution. The PRC is a multivariate approach specifically designed to analyse experimental community response to treatments over time by concentrating on the taxonomic composition of the treatment (NP and PD) and control (NS) gathered concurrently (van den Brink & ter Braak, 2009). The PRC model divides variance by time (sample date) and treatment (substrate). The summary of procedure and interpretation of the PRC analysis has been given in the previous chapter (Chapter 3, section 3.3.3).

Two-way multivariate permutational analysis of variance (PERMANOVA) was used to examine the influence of hydraulic biotopes on macroinvertebrate assemblage structure on macroplastics, by comparing macroinvertebrate community composition for the three substrate groups within a particular hydraulic biotope. Where applicable, multiple pairwise post-hoc comparison tests were performed to reveal significant differences in the macroinvertebrate community structure between substrates or period. Before PERMANOVA, Shapiro-Wilk and Levene tests confirmed the distribution of the data and homogeneity of variance, respectively. Similarity percentage (SIMPER) was used to indicate the percentage contributions of taxa to the (dis)similarity among different gradients of plastic substrate. Both analyses were conducted using the vegan package version 2.4.3 within the R statistical software version 4.2.0 (Oksanen, 2013; R Core Team, 2022).

4.4 Results

4.4.1 Spatio-temporal response of macroinvertebrate assemblage structure to substrate groups relative to hydraulic biotopes

The pool biotope had 40 macroinvertebrate taxa from a total of 2,611 macroinvertebrate individuals. The highest percentage relative composition of macroinvertebrates throughout the study was macroinvertebrates observed in 100% natural substrate NS (40.03%) with 28.07%

for 50% plastic substrate NP as the lowest contributor (Table 4.1). The relative abundance contributions of macroinvertebrates in NS were Chironomidae, Hydropsychidae, and Baetidae with 35.59%, 13.21%, and 9.38%, respectively. By contrast, in NP, the highest contributing taxa were Chironomidae (39.59%), Baetidae (14.87%), and Caenidae (10.5%). The families Chironomidae, Baetidae, and Lestidae dominated macroinvertebrate assemblage in 100% plastic substrates PD contributing 39.49%, 21.48%, and 8.88%, respectively. The least relative abundance of macroinvertebrates was recorded in the families Perlidae, Corduliidae, Nepidae, Leptoceridae, and Planaria with a contribution of 0.09% each.

At riffle, a total of 5,431 macroinvertebrate individuals belonging to 37 families was recorded with relative abundance contributions of 40.4%, 31%, and 27.8% by NP, PD, and NS, respectively (Table 4.1). In NS, macroinvertebrate assemblage was dominated relatively by Chironomidae (51.62%), Hydropsychidae (13.38%), Baetidae (12.13%), and Simuliidae (7.55%%). For NP, Chironomidae, Hydropsychidae, and Simuliidae contributed 30.75%, 21.1%, and 18.36%, respectively. The relative abundance of macroinvertebrates in PD was by dominating chironomids (34.11%), hydropsychids (24.1%), baetids (15.87%), and simuliids (11.28%). The families Pisuliidae, Naucoridae and Corduliidae, Pyralidae, and Sericostomatidae had the lowest dominance and contribution to relative macroinvertebrate abundance in riffles.

In runs, 9,406 macroinvertebrates belonging to 35 families were recorded (Table 4.1). Relatively, macroinvertebrate assemblage contribution was 38.6% (NP), 32.3% (PD), and 29.2% (NS). Hydropsychids, chironomids, baetids, and simuliids dominated the assemblage structure in all substrate groups. They contributed 29.1%, 30.74%, 14.13%, and 9.54% in NS; 38.24%, 19.35%, 13.15%, and 11.17% in NP; and in PD, 27.88%, 27.36%, 15.16%, and 15.39%, respectively.

In general, there were no significant differences in the number of taxa between substrate groups (NS, NP, and PD) and substrate groups at any period for all hydraulic biotopes (Figure 4.1; KW-W values having $P > 0.05$). Temporally, the number of taxa only differed significantly per period in riffles. The first three periods (30–90 days) in riffles had significantly higher number of taxa than the days 120–180 (Figure 4.1; $P < 0.05$). In pool, riffle and run, more macroinvertebrate taxa occurred on day 60 than any other day.

Table 4.1: Percent relative abundance of macroinvertebrates on substrate groups within hydraulic biotope during the study period (October 2021–April 2022). Abbreviations: NS = Natural substrate, NP = Natural + Plastic substrate, and PD = Plastic-dominated substrate.

		Pool			Riffle			Run		
Order	Family	NS	NP	PD	NS	NP	PD	NS	NP	PD
ANNELIDA	Oligochaeta	0.55	0.25	0.20	0.28	0.59	0.13	0.39	0.30	0.30
CRUSTACEA	Potamonautidae	0.34	0.37	0.29	0.57	0.19	0.24	0.50	0.13	0.38
PLECOPTERA	Perlidae	1.00			0.50	0.50		0.33	0.50	0.17
EPHEMEROPTERA	Baetidae	0.25	0.28	0.46	0.24	0.39	0.37	0.29	0.36	0.35
	Caenidae	0.41	0.38	0.21	0.28	0.41	0.31	0.26	0.41	0.32
	Leptophlebiidae	0.22	0.39	0.39	0.25	0.39	0.36	0.50	0.16	0.34
	Tricorythidae	0.83	0.17		0.07	0.75	0.18	0.25	0.57	0.18
	Heptageniidae	0.25	0.50	0.25	1.00			0.50		0.50
ODONATA	Aeshnidae	0.33	0.19	0.48	0.24	0.43	0.32	0.38	0.24	0.38
	Chlorosyphidae				1.00					
	Synlestidae		1.00						1.00	
	Coenagrionidae	0.85	0.08	0.08	1.00				1.00	
	Corduliidae		1.00							
	Gomphidae	0.23	0.23	0.54	0.27	0.27	0.45	0.29	0.53	0.18
	Lestidae	0.39	0.25	0.36	0.45	0.21	0.33	0.63	0.17	0.21
	Libellulidae	0.46	0.31	0.23	0.45	0.35	0.20	0.54	0.17	0.29
LEPIDOPTERA	Pyralidae				1.00					
HEMIPTERA	Belostomatidae							1.00		

	Pleidae	0.75		0.25						
	Corixidae		1.00		0.50	0.50				
	Gerridae	0.50	0.50			1.00				
	Nepidae			1.00						
	Naucoridae	0.47		0.53	0.67	0.33				1.00
	Veliidae			1.00						1.00
TRICHOPTERA	Ecnomidae	0.75	0.25							
	Glossosomatidae					0.67	0.33		1.00	
	Hydropsychidae	0.70	0.14	0.16	0.19	0.43	0.38	0.26	0.46	0.28
	Philopotamidae	1.00			0.03	0.38	0.59	0.07	0.40	0.52
	Sericostomatidae	1.00				1.00		0.09	0.55	0.36
	Leptoceridae	1.00					1.00			
	Pisuliidae					1.00				1.00
COLEOPTERA	Dytiscidae	0.40		0.60	0.22	0.56	0.22		1.00	
	Elmidae	0.80		0.20	0.38	0.25	0.38	0.35	0.59	0.06
	Gyrinidae	0.50		0.50	0.26	0.29	0.46	0.42	0.28	0.30
	Helodidae							1.00		
	Psephenidae			1.00		0.67	0.33		0.06	0.94
	Hydraenidae			1.00						
	Hydrophilidae				1.00					
DIPTERA	Athericidae	0.38	0.38	0.25		0.34	0.66	0.11	0.49	0.40
	Chironomidae	0.37	0.29	0.33	0.38	0.33	0.29	0.36	0.30	0.35

	Ceratopogonidae	0.25	0.50	0.25			1.00	1.00		
	Ephydridae	1.00								
	Tabanidae	1.00			0.56	0.33	0.11	0.48	0.43	0.09
	Simuliidae	0.85		0.15	0.16	0.57	0.27	0.23	0.36	0.41
	Tipulidae				1.00					
GASTROPODA	Ancylidae	0.22	0.22	0.56	0.25		0.75		0.36	0.64
	Physidae	1.00								
TURBELLARIA	Planaria	1.00				1.00			1.00	
Total Abundance		1045	733	833	1509	2195	1727	2745	3627	3034
Percent Relative Abundance		40	28.1	31.9	27.8	40.4	31.8	29.2	38.6	32.3
No. of Taxa		32	22	26	27	28	24	24	27	26

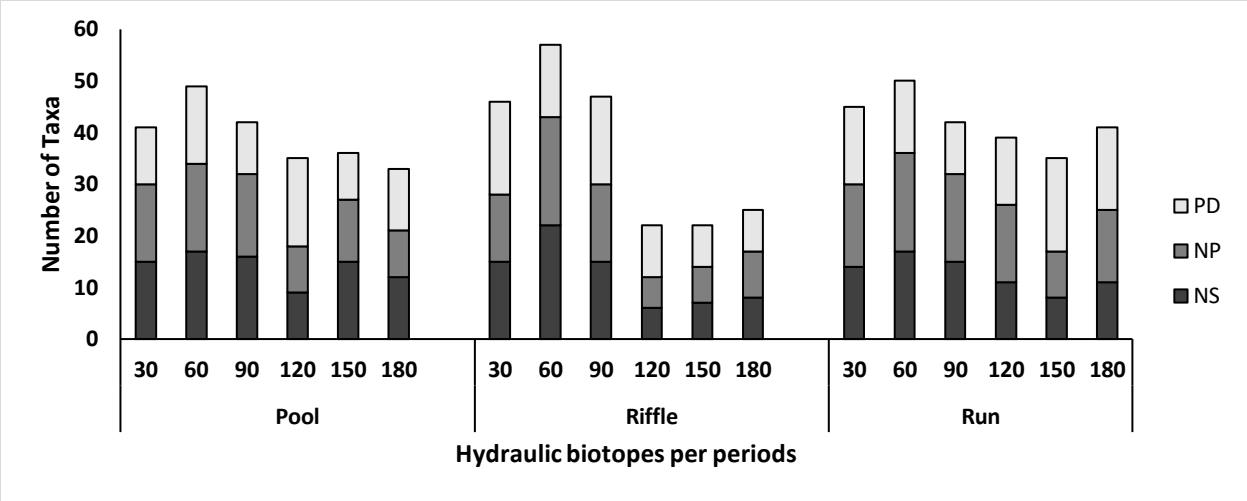

Figure 4.1: Clustered column graphs indicating number of taxa recorded on substrate groups within hydraulic biotopes per period during the study (October 2021–April 2022). Substrates: NS = Natural substrate, NP = Natural + Plastic substrate, PD = Plastic-dominated substrate.

In terms of summary metrics for pools, the NS substrate had significantly higher Shannon and Simpson index values of macroinvertebrates than NP and PD (Figure 4.2C and D; KW-H values with P <0.05). No significant difference was observed for substrate groups per period and between periods for all other summary metrics (Figure 4.2A-D; KW-H values all had *p-value* >0.05).

In riffles, there were no significant differences in substrate groups and the comparison of substrate groups per period for all summary metrics (both KW-H values at P>0.05; Figure 4.2A-D). However, there were significant temporal differences for all metrics with post-hoc tests revealing that on the days 30–90, the macroinvertebrate structure was richer, more even, diverse, and dominant than days 120–180 (Figure 4.2A-D; P <0.05).

Summary metrics in runs showed a similar trend to the riffle biotope with no difference between overall substrate groups and substrates by period (both KW-H values at P>0.05; Figure 4.2A-D). Temporal differences were observed for all metrics in runs (Figure 4.2A-D; P <0.05). The post-hoc test revealed higher richness, evenness, diversity and dominance on days 30 and 60 than days 150 and 180 (Figure 4.2A-D; P <0.05).

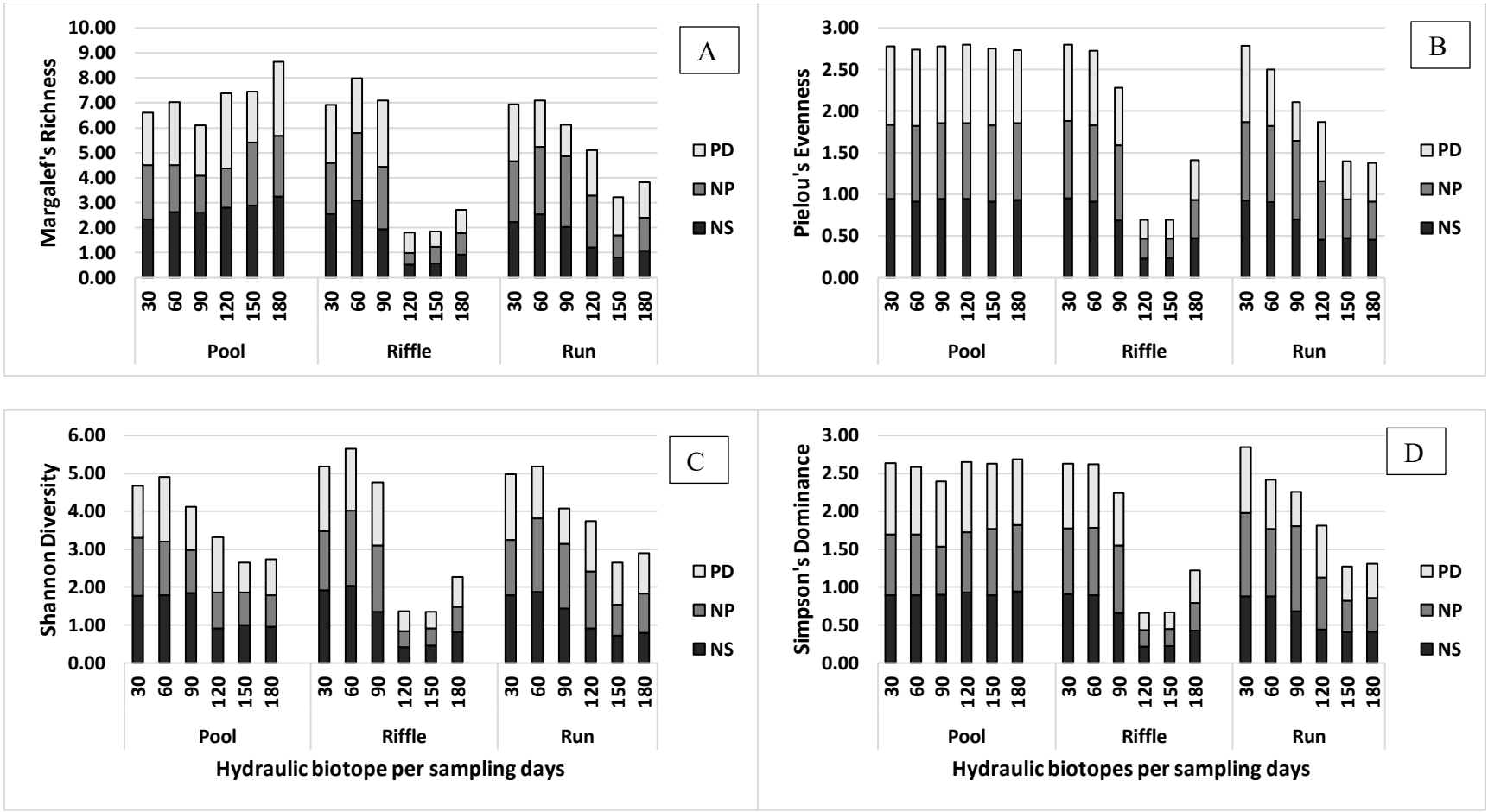

Figure 4.2: Mean values for Margalef's richness (A), Pielou's evenness (B), Shannon index (C), and Simpson's index (D) for substrate groups per sampling day across hydraulic indicating the spatiotemporal summary metrics value during the experiment (October 2021 to April 2022). Substrate groups: NS (Natural substrate); NP (Natural + Plastic substrate); PD (Plastic dominated substrate).

4.4.2 Taxa preferences for particular hydraulic biotope across the gradients of plastic substrate

Macroinvertebrate samples from the pool were subjected to Pearson's point-biserial correlation analysis to elucidate the association between macroinvertebrate taxa and substrate groups. Six macroinvertebrate assemblage types were outlined, based on the association with substrate groups (Table 4.2A). The highest association was recorded in the first assemblage type with 17 families, of which only the family Tabanidae was significantly associated with the NS substrate ($p < 0.05$). No other taxa showed significant preference for any particular substrate or combination of substrate groups (Table 4.2A).

Table 4.2A: Macroinvertebrate assemblage preference for the particular substrate group in pools during the study (October 2021–April 2022). Substrates: NS = Natural; NP = Natural + Plastic, and PD = Plastic-dominated. Taxa preference is indicated by the numbers 1 and 0 under the substrate indicating a family preferring a particular substrate or not preferring a specific substrate, respectively. Index (substrate preference index) indicates families preferring the NS (1), NP (2), PD (3), both NS and NP (4), both NS and PD (5), and both NP and PD (6). Significant associations at $p < 0.05$ are bolded.

Family	NS	NP	PD	Code	Point-Biserial Co-efficient	*p*-value
Tabanidae	1	0	0	1	0.309	**0.049**
Elmidae	1	0	0	1	0.263	0.15
Simuliidae	1	0	0	1	0.273	0.15
Ephydridae	1	0	0	1	0.277	0.20
Philopotamidae	1	0	0	1	0.209	0.22
Hydropsychidae	1	0	0	1	0.213	0.24
Ecnomidae	1	0	0	1	0.173	0.41
Tricorythidae	1	0	0	1	0.187	0.43
Libellulidae	1	0	0	1	0.156	0.51
Planaria	1	0	0	1	0.194	0.62
Leptoceridae	1	0	0	1	0.194	0.65
Perlidae	1	0	0	1	0.194	0.68
Physidae	1	0	0	1	0.194	0.68

Sericostomatidae	1	0	0	1	0.194	0.68
Pleidae	1	0	0	1	0.154	0.75
Coenagrionidae	1	0	0	1	0.176	0.77
Oligochaeta	1	0	0	1	0.134	0.84
Corixidae	0	1	0	2	0.270	0.31
Chlorolestidae	0	1	0	2	0.255	0.32
Ceratopogonidae	0	1	0	2	0.073	1.00
Corduliidae	0	1	0	2	0.189	1.00
Heptageniidae	0	1	0	2	0.073	1.00
Veliidae	0	0	1	3	0.277	0.22
Hydraenidae	0	0	1	3	0.277	0.22
Baetidae	0	0	1	3	0.164	0.51
Gomphidae	0	0	1	3	0.144	0.59
Nepidae	0	0	1	3	0.194	0.66
Psephenidae	0	0	1	3	0.194	0.68
Ancylidae	0	0	1	3	0.112	0.91
Caenidae	1	1	0	4	0.135	0.64
Athericidae	1	1	0	4	0.064	0.92
Potamonautidae	1	1	0	4	0.057	0.94
Gerridae	1	1	0	4	0.137	1.00
Dytiscidae	1	0	1	5	0.226	0.19
Naucoridae	1	0	1	5	0.195	0.34
Aeshnidae	1	0	1	5	0.182	0.35
Gyrinidae	1	0	1	5	0.139	0.53
Lestidae	1	0	1	5	0.123	0.64
Chironomidae	1	0	1	5	0.096	0.80
Leptophlebiidae	0	1	1	6	0.148	0.56

The riffle had six macroinvertebrate assemblage types based on their preference for substrate groups as revealed by Pearson's point-biserial correlation coefficient (Table 4.2B). The first assemblage type comprised 10 families with none having significant preference for the NS

substrate. No significant association was observed within the different assemblage types outlined in riffle.

Table 4.2B: Macroinvertebrate assemblage preference for the particular substrate group in riffles during the study (October 2021–April 2022). Substrates: NS = Natural; NP = Natural + Plastic, and PD = Plastic-dominated. Taxa preference is indicated by the numbers 1 and 0 under the substrate indicating a family preferring a particular substrate or not preferring a specific substrate, respectively. Index (substrate preference index) indicates families preferring the NS (1), NP (2), PD (3), both NS and NP (4), both NS and PD (5), and both NP and PD (6). Significant associations at $p < 0.05$ are bolded.

Family	NS	NP	PD	index	Point-Biserial Co-efficient	*p*-value
Tipulidae	1	0	0	1	0.316	0.21
Potamonautidae	1	0	0	1	0.239	0.32
Naucoridae	1	0	0	1	0.154	0.53
Coenagrionidae	1	0	0	1	0.221	0.64
Chironomidae	1	0	0	1	0.150	0.64
Tabanidae	1	0	0	1	0.149	0.65
Heptageniidae	1	0	0	1	0.221	0.65
Chlorocyphidae	1	0	0	1	0.221	0.65
Hydrophilidae	1	0	0	1	0.221	0.66
Pyralidae	1	0	0	1	0.221	0.67
Tricorythidae	0	1	0	2	0.252	0.25
Oligochaeta	0	1	0	2	0.194	0.49
Simuliidae	0	1	0	2	0.155	0.72
Gerridae	0	1	0	2	0.213	1.00
Sericostomatidae	0	1	0	2	0.213	1.00
Pisuliidae	0	1	0	2	0.213	1.00
Planaria	0	1	0	2	0.213	1.00
Athericidae	0	0	1	3	0.238	0.24
Ancylidae	0	0	1	3	0.230	0.37
Ceratopogonidae	0	0	1	3	0.221	0.65
Leptoceridae	0	0	1	3	0.221	0.66

Gyrinidae	0	0	1	3	0.127	0.72
Gomphidae	0	0	1	3	0.109	0.77
Libellulidae	1	1	0	4	0.170	0.50
Perlidae	1	1	0	4	0.176	0.77
Corixidae	1	1	0	4	0.155	1.00
Lestidae	1	0	1	5	0.184	0.47
Elmidae	1	0	1	5	0.085	0.86
Dytiscidae	1	0	1	5	0.081	0.86
Hydropsychidae	0	1	1	6	0.178	0.53
Baetidae	0	1	1	6	0.129	0.70
Philopotamidae	0	1	1	6	0.180	0.72
Leptophlebiidae	0	1	1	6	0.091	0.83
Aeshnidae	0	1	1	6	0.096	0.83
Caenidae	0	1	1	6	0.032	0.97
Glossosomatidae	0	1	1	6	0.147	1.00
Psephenidae	0	1	1	6	0.147	1.00

In run, Pearson's point-biserial correlation coefficient identified six community types (Table 4.2C). The second assemblage type preferred the NP substrate and had the highest assemblage of nine families, of which only the family Glossosomatidae had significant preference for the NP substrate. Of the other substrate community types, none indicated a significant association with any of the observed macroinvertebrate families.

Table 4.2C: Macroinvertebrate assemblage preference for the particular substrate group in run during the study (October 2021–April 2022). Substrates: NS = Natural; NP = Natural + Plastic, and PD = Plastic-dominated. Taxa preference is indicated by the numbers 1 and 0 under the substrate indicating a family preferring a particular substrate or not preferring a specific substrate, respectively. Index (substrate preference index) indicates families preferring the NS (1), NP (2), PD (3), both NS and NP (4), both NS and PD (5), and both NP and PD (6). Significant associations at $p < 0.05$ are bolded.

Family	**NS**	**NP**	**PD**	**index**	**Point-Biserial Co-efficient**	***p*-value**

Lestidae	1	0	0	1	0.233	0.20
Helodidae	1	0	0	1	0.206	0.28
Ceratopogonidae	1	0	0	1	0.206	0.32
Belostomatidae	1	0	0	1	0.206	0.33
Libellulidae	1	0	0	1	0.196	0.37
Leptophlebiidae	1	0	0	1	0.177	0.52
Oligochaeta	1	0	0	1	0.084	0.87
Gyrinidae	1	0	0	1	0.089	0.93
Glossosomatidae	0	1	0	2	0.316	**0.03**
Tricorythidae	0	1	0	2	0.158	0.58
Gomphidae	0	1	0	2	0.124	0.68
Hydropsychidae	0	1	0	2	0.117	0.73
Caenidae	0	1	0	2	0.079	0.87
Chlorolestidae	0	1	0	2	0.194	1.00
Coenagrionidae	0	1	0	2	0.194	1.00
Dytiscidae	0	1	0	2	0.194	1.00
Planaria	0	1	0	2	0.194	1.00
Psephenidae	0	0	1	3	0.239	0.10
Naucoridae	0	0	1	3	0.200	0.61
Pisuliidae	0	0	1	3	0.200	0.64
Veliidae	0	0	1	3	0.200	0.65
Elmidae	1	1	0	4	0.256	0.15
Tabanidae	1	1	0	4	0.190	0.42
Perlidae	1	1	0	4	0.108	0.85
Potamonautidae	1	0	1	5	0.219	0.24
Heptageniidae	1	0	1	5	0.145	0.52
Aeshnidae	1	0	1	5	0.131	0.62
Chironomidae	1	0	1	5	0.100	0.78
Ancylidae	0	1	1	6	0.183	0.49
Philopotamidae	0	1	1	6	0.169	0.52
Athericidae	0	1	1	6	0.155	0.59

Simuliidae	0	1	1	6	0.098	0.84
Baetidae	0	1	1	6	0.047	0.95
Sericostomatidae	0	1	1	6	0.107	1.00

4.4.3 Macroinvertebrate community response to substrate groups within hydraulic biotopes

The principal response curve (PRC) analysis of data from pools displayed marked fluctuating deviations between the macroplastic and natural substrate macroinvertebrate communities with a similar pattern for NP and PD macroinvertebrate communities (Figure 4.3A). The greatest deviation of macroinvertebrate communities from the natural substrate by macroinvertebrates in the macroplastic substrates was observed on day 150 in both NP and PD (Figure 4.3A). The PRC analysis indicated that the substrate group effect accounted for 11.34% of the total variance. The variance attributed to sampling time differences accounted for only 11.7%. However, the remaining 85.50% was due to variability between replicates over time. Based on the Monte Carlo permutation test, the PRC test result was not statistically significant (F = 0.66, pr (>F) = 0.99). The first axis of the PRC with an eigen value of 0.41 explained 37.18% of the variance and was plotted as Figure 4.3A.

The PRC taxa scores in pools indicated that the abundance of families Hydropsychidae, Lestidae and Simuliidae were substantially reduced in macroplastic substrates (NP and PD). However, these taxa abundances in NS increased over time (Figure 4.3A). Figure 4.3A shows the remaining taxa had weights close to zero and were determined as non-influential to the PRC.

The PERMANOVA results of pool macroinvertebrate data indicated no significant differences (*P(perm)* > 0.05) in macroinvertebrate community composition of substrate group pair and period pair. At no time were there differences between substrate group pair per period (Table 4.4). The SIMPER analysis revealed macroinvertebrate community dissimilarities between the substrate groups, with the highest dissimilarity of 75.03% observed for NS and PD substrates, 74.3% between the NP and PD substrates, and 73.44% for the NS and PD substrates (Figure 4.3A). With the exception of Simuliidae revealed in the pool PRC, the SIMPER result also reported the main contributors to the dissimilarities between the substrate groups were mainly Chironomidae, Lestidae, Hydropsychidae, and Baetidae across all substrate dissimilarity pairs (Figure 4.3A).

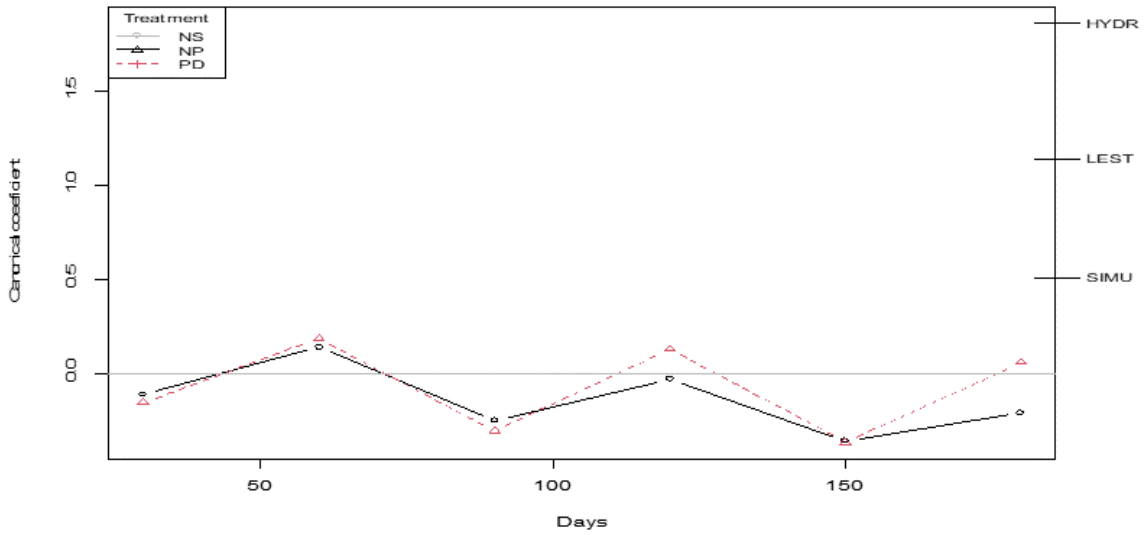

Figure 4.3A: Principal response curve (PRC) of pool with taxa weights indicating the macroinvertebrate community response to the plastic substrates per period. Taxa include Hydropsychidae (HYDR), Lestidae (LEST), and Simuliidae (SIMU). Taxa whose weights were between 0.5 and -0.5 were omitted for clarity.

The riffle data PRC taxa scores indicated that the most affected taxa were Oligochaeta and Lestidae. The abundance of Oligochaeta and Lestidae declined in macroplastic substrates (NP and PD; Figure 4.3B). However, Tricorythidae, Hydropsychidae and Simuliidae abundances increased in NP and PD relative to NS over time as they were the taxa less affected by macroplastic substrates in riffles (Figure 4.3B). The remaining taxa had weights close to zero and were determined as non-influential to the PRC.

The principal response curve (PRC) analysis of data from riffles showed a fluctuating deviation between the macroplastic and natural substrate macroinvertebrate communities, with the greatest deviation of macroinvertebrate community on day 90 by NP macroinvertebrate community (Figure 4.3B). The PRC analysis indicated that the substrate group effect accounted for 8.48% of the total variance. The variance attributed to sampling time differences accounted for only 17.3%. The variability between replicates over time accounted for the remaining 74.26%. The Monte-Carlo permutation test revealed that the PRC test result was not statistically significant ($F = 0.37$, $pr(>F) = 0.73$).

In the riffles, PERMANOVA results indicated no significant differences ($P(perm) > 0.05$) in macroinvertebrate community composition of substrate group pair and period pair. At no time

were there differences between substrate group pair per period (Table 4.4). The SIMPER analysis revealed macroinvertebrate community highest dissimilarities between NP and PD substrates (62.7%), followed by NS and NP substrates (62.32%), and NS and PD substrates (61.28%; Figure 4.6). Chironomidae, Hydropsychidae, Baetidae, and Simuliidae were the main contributors to dissimilarity in substrate communities in riffles as revealed by SIMPER analysis.

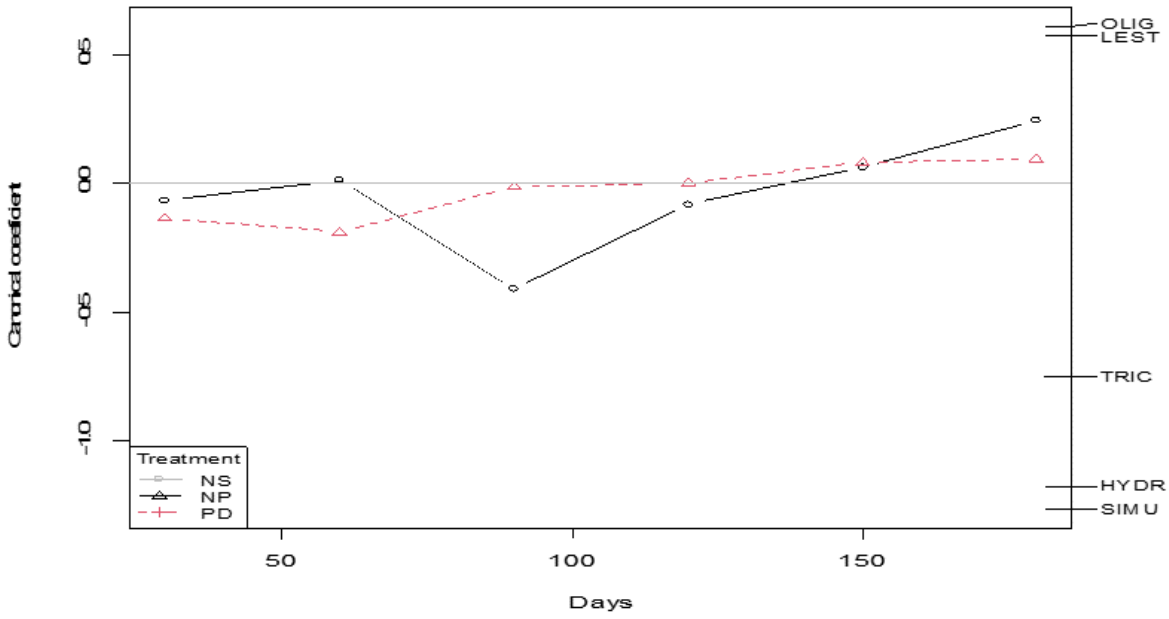

Figure 4.3B: Principal response curve (PRC) of riffles with taxa weights indicating the macroinvertebrate community response to the macroplastic and natural substrates on the right. Taxa are Oligochaeta (OLIG), Lestidae (LEST), Tricorythidae (TRIC), Hydropsychidae (HYDR), and Simuliidae (SIMU). Taxa whose weights were between 0.5 and -0.5 were omitted for clarity.

The macroinvertebrate PRC taxa weights for runs showed slight deviations between the macroplastic and natural substrate macroinvertebrate communities, with a similar pattern for NP and PD macroinvertebrate communities (Figure 4.3C). The greatest deviation of macroinvertebrate community from the natural substrate by macroinvertebrates in the macroplastic substrates was observed on day 150 for both NP and PD (Figure 4.3C). The PRC analysis indicated that the effect of substrate groups accounted for 13.32% of the total variance. The variance attributed to sampling time differences accounted for only 23.51%. However, the remaining 63.17% was due to variability between replicates over time. Based on the Monte Carlo permutation test, the PRC test result was not statistically significant ($F = 0.615$, $pr(>F) = 0.99$).

The PRC taxa scores in runs indicated that the abundance of families Baetidae, Chironomidae, Tricorythidae, Hydropsychidae, and Simuliidae were substantially supported in macroplastic substrates (NP and PD). These taxa abundances in NS decreased over time (Figure 4.3C). In Figure 4.3C, the remaining taxa had weights close to zero and were determined as non-influential to the PRC.

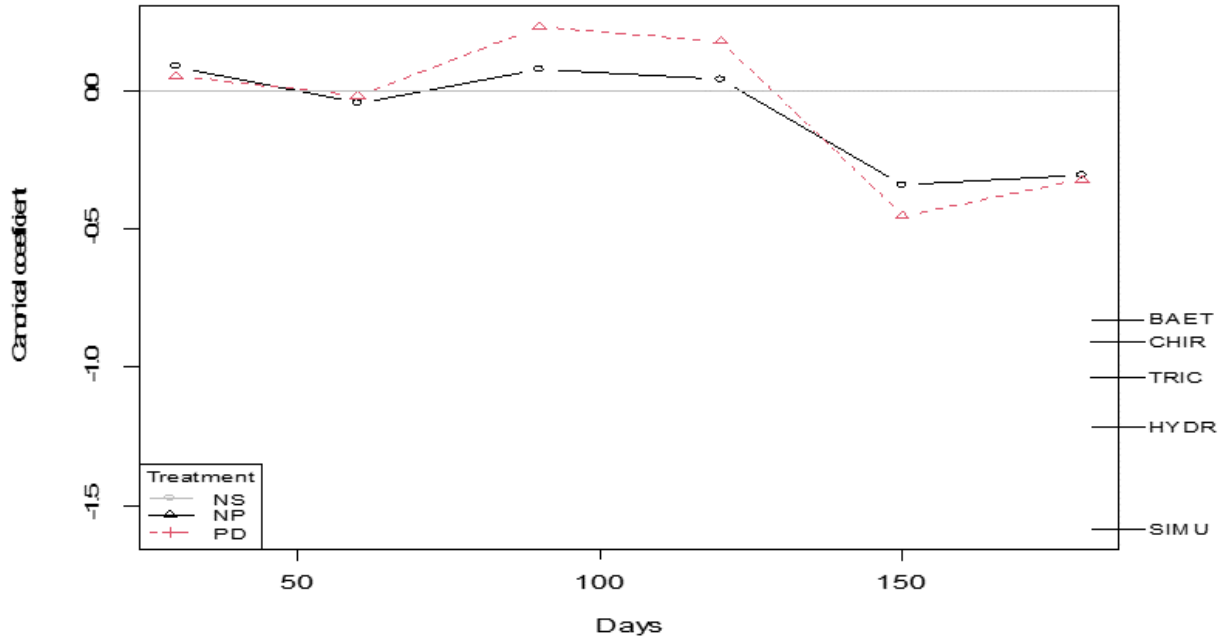

Figure 4.3C: Principal response curve (PRC) of riffles with taxa weights indicating the macroinvertebrate community response to the macroplastic and natural substrates on the right. Taxa are Oligochaeta (OLIG), Lestidae (LEST), Tricorythidae (TRIC), Hydropsychidae (HYDR), and Simuliidae (SIMU). Taxa whose weights were between 0.5 and -0.5 were omitted for clarity.

The macroinvertebrate data for runs indicated significant differences in the period of sampling (PERMANOVA $P = 0.001$; Table 4.5). Of the 15 day pairs, five (day 30 versus 60, 90, 150 and 180, and day 60 versus 180) macroinvertebrate community compositions were distinct in runs (Table 4.5). However, no significant differences ($P(perm) > 0.05$) were apparent in macroinvertebrate community composition of substrate groups, and between substrate group per period (Table 4.5). The trend in descending dissimilarity in substrate pairs was in NS and NP, NP and PD, and NS and PD macroinvertebrate community with 53.15%, 51.61%, and 49.51%, respectively (Figure 4.6). Similar to the PRC result for runs (Figure 4.3C), the SIMPER result also

revealed Chironomidae, Hydropsychidae, Baetidae, Simuliidae and Tricorythidae as the main contributors to the dissimilarities between the substrate groups in runs.

Table 4.5: PERMANOVA and pairwise results for macroinvertebrate assemblages across the gradient of plastic substrates and periods conducted separately for each hydraulic biotope. *P*-values in bold indicate differences under P(perm). Macroplastic substrates gradients: NS: natural substrates; NP: natural + plastic substrate; and PD: plastic-dominated substrate. Only pairs with significant differences were presented for pairwise results.

PERMANOVA table of results			
Source (Pools)	Pseudo-F	P(perm)	Perms
Substrates	0.636	0.81	998
Period	6.2797	0.13	997
Substrates x Period	0.38926	0.9	999
Source (Riffles)	Pseudo-F	P(perm)	Perms
Substrates	0.111	0.99	999
Period	1.504	0.052	998
Substrates x Period	0.454	0.99	997
Source (Runs)	Pseudo-F	P(perm)	Perms
Substrates	0.775	0.736	998
Period	2.295	**0.001**	998
Substrates x Period	0.464	0.99	997
Pairwise of significant periods in run			
Groups (Days)	t	P(perm)	Perm
30 versus 60	1.58	**0.012**	998
30 versus 90	1.87	**0.002**	999
30 versus 150	1.66	**0.01**	999
30 versus 180	1.91	**0.002**	999
60 versus 180	1.72	**0.018**	999

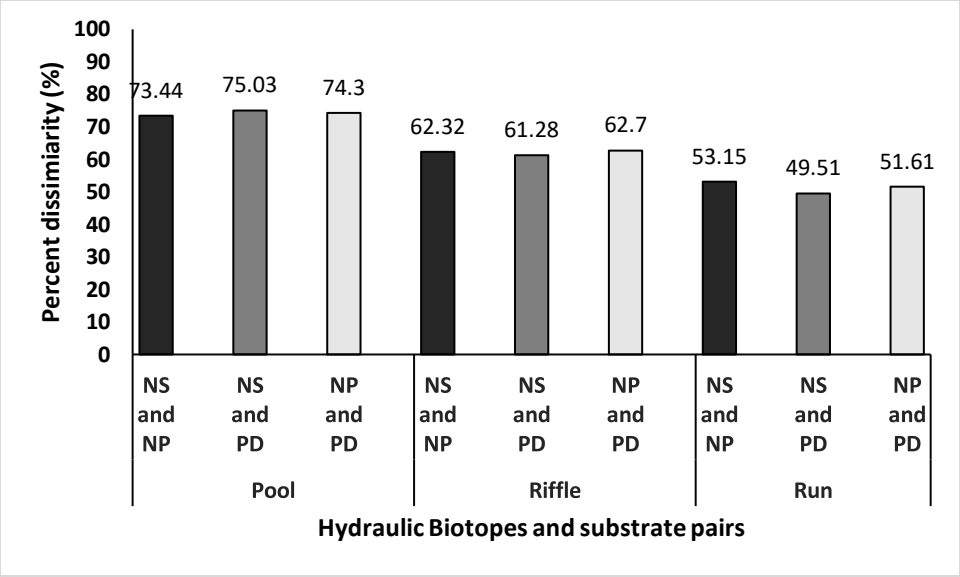

Figure 4.4: Similarity percentage (SIMPER) analysis result showing percent average dissimilarity (bold numbers on top of bars) between substrate groups and macroinvertebrate taxa contributing to dissimilarity during the study period (October 2021–April 2022).

Responses of the taxa indicated by hydraulic biotope PRC analyses that discriminated between substrate groups

Hydropsychidae, Oligochaeta, Lestidae and Simuliidae

Three taxa (Hydropsychidae, Lestidae and Simuliidae) responded positively to natural substrates in pools. Hydropsychids were poorly represented in NP and PD in the experiment pools. The highest abundance of hydropsychids in NS was recorded on day 150 (Figure 4.5). Lestidae showed a fluctuating trend in pools, with significantly high abundance recorded in NS on days 30, 90 and 150 (Figure 4.5). Although no individual simuliid was recorded on NP in pools, they did occur on day 180 in PD. The few recorded simuliids in pools were recorded on days 90, 120 and 180 on NS (Figure 4.5).

In riffles, Oligochaeta increased in abundance from day 30 to 60, and then reduced on day 90 in NS. No individual oligochaete was further recorded in all substrates from day 120 onward (Figure 4.5). Although no individual Lestidae were recorded on NP and PD; their abundance was similar in NS and PD on all other sampling days in riffles (Figure 4.5).

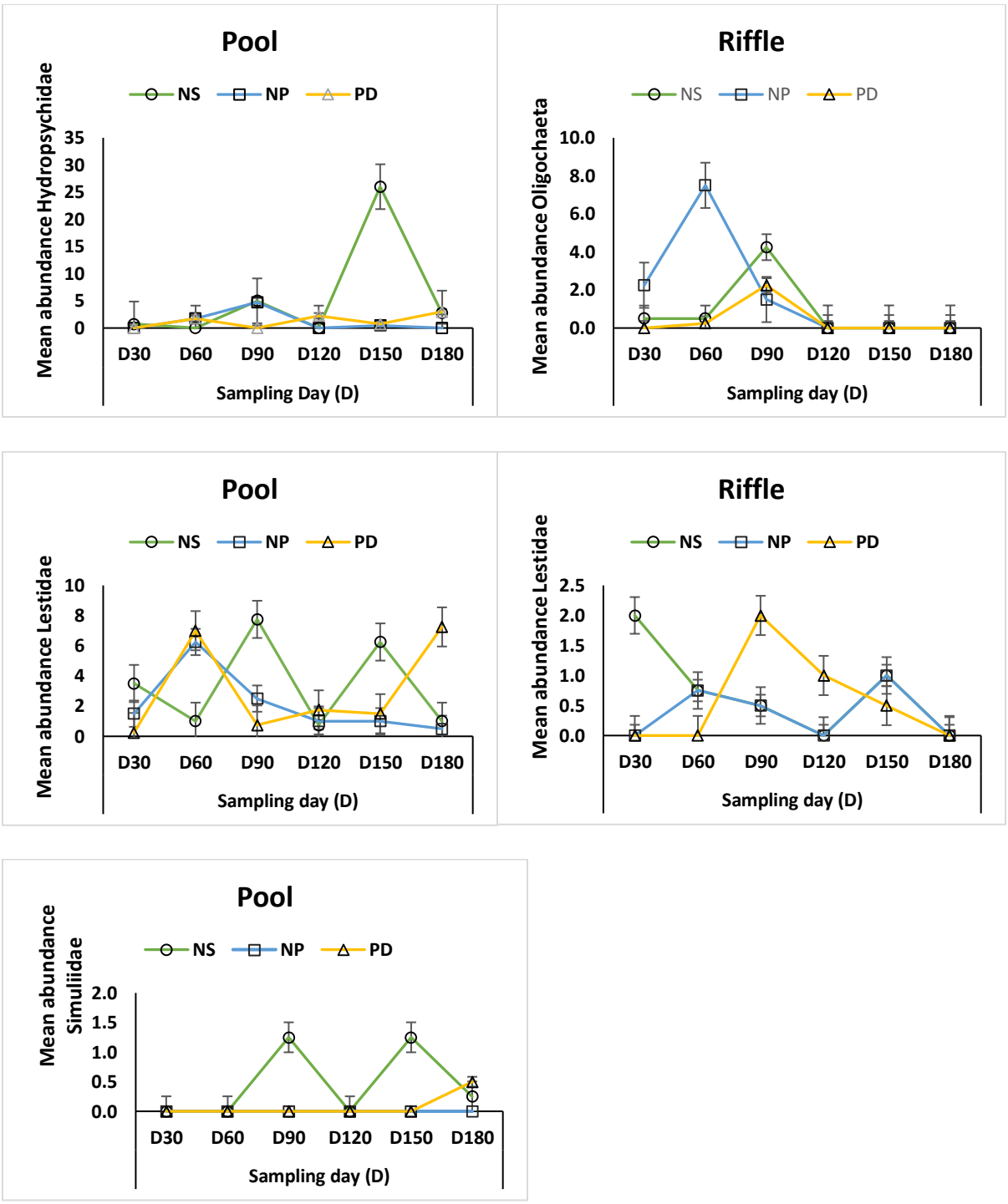

Figure 4.5: Mean abundance ± standard error (bars) of Hydropsychidae, Lestidae and Simuliidae recorded in pools, and Oligochaeta and Lestidae recorded in riffles on all substrate groups per period, showing their fluctuating responses to substrate groups during the experiment (October 2021–April 2022).

Tricorythidae, Hydropsychidae, Simuliidae, Baetidae and Chironomidae

Tricorythidae were only recorded on days 60 and 90 in riffles, with the greatest abundance in NP (Figure 4.6), while in runs, although tricorythids were highly abundant on NS on day 60, greater abundances were recorded on NP and PD from days 90, 150–180 (Figure 4.6).

The greatest abundance of Hydropsychidae on a substrate in riffles was on PD on day 60. Consistently, ANOVA, followed by the Tukey's HSD post-hoc test, indicated significantly higher abundance of Hydropsychidae on PD and NP compared to NS substrate from days 30–90 (Figure 4.6; $P < 0.05$).

The Simuliidae were more abundant in riffles on days 60 and 90 in NP and PD, respectively (Figure 4.6). On day 90, NS had a greater abundance of simuliids than NP and PD in runs. However, on days 150 and 180, greater simuliid abundance were recorded on PD and NP (Figure 4.6).

In runs, Baetidae had more abundance in NS on day 30, and then showed a linear decline from day 60 onward. On the other hand, Baetidae abundance was highest in NP, PD, and NP and PD on days 120, 150, and 180, respectively (Figure 4.7).

Chironomidae were recorded throughout the experiment and were significantly more abundant in PD, NS and NP on days 60, 90 and 150, respectively ($P < 0.05$). The abundance of the chironomids on sampling days 30, 120 and 180 was similar for all substrate groups (Figure 4.7)

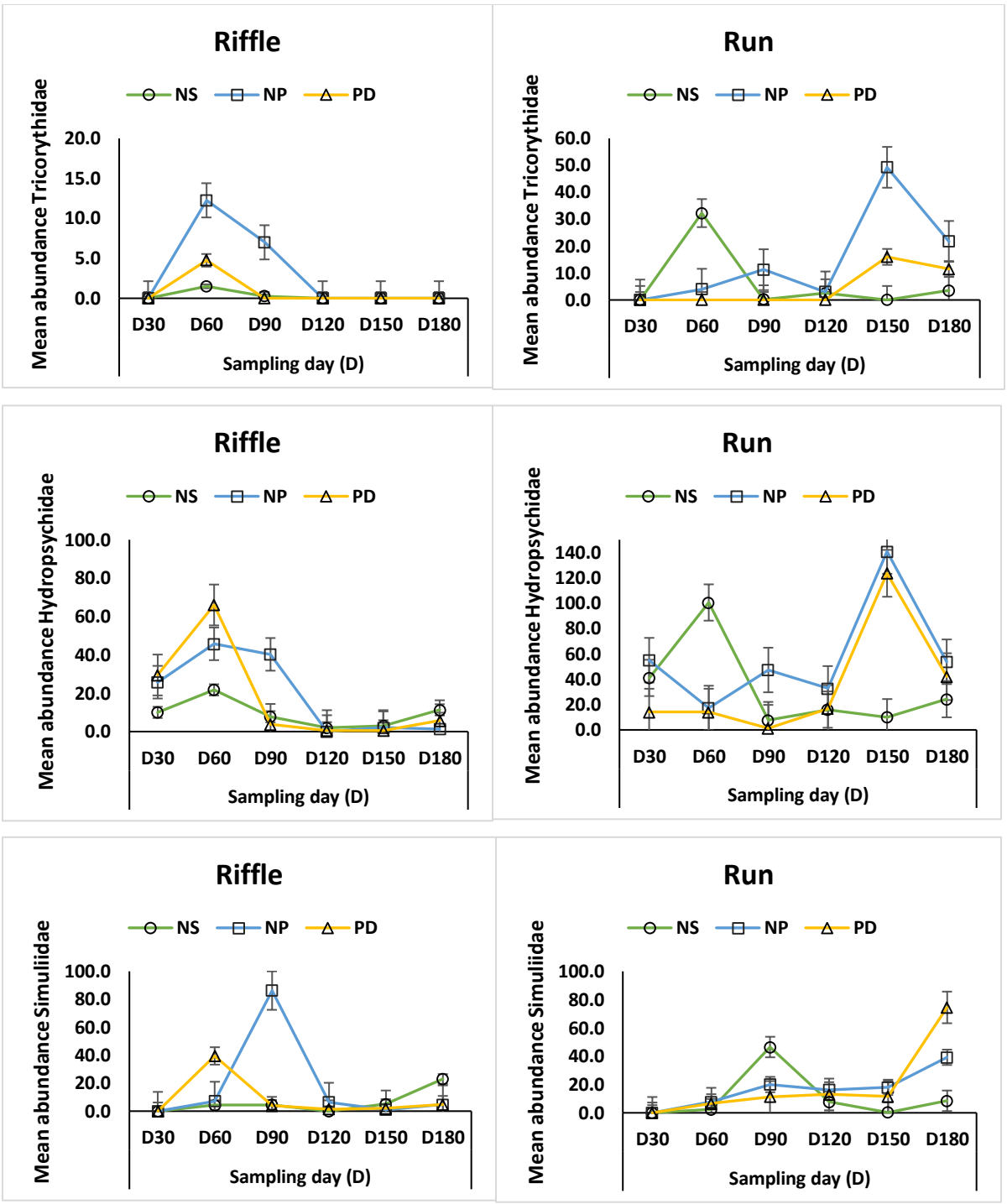

Figure 4.6: Mean abundance ± standard error of Tricorythidae, Hydropsychidae and Simuliidae recorded in riffles and runs on all substrate groups per period showing their high abundance responses to macroplastic substrate groups during the experiment (October 2021–April 2022).

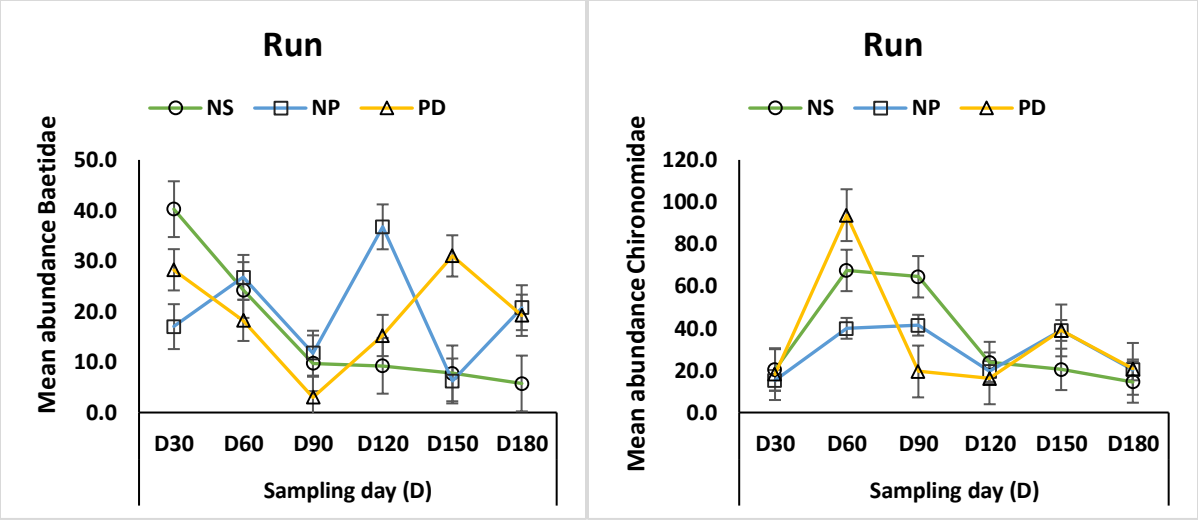

Figure 4.7: Mean abundance ± standard error of Baetidae and Chironomidae recorded in runs on all substrate groups per period showing their high abundance responses to macroplastic substrate groups during the experiment (October 2021–April 2022).

4.5 Discussion

4.5.1 Macroinvertebrate assemblage structure of substrate groups across hydraulic biotopes

The results outlined in Chapter 3 of this study indicated no differences in macroinvertebrate assemblage structures between the different gradients of plastic substrates, regardless of hydraulic biotopes (Figure 3.2). This chapter seeks to examine how different hydraulic biotopes influenced the colonisation of macroinvertebrates on natural and macroplastic substrates. The results show that, within the different hydraulic biotopes, the only differences observed in macroinvertebrate taxa diversity and dominance were from pools. The 100% natural substrate (NS) macroinvertebrate assemblages were significantly more diverse, with a higher number of dominant taxa than the 50% plastic (NP) and 100% plastic (PD) substrates (Figure 4.2 C-D). The Shannon diversity index reflected habitat stability and hence, its integrity (Daly et al., 2018). The Simpson's dominance index, on the other hand, was highly influenced by the commonest taxa, the numerical value of which reflects ecological factors influencing taxa abundance and diversity (Gray & Delaney, 2008; Morris et al., 2014). This result implies that within pools, macroplastic substrates (NP, and PD) were relatively unstable compared to the NS substrate, although these macroplastic substrates instability may have been influenced by external disturbances. One such explanation could be the flow dynamics of the environment in which the substrates were located. For instance, flow

competence and capacity for transporting sediment decrease under low flow conditions peculiar to pools (Graeber et al., 2017). As a result, fine deposits accumulate over the macroplastic and natural substrates in the pool. In this way, the habitat is simplified, and much of the variability in the microenvironment is removed (Mathers & Wood, 2016). In their colonisation experiment using PVC cylinders to determine the influence of sediment deposition on associated macroinvertebrates, Mathers and Wood (2016) reported a lower diversity of taxa on PVC cylinders with accumulated sediment than on sediment-free cylinders. The low values of diversity and dominance of macroplastic substrate macroinvertebrate assemblage relative to the natural substrates may be an indication of environmental stress on macroplastic habitats within pools.

Similar macroinvertebrate assemblage structure has been recorded in identical substrates from different hydraulic biotopes with varying flow rates (Principe et al., 2007). This finding is congruent with the result of riffles and runs described in this chapter, which showed that macroinvertebrate assemblage structure for all substrate groups and the comparison of substrate groups per sampling period were statistically similar. However, temporal variation in macroinvertebrate assemblages were observed in riffles and runs for all substrates (Figure 4.2 A-D). For example, the summary metrics indicated that macroinvertebrate assemblages on days 30–90 were more diverse and richer than days 120–180, suggesting a periodic change in the macroinvertebrate community structure (Morris et al., 2014). Flow type introduces a number of variabilities at a reach level and over hydrological periods. Higher hydraulic stress and substrate movement during high-flow events alter community compositions and shape diversity patterns at low spatio-temporal scales (Jacobsen et al., 2005; Gonzalez-Trujillo & Alonso-Moreno, 2020). The decline in diversity and equitable distribution of macroinvertebrates over time may be due to such changing environmental conditions within the hydraulic biotopes, which in turn lead to habitat degradation or shifts in resource availability for macroinvertebrates (Biggs et al., 2005; Reid & Thoms, 2008). These results correspond with the spatio-temporal variation reported in Chapter 3 (Figure 3.2), potentially highlighting the influence of hydraulic biotopes on the overall macroinvertebrate structural patterns on natural and macroplastic substrates. Combined with the evidence given in Chapter 3, these results support the conclusion that external conditions, such as hydraulic regimes, were responsible for the lower values of diversity, richness, evenness, and dominance recorded on all substrates in the second half of the experiment.

4.5.2 Macroinvertebrate preference for a particular substrate across hydraulic biotopes

Flow biotopes are of fundamental importance to biota, as they modify physical habitat and levels of hydraulic stress, influence the concentration of dissolved gases and nutrients (Reid & Thoms, 2008), and impact sediment settling (Graeber et al., 2017). Macroinvertebrate taxa were associated with specific substrate groups, but most of these preferences were not statistically significant, as revealed by the Pearson's point-biserial correlation coefficient of all individual hydraulic biotope macroinvertebrate data (Table 4.2 A–C). The preference of macroinvertebrate taxa for particular substrate groups within different hydraulic biotopes showed similarities with the evidence in Chapter 3 taxa-substrate association (Figure 3.5). For example, the highest number of taxa associated with a particular substrate were mostly associated with the 100% natural substrate (NS) in pools and riffles, followed by 50% plastic (NP) substrate in runs (Table 4.2 A – C). The NS and NP substrates are morphologically complex and provide more ecological niches than the 100% plastic PD substrate, and are deemed likely to support more available refuge and food resource, and thus support more macroinvertebrate taxa. These results are in concordance with previous studies that report greater association of macroinvertebrate taxa and high diversity values with the most heterogenous habitats rather than homogenous habitats (Milesi et al., 2016; Porst et al., 2019). However, as stated in Chapter 3, the lack of significant association of two or more unique taxa to a substrate group from any hydraulic biotope also means that there was a high degree of plasticity in macroinvertebrate taxa colonising macroplastic and natural substrate. This plasticity in macroinvertebrates could also be due to the taxonomic resolution (family-level) use, as most of the colonising taxa were large families with many species. Thus, macroinvertebrates may find macroplastics as suitable habitats, regardless of hydraulic biotope.

4.5.3 Macroinvertebrate community response to substrate groups within hydraulic biotopes

The principal response curve (PRC) analysis conducted separately at the hydraulic biotope level (run, riffle and pool), revealed similar macroinvertebrate community responses for macroplastic and natural substrates (Figure 4.3A–C). The results indicated that a greater variability in macroinvertebrate composition is owed to sampling times rather than between substrate groups or replicates. Thus, the substrate groups played a minimal role in the community response of macroinvertebrate community to macroplastic and natural substrates. The PERMANOVA result

of runs indicated a significant effect of period in shaping macroinvertebrate community composition on all substrate groups (Figure 4.3). The community response was driven by the most common and dominant taxa (Hydropsychidae, Chironomidae, Simuliidae, Baetidae, Tricorythidae, Lestidae and Oligochaeta; Figure 4.3A–C) that showed an alternating effect to macroplastic substrate gradient within different hydraulic biotopes. For example, the abundance of Hydropsychidae and Simuliidae increased markedly in NS substrates compared to NP and PD substrates throughout the experiment in pools (Figure 4.3A). In contrast, in riffles and runs, Hydropsychidae, Simuliidae, Tricorythidae, Chironomidae, and Baetidae interchangeably increased in macroplastic and natural substrates over time (Figure 4.3B and C). These taxa are mainly habitat generalists, able to colonise and exploit a diverse range of habitats and resources (Wallace & Anderson, 1995; Carvalho et al., 2013) and were shown in Chapter 3 to be responsible for the similarity in macroplastic and natural substrate macroinvertebrate communities (Figure 3.3). Similar to the macroinvertebrate assemblage structure, the correspondence between this result and the findings in Chapter 3 regarding macroplastic and natural substrate macroinvertebrate community composition suggests that external factors, such as periodic hydraulic conditions, were primarily responsible for the similarity in community composition. Therefore, hydraulic biotopes have an influence on the macroinvertebrate colonisation on both macroplastic and natural substrates. This is in agreement with several studies that have established that hydraulic biotopes influence macroinvertebrate colonisation of substrates (Lyon et al., 2009; Principe et al., 2007). However, an increase or decrease in current could potentially influence the hydraulic biotope habitat characteristics from a pool to run and vice versa (Dallas, 2007), and this could have a confounding effect on macroinvertebrate communities on both natural and macroplastic substrates.

4.6 Conclusion

In freshwater systems, plastic endpoints depend primarily on the morpho-hydrological dynamics and characteristics of rivers that are vital for the interaction of macroinvertebrates with their habitat and the stability of those habitats. The results from this chapter indicated that hydraulic biotopes have significant influence on macroinvertebrate colonisation of natural and macroplastic substrates, and this may depend on the spatio-temporal hydrological conditions of the hydraulic biotope. The significantly reduced macroplastic macroinvertebrate diversity and dominance value relative to natural substrate in pools, as well as the temporal variability in macroinvertebrate

assemblage structure and composition in riffles and runs which corresponded to the overall macroinvertebrate community pattern observed in Chapter 3, provided evidence that factors such as hydraulic biotopes influence the macroinvertebrate colonisation of macroplastics. The separate examination and comparison of macroplastic and natural substrate macroinvertebrate communities from different hydraulic biotopes may help unravel the factors driving the potential impact of macroplastics on macroinvertebrates and riverine habitats under varying natural field conditions. More ecological research incorporating the direct influence of hydraulic biotopes on macroinvertebrate colonisation of macroplastics is imperative to adequately understand macroinvertebrate responses to macroplastic pollution in freshwater systems. Although the results contribute to reducing the knowledge gap about the confounding factors influencing macroinvertebrate community responses to macroplastic pollution, the use of a trait-based approach may reveal the mechanistic linkage in the response of macroinvertebrates to macroplastic pollution in riverine systems.

CHAPTER 5: TRAITS AND ECOLOGICAL PREFERENCES MEDIATING THE POTENTIAL COLONISATION OF MACROINVERTEBRATE ASSEMBLAGES ACROSS GRADIENTS OF MACROPLASTIC SUBSTRATES.

5.1 Introduction

The traits possessed by macroinvertebrates reflect their survival strategies, which have developed over time due to selection pressures and evolutionary processes (Townsend & Hildrew, 1994). These traits determine how species adapt to their environment, and include preferred physical habitats, food acquisition, and water quality (Frainer et al., 2018). For instance, Dinh and Death (2018) found that modifying or disturbing substrates can reduce the abundance of macroinvertebrates with specific traits. The authors reported a positive correlation between the abundance of burrowers and increasing substrate disturbances and proposed that pool habitats could serve as a crucial refuge for burrowers during periods of high flow. Another study by Brooks et al. (2005) demonstrated a stable substrate as a requirement for attachment sites by filter-feeders, thus their remarkable presence in areas of high flow. Analysing traits allows one to assess macroinvertebrate adaptation to their environments.

The proliferation of macroplastics in the environment has created novel habitats for macroinvertebrates (Wilson et al., 2021; Taurozzi et al., 2023; Gallitelli et al., 2023; Chapters 3 and 4). Macroinvertebrates that are able to cope in these habitats must have the necessary traits and adaptive features because traits mediate the interaction between the organism and its external environment. By analysing the trait distribution of macroinvertebrates across the substrate groups, it is possible to determine how the different organisms utilise the resources provided by the macroplastic-created habitats. The use of traits in this regard is important because it i) enables analysis of resource utilisation by different macroinvertebrates, ii) allows elucidation of the adaptive features necessary to survive in a plastisphere, and iii) provides insights into the predictive role of traits – predicting organisms likely to colonise macroplastic substrates based on the combination of traits possessed.

The trait-based approach (TBA) has been gradually incorporated and successfully applied in the Afrotropical region. In studies such as those by Odume, (2020), Matomela et al. (2021), and Akamagwuna et al. (2022), the trait-based approach to macroinvertebrate response to different

levels of disturbance, such as urban, agricultural, and industrial pollution, was applied. Matomela et al. (2021) investigated the spatio-temporal dynamics of taxonomic and traits responses to different land use disturbances in three Afromontane headwater tributaries of the Kat River. However, despite the potential impacts of plastic pollution on riverine habitats, the use of traits to explain the effects of plastic-created habitat on macroinvertebrate communities has not been investigated in the Afrotropical region. This chapter explores the potential of using a trait-based approach to understand how macroinvertebrates colonised plastic substrates in the four selected riverine systems.

The four selected riverine systems (Kowie, Buffalo, Kat, and Swartkops) are urban rivers that drain catchments impacted by a range of anthropogenic activities (Odume et al., 2012, Dalu et al., 2014; Odume et al., 2015; Odume & Mgaba, 2016; Matomela et al., 2021; Akamagwuna et al., 2022). The dominant sources of impacts within these catchments include effluents from municipal wastewater treatment works, run-off from informal settlements, industrial facilities and agricultural farmlands, urban stormwater return flow, and indiscriminate waste disposal. These anthropogenic activities are mainly in the middle and lower reaches of the rivers and are important contributors of plastic debris in the studied systems (Jambeck et al., 2015; Baldwin et al., 2016). The objective of this chapter was to analyse the trait distribution patterns of macroinvertebrates across the substrate groups and to identify traits that mediate the interactions between macroinvertebrates and plastic substrates. This chapter thus addresses Objective 3 of the study outlined in Chapter 1, Section 1.6.2.

5.2 Methods

During the experimental period, a total of 54 bags were deployed per site, with 18 bags each for NS, PD, and NP. Six bags were deployed for each hydraulic habitat (riffles, runs, and pools) for every category of substrate, as shown in Figure 2.7. The bags were randomly placed in each hydraulic habitat to cover all seasons from October 2021 to April 2022, including spring, summer, and autumn. A total of 216 bags were deployed in a similar fashion across the four rivers. To account for substrate loss during the experiment, the retrieved substrates (NS: 48; NP: 52 and PD: 49) from all sites were utilised as replicates for statistical analyses.

5.2.1. Trait selection

Nine traits, comprising 36 attributes, were selected. The traits include behavioural (mobility), morphology and physiology (body size, body shape, and respiration), and ecological preference (attachment strategies, substrate preference, velocity preference, feeding preference, and food materials; Table 5.1). The selected macroinvertebrate traits were compiled at the family-level taxonomic resolution. The trait categories and attributes are based on their mechanistic relationships with macroplastic-mediated stressors in river systems. Plastic in rivers affects aquatic organisms through variously mediated stressors such as abrasion, entanglement, particle ingestion, chemical leaching, habitat disturbance, and interaction with faunal and floral microbial assemblages (Thompson et al., 2009; Koelmans et al., 2014; Green et al., 2015).

Availability of trait information in the literature was a crucial consideration as a criterion for trait selection because the scarcity of trait data has remained a critical challenge to developing the trait database both in Africa (Akamagwuna et al., 2019; Odume, 2020; Odume et al., 2023). This study used the invertebrate trait database for southern Africa as the primary source of trait information in this study (Odume et al., 2023), as previous regional studies have successfully used this database in trait-based biomonitoring (Akamagwuna et al., 2019, 2021, 2022; Odume, 2020; Matomela et al., 2021; Ntloko et al., 2021). Supplementary trait information was retrieved from published literature elsewhere (e.g., Merritt et al., 2008; Statzner & Bêche, 2010; Tachet et al., 2010).

To link trait attributes to each taxon, a fuzzy coding method was used (Appendix 1, Table 5A). In this method, affinity scores are used to account for possible functional differences between taxa (Chevenet et al., 1994). In addition, fuzzy coding compensates for variability in confidence levels among various traits obtained from the literature (Chevenet et al., 1994). It eliminates the problems associated with assigning a taxon directly to a single trait attribute, which may result in inaccurate organism profiles and interactions (Mondy et al., 2014). Scores ranging from 0 to 5 were used to describe organism affinities to trait attributes. A score of 0 indicates the lowest affinity of an organism to a particular trait attribute, and a score of 5 indicates the highest affinity. Low affinity was indicated by 1, and a score of 3 indicated a moderate affinity.The selected traits, ecological preferences, and rationale behind the selection are discussed below.

i. Mobility

Locomotory traits determine the ability of an animal to migrate either to search for food, seek refuge or evade predation. As such, the locomotory traits can influence the vulnerability and resilience of animals in river systems. For example, semi-sessile animals, such as the family Simuliidae can be highly vulnerable during increased pollution (Gjerløv et al., 2003), as they cannot readily move when their immediate habitat becomes unfavourable. In this study, mobility traits, including burrowing, clinging /climbing, crawling, skating, sprawling, and swimming were selected, as they can influence the ability of macroinvertebrate taxa to either colonise plastic substrates or move away from plastic-dominated substrates when conditions change. This is important as previous studies have demonstrated that the impact of macroplastics on habitats can be linked to the long retention time of plastics in storage zones in rivers. For example, studies have shown that plastics degrade/fragment into microplastics within fluvial systems, causing more animal harm (e.g.Williams & Simmons, 1996; Corcoran et al., 2009; Andrady et al., 2022).

ii. Attachment strategies

Macroinvertebrates use different attachment strategies to anchor themselves onto substrates and generally prefer stable substrates to unstable substrates. Filter feeders may attach themselves to stable substrates while collecting particulate organic materials from the water column. The introduction of macroplastic may alter stability and suitability of natural substrates and, in turn, structure macroinvertebrate assemblages as the macroplastic substrates provide surfaces for macroinvertebrate attachments (Wohl, 2013; Chapters 3 and 4). In this study, attachment strategy was selected for analysis and categorised into three groups based on macroinvertebrate ability to attach permanently, attach temporarily , or not to attach (free-living) to substrates.

iii. Substrate preference

Macroplastics in river systems have variable structures and functions; organisms may use these plastics differently within aquatic ecosystems. For example, hard textured plastics, such as PET and PVC, can function in ways similar to rocks and pebbles (Thompson et al., 2009; Wohl, 2013), providing a stable substrate for attachment. Filter-feeding macroinvertebrates may prefer the smooth surfaces of plastic to the rough surfaces of rock particles, which may have epilithic growth. Okano et al. (2012) reported a high correlation between filter-feeding simuliids, and hydropsychids with newly introduced substrates, and smoother rock surfaces than with existing and rough rock

substrates. In terms of substrate preference, the preference for natural substrates (NS), natural + plastic (NP), and plastic dominated (PD) substrates provided in Chapter 3 (Table 3.5) were used for analysis.

iv. Respiration

As plastic litter accumulates on the riverbed, it can alter the microchemical environments critical to organisms that dwell in interstices (Katsanevakis et al., 2007; Akoumianaki et al., 2008; de Souza Machada et al., 2018). Macroinvertebrates surviving and thriving in such habitats must possess specialized respiratory apparatus such as breathing tubes, elytra, etc. Respiration is a critical trait for many macroinvertebrates because it impacts several physiological functions, and the survival of the organism. In this study, gills, teguments, plastron, and spiracle were selected for analysis in terms of respiration.

v. Flow velocity preference

Macroinvertebrate assemblages are often characterised by their preferences to flow velocity (Reid & Thoms, 2008) which is one of the factors that characterises different hydraulic biotopes and determines the nature of the available substrates for organisms. For instance, low-flow velocity zones such as a pool is often dominated by sediments and is regarded as a less stable habitat (Brooks et al., 2005). The preference for flow velocity is pertinent as macroplastics may be buried by sediment accretion and remain buried for a long period within a low-flow velocity zone. However, within high-flow velocity zones, macroinvertebrates colonising macroplastic may undergo stochastic disturbances, washing away fauna and flora on the surface. Velocity was used as a surrogate for hydraulic biotopes, and macroinvertebrate preferences for the different velocity regimes were thus determined using the MIRAI model (Thirion, 2007; Odume et al., 2023): fast flowing (run = 0.3-0.6 m/s), moderate flow (riffle = 0.1-0.3 m/s), and slow/still flow (pool = <0.1 m/s) based on the recorded average of the hydraulic biotopes were used in this study.

vi. Body size

The body size of an organism can affect its use of habitat resources, competitive ability, and vulnerability to predation. Milesi et al. (2016) found that smaller organisms were more likely to resist drag (Lamouroux et al., 2004; Forcellini et al., 2022) and reduce eviction (Statzner et al., 1988) by inhabiting homogenous habitats. The size of the organism may influence its interaction

with macroplastics. Within plastiphere, macroinvertebrates with smaller bodies or streamlined shapes may be better able to navigate and utilise microhabitats. In the UK, large freshwater decapods were more often trapped in ghost nets at Lake Ohrid than smaller sized species (Spirkovski et al., 2019). This insight was used to deduce that macroinvertebrates, such as potamonautids with average to large bodies, can become entangled in plastics such as PET bottles or bottle rings. Using relevant literature and databases (Tachet et al., 2010; Odume et al., 2023), macroinvertebrates were classified into four classes, based on the length from head to tip: <5 mm, >5-10 mm, >10-20 mm, and >20 mm.

vii. Body Shape

Macroinvertebrates have specialised body shapes that enable them to colonise specific habitat niches. In conjunction with body size, both body size and shape traits influence whether an organism can utilise a microhabitat while avoiding predation and adapting to high-flow conditions (Townsend & Hildrew, 1994). For example, a dorsoventrally flat body allows macroinvertebrates, such as leptophlebiids, to cling to the substrate while resisting fast-flowing water (Christidis & Dean, 2008). Body shape was categorised into four modalities: cylindrical, flattened, spherical, and streamlined (Gerber & Gabriel, 2002; Odume et al., 2023).

viii. Feeding habit

In the various gradients of plastic substrate deployed, macroinvertebrates will readily use the food quality and quantities available depending on their feeding group, habitat preference, and biomass. The introduction of macroplastics may reduce available food by covering the available detritus and limiting sediment-water-column interchange of nutrients needed for primary production (Uneputty & Evansh, 1997; Akoumianaki et al., 2008). However, with time, epilithic algal biomass growth will develop on plastic surfaces and facilitate macroinvertebrate colonisation, depending on environmental conditions (Kuo et al., 2016) and the resident time of such plastic substrate (Taurozzi et al., 2023; Gallitelli et al., 2023). Higher algae biomass will increase herbivory by macroinvertebrate families like Baetidae that graze on algae. Also, macroplastics trapped in different riverine compartments have been reported to accumulate sediment and detritus, resulting in rich, heterogeneous habitats for macroinvertebrates (van Emmerik & Schwarz, 2020). The feeding preferences of macroinvertebrates could impact their utilisation of plastic habitats as shelter or resource locations in response to changes in temporal macroplastic habitats (Frainer et

al., 2018). Five feeding methods: filter feeding, collecting, scraping/grazing, shredding, and predating, were selected using published information for feeding habits (Palmer & O'Keeffe, 1992, Merritt et al., 2008; Odume et al., 2023).

ix. Food type

The environment in which an organism finds itself and its preferred feeding mechanism determines the food material it consumes. Food availability depends on various factors, such as the complexity of an organism's microhabitat and the accumulation of food resources within it. For example, Chen et al. (2019) reported the growth of biofilms on rafting plastics, which contributes to their density and subsequent sinking. Macroinvertebrates belonging to the family Glossosomatidae, which are scrapers, can feed on biofilms growing on plastic substrates. Moreover, plastic mimics natural substrates by providing a suitable surface for filter feeders that attach to rigid substrates while filtering seston off the water column. Four food types were selected, based on observation and relevant literature (Palmer & O'Keeffe, 1992; Merritt et al., 2008): detritus (coarse particulate organic matter CPOM, and fine particulate organic matter FPOM), macrophytes, periphytic biofilms, and animal prey.

Table 5.1: Summary of three trait groups (bolded face), comprising 36 trait attributes applied to 48 macroinvertebrate taxa at the family level across a macroplastic substrate gradient in the four selected river systems during the study period (October 2021– April 2022).

Traits	Trait attributes	Code
Behavioural		
Mobility	Burrowers	BURR
	Clingers/Climbers	CLIM
	Crawlers	CRAW
	Skaters	SKAT
	Sprawlers	SPRA
	Swimmers	SWIM
Morphology and Physiology		
Respiration	Gills	GILLS
	Tegument	TEGU
	Spiracle	SPIR

	Plastron	PLAS
Body size	Very small (< 5 mm)	BS_1
	Small (5 > 10 mm)	BS_2
	Medium (10 > 20 mm)	BS_3
	Large (> 20 mm)	BS_4
Body shape	Cylindrical	CYLI
	Flattened	FLAT
	Oval	OVAL
	Streamlined	STRE
Ecological preference		
Flow velocity preferendum	Slow/still flow (<0.1 m/s)	S_VEL
	Moderate flow (0.1-0.3 m/s)	M_VEL
	Fast flow (>0.3-0.6 m/s)	F_VEL
Attachment strategy	Permanent attached	P_ATTA
	Temporary attachment	T_ATTA
	Free-living	FRE_L
Substrate preference	Natural substrates	NS
	Natural + plastic substrates	NP
	Plastic dominated substrates	PD
Feeding habit	Collector-gatherers	COL_G
	Filter-feeders	FIL_F
	Predators	PRED
	Scrapers/grazers	SCRA
	Shredders	SHRE
Food type	Coarse particulate organic matter	CPOM
	Fine particulate organic matter	FPOM
	Plant materials	HB
	Prey (animals)	PREY

5.3 Statistical analyses

5.3.1 Spatial and temporal differences of traits and ecological preference across substrate groups

The differences in ecological preference and trait distribution of macroinvertebrates across the substrate groups were statistically tested using a two-way Permutational Multivariate Analysis of Variance (PERMANOVA) with substrate groups (NS, NP, PD) and sampling days (Days: 30, 60, 90, 120, 150 and 180) as factors. Where applicable , multiple post-hoc pairwise comparison tests were conducted to identify significantly different substrate effects, day effects, and/or substrate per day interaction pairs. Shapiro-Wilk and Levene's tests was conducted to ascertain if the macroinvertebrate trait data met the assumption of normal distribution and homogeneity of variance before PERMANOVA using the Statistica Software (Tibco Software, 2018). Bray-Curtis resemblance index and PERMANOVA were conducted using PRIMER 6 with PERMANOVA+ add-ins (Anderson et al., 2008).

5.3.2 Identifying traits distribution structure of macroinvertebrate communities to substrate groups

To evaluate trait distribution structure of macroinvertebrate communities, descriptive and inferential statistics and multivariate methods were used to find and compare macroinvertebrate traits and ecological preferences (at the family level) and to measure substrate use by trait mode. Multivariate analysis (correspondence analysis; CA) of taxa by substrate distribution matrix, fuzzy CA (FCA) of taxa by trait matrix were analysed and plotted simultaneously using co-inertia analysis (COIA) ordination to check for a potential relationship between taxa traits and substrate utilisation of the CA and FCA. The RV-coefficient embedded with Monte-Carlo permutational test was used to evaluate the correlation and significance of various trait modality distribution and COIA co-structure.

Fuzzy correspondence analysis (FCA) was used to describe and compare the trait composition of the natural and macroplastic macroinvertebrate communities as it allows for identification of the traits that contributed more to the ordination of taxa (Chevene et al., 1994). The FCA aims to compute numerical codes for species to separate the modalities of trait categories or to compute numerical codes for variables to discriminate the species affinity distribution among modalities for

different substrate groups. The calculation of correlation ratio enables a rapid check of which variables are involved in the separation of species profiles on substrate groups. The result of the FCA output was plotted on the first two axes, which present two independent structures: (i) axis 1, which shows a clear arrangement of modalities along a gradient, and (ii) axis 2, the separation of different groups of modalities (Chevene et al., 1994; Bis & USseglio-Polatera, 2004). Prior to FCA, correspondence analysis (CA) of taxa-substrate matrix data was analysed to visualise taxa distribution among substrate groups.

Co-inertia analysis was used to check for potential relationships between spatial distribution of species and trait structure of macroinvertebrate assemblages described by their trait combinations. Co-inertia analysis defined axes which simultaneously explained the highest possible variance in each of the two data sets and described the closest possible common structure (i.e., optimisation of the correlation between each set of the coordinates; Bis & Usseglio-Polatera, 2004).

The RV-coefficient correlation was used to ascertain the relation of (i) the co-structure of the co-inertia analysis and (ii) the distribution of modalities of specific traits and ecological preference categories to that of others. The RV-coefficient calculates the extent to which two sets of data matrices (eigen values of taxa-traits FCA and taxa-substrates CA) or variables (e.g., fuzzy feeding mode and food type modalities) give similar images of taxa utilisation of substrate habitat (Robert & Escoufier, 1976; Smilde et al., 2009). This analysis provides the measure of closeness of the corelated matrices and modalities within two trait categories and gives an RV-value of 0 and 1, defining a scale of similarity between two matrices (Smilde et al., 2009). The scale can be interpreted in much the same way as the absolute value of the Pearson correlation coefficient, with an RV-value closer to 1 indicating that the patterns are close and one can be substituted for the other to characterise the taxa of substrate groups (Robert & Escoufier, 1976, Smilde et al., 2009). The significance of the RV-coefficient correlations were tested using the Monte-Carlo permutation test (999 permutation; $p < 0.05$). The "*ade4"* package within R statistical software version 4.2.2 was used to conduct all multivariate and inferential statistics (Dray & Dufour, 2007; R Core Team, 2022).

5.4 Results

5.4.1 Macroinvertebrate traits and ecological preference responses across plastic substrate gradients

The PERMANOVA result highlighted that trait composition did not differ significantly between substrate groups, and that the interaction between substrate and days in terms of trait composition was not statistically different (*P(perm)* > 0.05; Table 5.2). In contrast, sampling days indicated significant differences in the distribution of macroinvertebrate traits (*P(perm)* < 0.05). Five of the 15 day pairs revealed significant differences in traits and ecological preferences of macroinvertebrates colonising the plastic substrate gradients. Of these, day 30 significantly differed from days 60, 90, 150, and 180, and day 60 was also distinct from day 180 (*P* <0.05; Table 5.2).

Table 5.2: Two-way PERMANOVA result for traits and ecological preference across substrate groups, retrieval days, and between the interaction of substrate and retrieval days in the Bloukrans, Buffalo, Kat, and Swartkops Rivers from October 2021 to April 2023. Bolded are significant levels (p <0.05), permutational (perm).

PERMANOVA Source	Pseudo-*F*	*P* (perm)
Substrates	0.6793	0.696
Days	2.5683	**0.001**
Substrate x Days	0.541	0.99
Pairwise groups (Days)	*F*	*P* (perm)
30, 60	1.95	**0.003**
30, 90	2.44	**0.001**
30, 120	0.97	0.42
30, 150	1.84	**0.008**
30, 180	2.31	**0.003**
60, 90	1.17	0.23
60, 120	1.06	0.33
60, 150	1.23	0.17
60, 180	1.80	**0.02**
90, 120	1.53	0.05

90, 150	1.21	0.19
90, 180	1.44	0.07
120, 150	1.00	0.38
120, 180	1.43	0.08
150, 180	0.83	0.52

5.4.2 Identifying traits distribution structure of macroinvertebrate communities to substrate groups

All trait and ecological preferences, except food type and feeding habit (RV = 0.77; $p < 0.001$) were weakly correlated (i.e., RV-values less than 0.3), with 30% of the RV coefficients being statistically significant ($P<0.05$; Table 5.3). The FCA of the taxa by trait data allowed a description of similarities between taxa on the basis of their traits and of the relationship between trait categories (Table 5.3; Figure 5.1). Table 5.4 summarises the contribution of each trait to the ordination of the taxa along the first three axes of the FCA, and the percent contribution of each axis to the FCA ordination. Respiration, food type and feeding habit were distributed along axis 1, while, body size, body shape, attachment mechanism, feeding habit, substrate preference and velocity preferences were distributed along axis 2 and were the traits that contributed most in the ordination of the taxa. The third axis discriminated the taxa according to their mode of movement, and substrate and velocity preferences; however, the third axis did not add to the ordination as these traits were already accounted for by the first two axes. The traits with corresponding correlation ratios higher than the average on axes 1 and 2 were further used to interpret the distribution of trait modalities and the utilisation of substrate groups by macroinvertebrates.

The first two axes of the COIA ordination explained 80.8% of the total variability in taxa abundance, trait, and substrate groups (Table 5.5). The COIA was not significant ($p > 0.05$), with a total inertia of 2.99 and RV-value of 0.95. In the COIA ordination, the different modalities clearly demonstrated that the taxa were arranged according to their local conditions and substrate-type utilisation. Taxa of the first group of modalities (Figure 5.2; preferring the NS substrate, bottom right of the co-structure) showed a preference for slow or still flow, large body size (> 20 mm), and were free-living. The second group of modalities (Figure 5.2; upper left of the co-structure;

NP region) were filter feeders that permanently attach to substrate and had an affinity for fast-flow velocity (0.3 – 0.6 m/s), crawling behaviour and fed on particulate organic matter. While taxa of the third group of modalities with the highest number of attributes (Figure 5.2; upper right of the co-structure, PD related) had an oval body shape, medium-sized (>10 – 20 mm) body, were predators or prey, flat, were temporarily attached, shredders, had a preference for plants and could respire using spiracles and plastrons.

Table 5.3: RV coefficient correlation result between traits and ecological preference, and estimated *p*-values (in parenthesis). Bolded are significant levels (p <0.05) based on Monte-Carlo permutation tests.

	Body shape	Mobility	Respiration	Attachment mechanism	Feeding habit	Food type	Substrate preference	Velocity preference
Body Size	0.09 (0.07)	0.04 (0.89)	0.05 (0.37)	0.10 **(0.04)**	0.05 (0.42)	0.08 (0.11)	0.03 (0.67)	0.04 (0.46)
Body Shape		0.05 (0.38)	0.07 (0.14)	0.02 (0.62)	0.05 (0.33)	0.05 (0.22)	0.02 (0.71)	0.06 (0.13)
Mobility			0.16 **(<0.01)**	0.23 **(<0.001)**	0.27 **(<0.001)**	0.22 **(<0.001**)	0.08 (0.16)	0.10 (0.11)
Respiration				0.12 **(0.02)**	0.18 **(0.002)**	0.16 **(0.005)**	0.03 (0.55)	0.05 (0.31)
Attachment mechanism					0.20 **(0.001)**	0.14 **(0.01)**	0.03 (0.5)	0.05 (0.22)
Feeding habit						0.77 **(<0.001)**	0.06 (0.27)	0.05 (0.40)
Food type							0.07 (0.14)	0.05 (0.28)
Substrate preference								0.09 (0.06)

Table 5.4: Contribution (expressed as correlation ratios) of each trait to the ordination of the taxa along the first three axes of the FCA. Ratios higher than the average per axis are bold.

Axes	Body size	Body shape	Mobility	Respiration	Attachment mechanism	Feeding habit	Food type	Substrate preference	Velocity preference	% variance explained
1	0.034	0.07	0.028	**0.411**	0.172	**0.525**	**0.503**	0.132	0.189	22.9
2	**0.171**	**0.193**	0.109	0.017	**0.283**	**0.278**	0.011	**0.203**	**0.216**	16.5
3	0.115	0.041	**0.272**	0.078	0.056	0.093	0.024	**0.257**	0.136	11.9

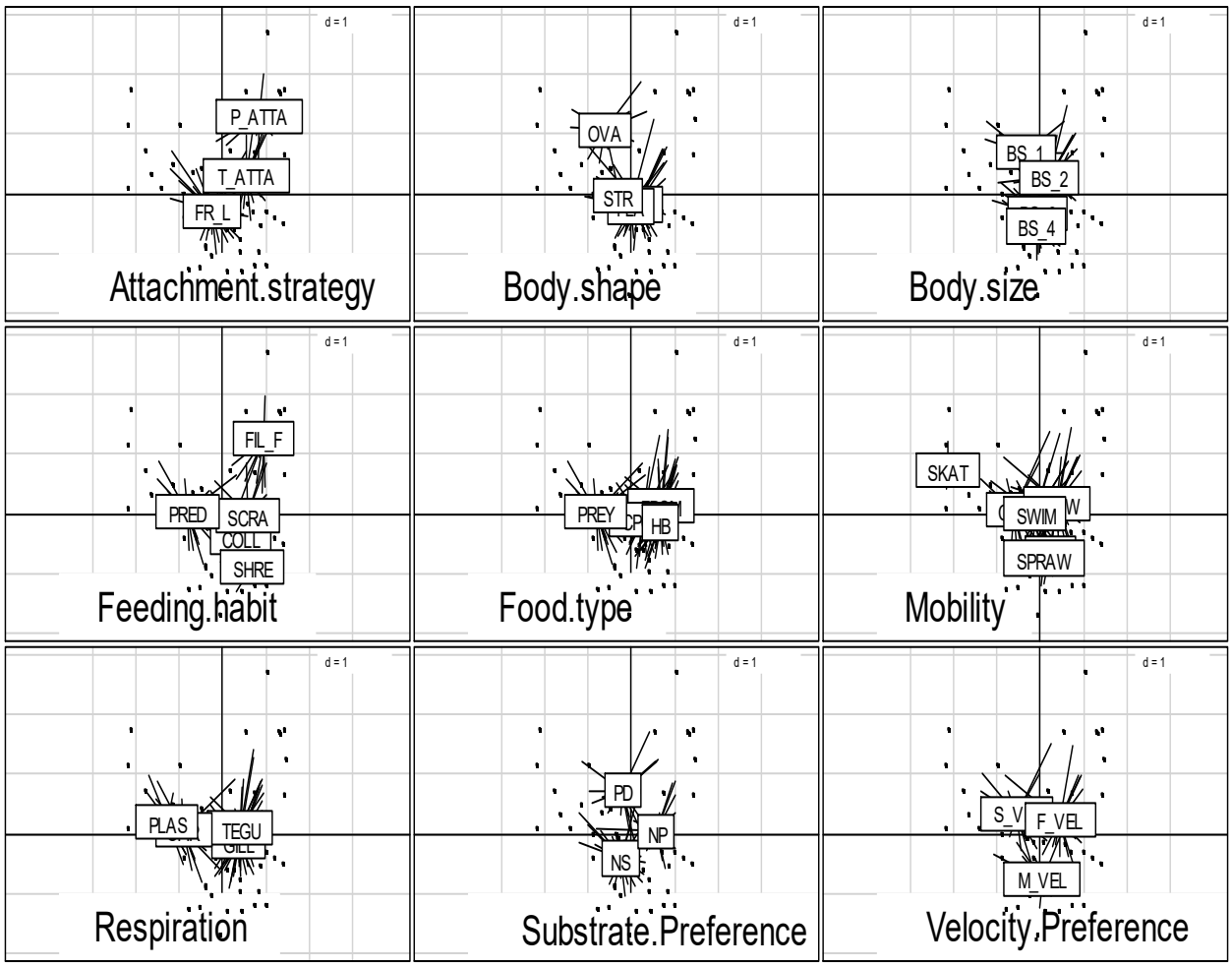

Figure 5.1: Ordination of individual FCA of traits and ecological preference distribution indicating their contribution to the first two axes of the FCA factorial plane. Axis 1 (horizontal plane), Axes 2 (vertical plane). Location of substrate may be interpreted in the light of the trait characteristics of macroinvertebrate community preferring substrate group. Abbreviations: plastic substrate gradients: natural substrate (NS), natural + plastic (NP), and plastic-dominated substrate (PD). Trait attributes: body size <5 mm (BS_1), >5–10 mm (BS_2), >10–20 mm (BS_3), >20 mm (BS_4); flattened (FLA), cylindrical (CYL), ovate (OVA), streamlined (STR); burrowers (BUR), climbers/clingers (CLIM), crawlers (CRAW), skaters (SKAT), sprawlers (SPRAW) and swimmers (SWIM). Collector-gatherers (COLL), predators (PRED), shredders (SHRE), scrapers (SCRA), gills (GILL), spiracle (SPIR), plastron (PLAS), tegument (TEGU), plants/alga material (HB), detritus (CPOM), detritus (FPOM), animal (PREY), free-living (FR_L), permanent attachment (P_ATTA), Temporal attachment (T_ATTA), slow velocity (S_VEL; <0.1 m/s), medium velocity (M_VEL; 0.1–0.3 m/s) and fast velocity (F_VEL; 0.3–0.6 m/s).

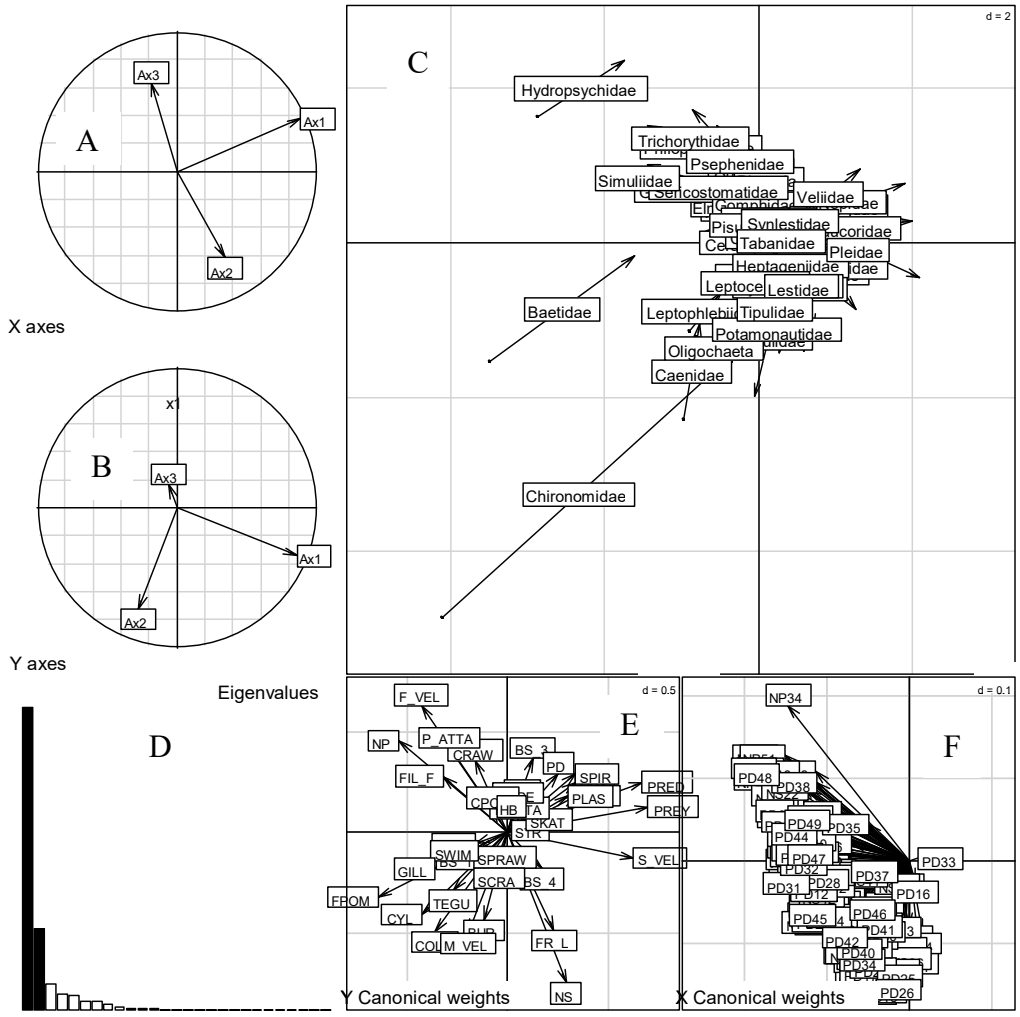

Figure 5.2: The graphical output of the result of co-inertia co-structure performed on trait data FCA and taxonomic assemblage data CA showing taxa and trait/ecological preference attributes during the study (October 2021– April 2022). Plots: a) components of the standardised correspondence of taxa dataset projected to the co-inertia axes; b) components of the standardised correspondence of taxa dataset projected to the co-inertia axes; c) position of taxa on the F1 x F2 co-inertia plane; d) histogram of eigenvalues; e) position of trait attributes within the co-inertia F1 x F2 axes, using trait co-inertia weights; and f) position of substrate plots on the co-inertia F1 x F2 axes, based on the taxa weights recorded in the plot. Abbreviations: substrate gradients: natural substrate (NS), natural + plastic (NP), and plastic-dominated substrate (PD). Trait modalities include body size <5 mm (BS_1), >5–10 mm (BS_2), >10–20 mm (BS_3), >20 mm (BS_4), flattened (FLA), cylindrical (CYL), ovate (OVA), streamlined (STR), burrowers (BUR), climbers/clingers (CLIM), crawlers (CRAW), skaters (SKAT), sprawlers (SPRAW) and swimmers (SWIM). Collector-gatherers (COLL), predators (PRED), shredders (SHRE), scrapers (SCRA), gills (GILL), spiracle (SPIR), plastron (PLAS), tegument (TEGU), plants/alga material (HB), detritus (CPOM), detritus (FPOM), animal (PREY), free-living (FR_L), and attached (ATTA).

Table 5.5: The Co-inertia co-structure ordination properties indicating the eigenvalues and percentage variation explained by the first two axes, correlations, and projected variance defined by the first two axes of log (x+1) taxa abundance (PCA) and traits (FPCA) matrices onto the first two co-structure axes during the study period (October 2021–April 2022).

RLQ properties	Axis 1	Axis 2
Eigenvalues	1.90	0.51
Covariance	1.37	0.72
Correlation	0.46	0.51
Variance	63.59	17.21
Variance CA/COIA (%)	82	92
Variance FCA/COIA (%)	81	81

5.5 Discussion

5.5.1 Spatial and temporal differences of traits and ecological preference across substrate groups

This chapter outlines the structural composition of macroinvertebrates traits and ecological preferences in relation to the substrate groups over the experimental duration. It identifies the traits and ecological attributes supporting macroinvertebrate colonisation of substrate groups in order to i) understand macroinvertebrate resource utilisation within macroplastic substrates; ii) elucidate the combination of modalities facilitating their adaption to macroplastic substrates; and iii) provide insight into the predictive role of traits – predicting macroinvertebrates likely to colonise macroplastics. The results revealed that macroinvertebrates colonising substrate groups were not significantly different in terms of their traits and ecological preferences (Table 5.2). These non-significant differences in trait and ecological preferences were also recorded across substrate groups on each sampling day. However, there was temporal variation in macroinvertebrate trait composition across periods (Table 5.2). These findings suggest that the distribution of taxa among the traits used indicates the existence of a diverse ecological community during the start of experiment for all substrate groups and were disturbed as the experiment progresses. These results further highlight the results from Chapter 3 and 4 which indicated rapid colonisation of different macroinvertebrate taxa of both natural and macroplastic substrates. Additionally, the results indicate the possibility of macroinvertebrates finding macroplastic an attractive habitat because

the surface is provided with morphological characteristics proper for colonisation (Taurozzi et al., 2023; Gallitelli et al., 2023). However, the result is in contrast with other studies on the colonisation of plastisphere. For instance, using a trait-based approach to elucidate the colonisation capacity of macroinvertebrates on Polystyrene (PS) tiles and PET bottles in Torre Flavia wetland, Italy, Taurozzi et al. (2023) reported temporal invariability in trait distribution over a period of ten months on both PS tiles and PET bottles. This contradictory result may be as a result of the hydrodynamics of these two ecosystems as the Italian studies were conducted in a lentic freshwater body.

The temporal differences in macroinvertebrate traits and ecological preferences in this study may be attributed to the changes in environmental conditions, such as hydrology, acting upon deployed substrate groups over time. On days 30 and 60, the combination of traits and ecological preferences, such as a medium and large body size, flat body, collector-gathering, predators, and free-living were more abundant. Taxa that are free-living and utilise the collector-gathering or predatory feeding mode may freely roam into newly introduced substrates, such as macroplastic, in search for food as organic matter builds up over time (Molokwu et al., 2014). However, intermittent hydrological variations are common in foothill rivers, which can lead to natural disturbances, like spates. Such disturbances can move substrates, lift deployed plastic litterbags, and eliminate epilithic food resources, and inhabitants not adapted to adverse habitat disturbance, such as the predators in rivers (Poff & Ward, 1990; Reid & Thoms, 2008). By contrast, specialist taxa with a preference for permanent attachment, filter-feeding mode, and higher velocity which are adapted for surviving stochastic disturbance, were observed to have thrived towards the later sampling days (120, 150, 180) of the experiment. This result is in agreement with several studies that posited that factors such as natural or anthropogenic disturbance, fluctuations in food resources, predation or competition for space drives changes in the proportion of specialists and generalists in a habitat over time (Shieh et al., 2012; Forcellini et al., 2022). At the onset of a new habitat and natural colonisation process, it is expected that an initial pool of generalists will quickly establish and be progressively replaced by specialists, such as filter feeders, that have specific habitat requirement.

5.5.2 Traits distribution structure of macroinvertebrate communities to substrate groups

The non-significant effect of substrate groups on trait distribution may be due to macroinvertebrate mass colonisation from the surrounding environment or habitat use. The co-inertia analysis showed a high correlation (RV-value of 0.95) with the total inertia of 2.99. The co-structure explained 80.8% of the total variability in taxa abundance, trait, and substrate groups. However, it was not significant ($P > 0.05$), although it did show a simultaneous variation in both structures due to the high correlation value (Dray et al., 2003). The results recorded and displayed associations between taxa-traits, traits-trait modalities, and traits-substrate groups (Table 5.3 and Figure 5.2). Based on the results from FCA and COIA (Table 5.4, Figures 5.1 and 5.2), the trait modalities that were displayed in two axes were classified into four groups. Of these four groups, three were found to be directly associated with substrate groups.

The first modality groups were associated with the 100% natural substrate (NS) with a combination of taxa with preference for slow/still flow velocity (<0.1 m/s), a large body size (> 20 mm), and being free-living. The major families contributing to this trait modality were mainly the Odonata, including Lestidae and Libellulidae and Gastropoda (Potamonautidae) that are large in size (Odume et al., 2023). This indicates that the NS substrates have a homogeneous community structure that is similar to the surrounding environment where the substrates were placed and suggests that free-living and large-body macroinvertebrates may range or use these substrates to avoid high-flow turbulence (Beisel et al., 1998; Reid & Thoms, 2008). The possession of a large body size and free-living by these taxa has been deemed beneficial during sediment accretion, which is a feature of areas with slow/still velocity. When flow discharge increases, these large-bodied organisms, which have a weight advantage, can move in between particles to avoid spate (Lamouroux et al., 2004; Forcellini et al., 2022). These findings are consistent with previous studies investigating the relationship between macroinvertebrate traits and habitats (substrates and hydraulic biotopes) (Lamouroux et al., 2004; Reid & Thoms, 2008). For example, Lamouroux et al. (2004) found that macroinvertebrate communities in habitats with still flow velocity were dominated by large-bodied, free-living taxa capable of moving through sediment load. The result of the combined COIA analyses also revealed this relationship, with large body size, free-living and preference for slow/still flow being indicators of NS substrate colonisation by macroinvertebrates in this experiment.

Duan et al. (2008) and Milesi et al. (2016) reported that heterogeneous substrates can offer refuge from flow disturbances and predation while providing diverse food sources. Thus, the complexity of a substrate influence may be linked to various traits, particularly those related to substrate selection, food and feeding habits, and locomotion. The second modalities include taxa that prefer fast-flowing water (0.3-0.6 m/s), permanent attachment, filter feeding, crawling, and coarse particulate organic matter. These modalities are linked to the 50% plastic (NP) substrate, the most diverse substrate used in this study due to its combination of equal proportions of natural and plastic substrates. Families of filter-feeders, such as Hydropsychidae, Simuliidae, Tricorythidae that are capable of attaching to hard surfaces in fast-flowing water were the major drivers of the association with NP substrate (Figure 5.2). These results reveal the mechanism responsible for the continual higher abundance of these taxa on the NP substrate reported in Chapter 3 and 4 (Figure 3.2; Figure 4.6 and 4.7). Thus, the preference for permanent attachment and filter feeding are traits that mediated macroinvertebrate colonisation and establishment on NP substrates in this experiment. This finding aligns with previous studies that suggested that the diversity of substrates allows macroinvertebrates to protrude from their complex habitat to feed on seston intermittently (Rempel et al., 2000) and indicates that macroplastic habitat heterogeneity may favour traits associated with foraging or maintaining a position in fast-flowing water such as the affinity for matter and filter feeding, permanent attachment, and crawling.

The development of epixylic biofilm on plastic substrates and its ability to trap detrital matter over time in the same way as debris patches makes it a rich ecological niche that may attract a diverse group of fauna and flora and initiate inter-specific interactions (Brouwer et al., 2020; Tarouzzi et al., 2023; Gallitelli et al., 2023). Temporary attachment, skating and clinging/climbing mobility, shredding, medium body sizes (>10 – 20 mm), oval and flat body shapes, being a predator or prey, and spiracle and plastron respiration were associated with the homogenous plastic-dominated (PD) substrate and represent the third modality group (Figure 5.2). This high number of trait modalities associated with the 100% plastic PD substrate suggests that the PD substrate provides an ecologically diverse community, as evidenced by the distribution of taxa according to traits used. This is surprising, especially as previous chapters showed that the macroinvertebrate community on the PD substrate were less diverse in terms of taxonomic richness and diversity (Chapters 3 and 4; Figures 3.2 and 4.2). An explanation of this trait complex PD substrate habitat may be owing to high inter-specific interactions as a result of predator-prey and shredders-detrital relation traits

expressed by taxa colonising PD substrates. This result confirms the findings of Booth et al. (2013), where the authors showed that the rapid colonisation of plastic substrates was likely influenced by predator-prey relationships. Furthermore, the reason behind the many traits attributed to inclination towards the PD substrate was due to families such as Naucoridae, Ancylidae, Athericidae, Pisuliidae, Hydraenidae, Corixidae, and Psephenidae (Figure 5.2). For instance, as reported in Chapter 3 (Table 3.5), psephenids with oval shapes were significantly correlated with plastic-dominated (PD) and exclusively found on macroplastic-related substrates. The psephenids were observed clinging to the tiny crevices on plastic substrates. This suggests that macroplastics may offer more intricate crevices that can serve as habitats for prey to avoid being preyed upon. Numerous studies have also documented the attachment of macroinvertebrates to plastic surfaces in freshwater environments (Booth et al., 2013; Gallitelli et al., 2023; Wilson et al., 2021).

5.6 Conclusion

The primary objective of this chapter was to analyse macroinvertebrate trait distribution patterns across substrate groups. Analysing macroinvertebrate trait distribution is crucial because it helps us understand how they utilise available resources and adapt to their environment and also assists in predicting which taxa benefit or are most affected by the presence of macroplastics in rivers. The results showed that the substrate groups had little impact on the distribution of macroinvertebrate traits by highlighting that both natural and macroplastic substrates supported similar ecological functions, based on the traits used. There was a tendency for 100% plastic (PD) substrates to have a higher number of modalities than 50% plastic (NP) substrates and 100% natural substrate (NS), but this difference was not statistically significant. The PD substrate likely supports a wide range of traits and modalities because the substrate provided various ecological functions, such as a space for predator-prey interaction, entrapment of plant material for shredders, and a surface for macroinvertebrates to temporarily attach and cling onto. The trait analysis made it possible to diagnose the potential mechanisms that shape macroinvertebrate communities on macroplastic substrates. This study contributes to reducing the knowledge gap regarding the mechanistic responses of macroinvertebrate communities to plastic pollution in the Afrotropic region.

CHAPTER 6: GENERAL DISCUSSION, CONCLUSION, AND RECOMMENDATION

6.1 Introduction

Freshwater ecosystems are experiencing the highest rate of biodiversity loss on Earth due to habitat loss and degradation, flow alteration, pollution, invasive alien species, and climate change (Dudgeon et al., 2006; Darwall et al., 2009). Emerging pollutants like plastics remain a critical threat to freshwater ecosystems, with a significant ecological impact on riverine habitat and biological diversity (Blettler et al., 2019; Meijer et al., 2021). Once in the environment, plastic can have physical, toxic, and behavioural impacts, and its association with other pollutants may enhance its effects on biota (Li et al., 2022). Most importantly, plastic proliferation in aquatic environments can alter the natural flow patterns, modify substrate composition, and potentially impact the natural microhabitats of aquatic organisms (e.g. macroinvertebrates). This habitat modification by macroplastic can ultimately affect community distribution and ecosystem functioning (Honingh et al., 2020; Kolenda et al., 2021; Blettler & Mitchell, 2021). However, little information is available on the consequences of macroplastics on natural microhabitat quality and integrity, and the potential of macroplastics as suitable habitats for riverine macroinvertebrates. It is, therefore, imperative to build this knowledge to understand the current ecological implications of macroplastics on riverine systems better and make informed predictions about future changes resulting from plastic pollution.

In this study, taxonomic- and trait-based techniques were used to examine the response of macroinvertebrates to macroplastic habitat quality in four minimally impacted river sites in Eastern Cape, South Africa. The study addressed the knowledge gaps identified in the general introduction and literature chapter of this thesis (Chapter 1) regarding a) the assemblage distribution patterns of macroinvertebrate communities across a gradient of plastic substrate groups (Chapter 3); b) the role of hydraulic biotopes on the colonisation and establishment of macroinvertebrates on macroplastic, relative to natural substrates in river systems (Chapter 4); and c) the role of traits in the colonisation and establishment of macroinvertebrates on plastic substrates. To address these gaps, three macroplastic substrate gradients were formulated, including natural substrate (NS), natural + plastic substrate (NP), and plastic-dominated (PD) substrate to represent 0%, 50% and

100% macroplastic gradients, respectively. These substrates were put in bags of equal dimension and deployed at the same time into three different hydraulic biotopes (pool, riffle, run) in four minimally impacted headwater reaches. This is important because it allows for direct comparison of macroplastic substrates of the same proportion, origin and duration, under similar river hydrodynamics, and away from major land-use activities that could shape habitat integrity and biotic response. Thus, any effects of substrate groups used could be explicitly identified. The results of the present study provide insights into whether macroplastics are important habitats for macroinvertebrates and the role hydraulics and traits play in the so-called plastic-macroinvertebrate interaction in riverine systems. This chapter synthesises the key results, their ecological implications, and provides perspectives for future study.

6.2 Spatio-temporal distribution structure of macroinvertebrate across macroplastic substrate groups.

Macroinvertebrate assemblages are shaped by the stream substratum because it can provide food, refuge, and protection from high-flow discharge and predators (Dallas, 2007). Chapter 3 examined and compared macroinvertebrate assemblage structure and colonisation patterns on macroplastic substrate groups to elucidate whether they preferred macroplastic over natural substrates spatio-temporally. Expectations were that, heterogenous substrates such as the 100% natural substrates and 50% plastic substrates woud be more suitable habitats for macroinvertebrate colonisation and establishment than the homogenous 100% plastic substrates. The PRC and ANOSIM results showed that macroinvertebrate assemblage structures did not differ across substrate groups (Figure 3.3; Table 3.6). Pearson's point-biserial correlation analysis also highlighted this similarity by revealing a nonsignificant correlation of two or taxa for particular substrate groups, indicating a lack of niche differentiation among macroinvertebrate communities. This suggests that each substrate group provided similar ecological conditions, fostering identical taxonomic adaptations. Surprisingly, Simpson's dominance index value was significantly higher on 50% plastic and 100% plastic substrates relative to natural substrates ($P<0.05$), possibly indicating a high degree of specialisation by the dominant taxa to the specific habitat conditions provided by macroplastic substrates. An implication of this finding is that macroplastic substrates may provide suitable ecological niches for a few dominant taxa that are well adapted to these macroplastic habitat conditions. Higher dominance may sometimes suggest greater stability and resilience of the habitat

to external changes, and dominant taxa often play crucial role in ecosystem function, such as ecosystem engineers or bioindicators (Devictor et al., 2010). However, the stability of macroplastic habitat in rivers is questionable as these substrates are prone to remobilisation in high-flow regimes and thus reduced ecosystem stability and functionality. The dominant taxa recorded in this experiment were Chironomidae, Baetidae, Hydropsychidae, and Simuliidae. These taxa are generalist and opportunist taxa, which may explain their rapid colonisation of macroplastic substrates (Armitage & Pardo, 1995; Carvalho & Uieda, 2004; Leite-Rossi et al., 2015).

The evaluation of the macroinvertebrate colonisation and succession patterns on various substrate groups was crucial because it provided insight into the relative importance of community assembly mechanism at different stages of succession across spatial and temporal scales (Chang & HilleRisLambers, 2016). In all substrate groups, the cladistic parsimony tree result showed a diversified trajectory of assemblage succession (Figure 3.7), indicating the colonisation of specific taxa during the initial phase can impact subsequent colonisation and establishment of other taxa. This can affect the selectivity of macroinvertebrate communities that colonise substrate groups over different periods. For instance, habitat generalists like baetids and chironomids were present throughout the experiment. These taxa contributed to the formation of the pioneering community across all substrate groups. This is consistent with recent research on the succession of macroinvertebrates, which found that eurytopic and agile macroinvertebrates like simuliids, chironomids, and baetids are able to quickly colonise newly introduced substrates (Winking et al., 2014; de Donnova et al., 2022). The comparison of macroinvertebrate colonisation substrate groups indicated that plastic-dominated substrates had the least variety of macroinvertebrates with only a few dominant taxa. The results from the plastic-dominated substrate indicated how critical habitat structure is for macroinvertebrates because it is not as complex as heterogeneous substrates. The taxa spread on the parsimony tree made clear the variations in macroinvertebrate composition among substrates.

When compared, the colonisation pattern of the 50% plastic substrates was similar to that of the 100% natural substrate. This was evident for the different taxa that did not achieve the highest saturation density and implies that substrates that are a combination of plastic and natural river components can still support a diverse range of species and experience comparable biological succession to those seen in natural substrates. Hence, macroinvertebrates may perceive 50% plastic

substrates as natural substrates. For instance, keystone macroinvertebrates such as the coenagrionids and naucorids were recorded as early colonists of natural substrate and natural + plastic substrates (Donald & Anderson, 2003). These keystone odonates are important in maintaining diversity in aquatic environments by regulating dominant prey in habitats (Donald & Anderson, 2003).

Coenagrionids and naucorids were only recorded on 100% plastic substrates on day 120, indicating a likelihood of a weak predator-prey relation on the 100% plastic substrates. One possible explanation for this could be that plastic-dominated substrates again provided a specialised type of habitat which supported the adaptation of few dominant taxa, and thus predators such as the coenagrionids could not adapt to the plastic-dominated substrate habitat. The result is significant because it shows differences in the colonisation and establishment of substrate group macroinvertebrates, despite having a similar community structure. The dynamics and diversity of a community are reflected in predator-prey relationships, which were observed to be more structured in 100% natural substrates and 50% plastic substrates than in 100% plastic-dominated substrates.

These results provided subtle evidence that macroinvertebrates did not prefer macroplastic substrates over natural substrate; however, they find macroplastic substrates as suitable habitat and may colonise and establish onto these macroplastic habitats differentially, as seen with the cladistic parsimony analysis succession result. In addition to these findings, it is important to ask if environmental factors such as hydraulic biotopes, which are critical components of riverine ecosystems, influenced macroinvertebrate-macroplastic interactions differentially.

6.3 The influence of hydraulic biotopes on macroinvertebrates assemblages on plastic substrate groups

Empirical evidence based on the results in Chapter 3 suggested that substrate groups had a minimal effect on the structuring of macroinvertebrate assemblages. Chapter 4 examined the influence of hydraulic biotopes on macroinvertebrate colonisation of the substrate groups. An hydraulic biotope is an important factor that shapes the structuring of macroinvertebrates in rivers (Reid & Thoms, 2008; Graeber et al., 2017), and it was expected that macroinvertebrate colonisation of macroplastics would vary depending on the hydraulic biotope. The unique characteristics of each

biotope, such as channel morphology, substrate roughness, depth, and flow velocity influence the adaptation of macroinvertebrates to their habitat in rivers. Contrary to expectations, the result showed that macroinvertebrate community composition was similar for all substrate groups across hydraulic biotopes (Figure 4.3A-C; Table 4.4). This further suggests that, regardless of hydraulic biotope, macroplastics can serve as novel substrates that facilitate the colonisation and establishment of macroinvertebrates to a similar degree as natural substrates. These findings provide support to the previous section by indicating the ability of macroplastic to serve as a habitat that can compete with or replace natural substrates in the long run. This could lead to a decrease in the availability and integrity of natural riverine habitats for macroinvertebrates.

In contrast to the above findings, macroinvertebrate Shannon diversity ($P < 0.05$) and Simpson index ($P < 0.05$) values in pools were significantly higher in the 100% natural substrate (NS) relative to macroplastic substrates (NP and PD), indicating macroplastic substrates provided less of an ecological niche in pools. This may imply disturbance, or a decrease in ecological suitability of macroplastic habitats within pools. The evidence was seen in the abundance of the most dominating taxa which were numerically higher in natural substrates relative to macroplastic substrate substrates in pools (Figure 4.3). It is possible that the environmental conditions in the pool have negatively impacted the habitat properties of macroplastic substrates. These substrates, which provide hard surfaces for attachment and surfaces for the growth of biofilms, may have been affected. On the other hand, the natural substrate in the pool, which consists of rocks, logs, and vegetation, is rich in nutrients and attracts more macroinvertebrates despite the disturbance.

6.4 Trait and ecological preference distribution pattern on substrate groups

Substrate structure can serve as a filter by which species possessing appropriate traits that distinguish them from the nearby species collection can adapt and coexist (Heino et al., 2005). The substrate's layout affects macroinvertebrates' capacity to move and use resources, reflecting macroinvertebrates' feeding, behaviour, and food type present on the substrate (Verdenschot et al., 2012). Traits provide a foundation for explaining why specific families of macroinvertebrates were able to adapt to certain substrate impacts. Thus, Chapter 5 examined trait distribution and ecological preference across substrate types to uncover indicator traits mediating macroinvertebrate colonisation of macroplastic substrates. The result showed that

macroinvertebrates that have the ability to attach permanently to substrates, crawl, and filter their food from fast-flowing water were positively associated with natural + plastic substrates, while macroinvertebrates that attach temporarily and can cling onto substrates were increasingly associated with plastic-dominated substrates. These results suggest that macroinvertebrates with the combination of temporary or permanent attachment, crawling, clinging/climbing, and filter feeding are more likely to benefit from macroplastic habitats.

With respect to time, temporal changes regarding trait distribution were recorded on all substrate groups (Table 5.2). At the beginning of the experiment, trait modalities of medium and large body size, flat shape, free-living, predators, and collector-gathers were the main traits, but these changed over time to trait modalities of permanent attachment, filter-feeders, and the preference for fast velocity, as the experiment progressed. This indicated a shift in trait profiles of macroinvertebrates from the more generalist to specialists, which supports the overall colonisation pattern of macroinvertebrates reported in the previous section. This suggests that the response of a community to substrate colonisation is closely related to the characteristics of the different taxa that make up the community.

The response of traits offered a practical and systematic approach to interpreting and comprehending the presence of macroinvertebrates on macroplastic surfaces, shedding light on why specific organisms could inhabit and thrive on macroplastics. Studies have emphasised the importance of a robust attachment mechanism for filter feeders to attach and detach from litter items' smooth and solid biotic and abiotic surfaces (Kiessling et al., 2015; Gallitelli et al., 2023). The traits required for macroinvertebrates to colonise and establish themselves on the macroplastic substrate are permanent and temporal attachment, clinging, crawling, and filter-feeding mode. These traits are directly linked to the substrate and are essential for successful colonisation.

Studies must make it clear how macroplastic characteristics affect physical habitat homogeneity and macroinvertebrate distribution to sustain riverine systems. Traits determine how organisms use various substrate groups and thus, trait-based techniques could predict how plastic pollution would affect riverine habitats and macroinvertebrates. Riverine plastic pollution may be assessed and managed based on traits to assist taxonomic biomonitoring.

6.5 General conclusion,

This research examined how macroinvertebrate community responded to the gradient of plastic pollution in the least impacted upper regions of four rivers in Eastern Cape, South Africa. The research covered three essential questions: (a) The study findings suggest that plastic pollution does not significantly affect macroinvertebrate assemblage structure spatially; however, the choice of a plastic gradient may depend on specific taxa and their ecological preferences. (b) It emphasises the importance of considering hydraulic biotopes in examining biological assemblage communities in rivers, showing that specific hydraulic biotopes (riffles and runs) may reveal the colonisation of unique communities of plastics found within them. However, others (pools) may retain macroplastic through sediment burial, unless remobilised by a higher flow event. Furthermore, (c) trait distribution and ecological preference having an affinity for a particular substrate group were identified, further highlighting the mechanisms mediating macroinvertebrate colonisation across substrate groups. For instance, permanent and temporal attachment, clinging/climbing, and filter-feeding traits were associated with NP and PD substrates. This finding suggests that macroplastic substrates could also act like rock and plant materials in riverine systems. The study's findings significantly provide room for the investigation of the effects of plastic pollution on the heterogeneity of riverine habitats and their interaction with macroinvertebrates in Afrotropic riverine systems.

Implications and management recommendations

Macroplastics are abundant in freshwater systems. The presence of macroplastic can have severe ecological implications on riverine habitat and macroinvertebrates. The study suggests that macroplastics can serve as essential habitats for river macroinvertebrates. Although macroinvertebrates were found to be colonising the macroplastics, macroplastics can impact riverine habitat heterogeneity. Therefore, there is a need to control plastic input into riverine systems.

The hydraulic biotope played a significant role in indicating the importance of flow, bed materials, and substrate roughness. Therefore, preserving hydraulic diversity in river systems is crucial, as previous studies have shown the potential disruption of hydraulic habitats due to the proliferation of plastic.

Traits mediate organisms' interactions with their external environment. In this study, traits such as permanent and temporary attachment, clinging/climbing, crawling, and filter-feeding were primarily associated with the 50% plastic and 100% plastic substrate groups, suggesting that they were essential for colonising and establishing on plastic surfaces.

Overall, the results presented here demonstrate that the effect of macroplastics on riverine habitats and macroinvertebrate communities are a concern for both biodiversity conservation and habitat integrity with increasing proliferation of plastics worldwide. The issue at hand is quite intricate and will require the implementation or intensification of several actions. These actions may include recycling programmes, proper disposal methods, strict legislation, regular inspections, and ecological restoration. If these actions are taken appropriately, they may help to reduce plastic pollution in freshwater and oceans.

Strength of the study

This study is the first to incorporate the potential influence of hydraulic biotopes and traits in colonising and establishing macroinvertebrates on macroplastics for a prolonged duration. The provided insight is crucial in determining whether plastics could serve as a habitat, especially in light of the current era of the plastisphere.

Limitations and recommendations

The following are important limitations of this study and recommendations for further study:

- To better understand macroplastic effects on riverine habitats, it is advisable to conduct further studies within controlled stream systems. This will help to minimise the impact of natural and human disturbances that may arise during the experiment.
- The interpretation of the result presented in this study should be done with caution especially as plastics within the bags is less likely to serve as existing plastics in the field, which can be highly mobile.
- The analysis of traits at the family level revealed that they exhibit some degree of response to plastic substrate gradients. Future studies investigating the impact of plastics on river habitats should employ a more detailed taxonomic identification system. Additionally,

these studies should consider other traits, such as reproductive behaviour, life stages, and voltinism.

- While the study used mesh bags of the same size for all substrate groups to ensure standard distribution, future studies should aim to standardise the surface area ratio between macroplastic and natural substrates because these substrates come in different structures and dimensions.
- Future investigations should consider the full seasonal effects to compare how different seasons impact the colonisation of plastic substrates.
- Further studies should focus on different land-use types to better understand how macroinvertebrates colonise macroplastic substrates within them.
- Conducting further research using trophic indexes, toxicological tests, and molecular analyses such as environmental Deoxyribonucleic Acid (e-DNA) can help to understand the impact of plastics better and identify the organisms that have been exposed to plastic habitats since their introduction.

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APPENDIX

Appendix 5

Table 5: Fuzzy coding of macroinvertebrate traits in this experiment. Codings: plastic substrate gradients: natural substrate (NS), natural + plastic (NP), and plastic-dominated substrate (PD). Trait attributes: body size <5 mm (BS_1), >5–10 mm (BS_2), >10–20 mm (BS_3), >20 mm (BS_4); flattened (FLA), cylindrical (CYL), ovate (OVA), streamlined (STR); burrowers (BUR), climbers/clingers (CLIM), crawlers (CRAW), skaters (SKAT), sprawlers (SPRAW) and swimmers (SWIM). Collector-gatherers (COLL), predators (PRED), shredders (SHRE), scrapers (SCRA), gills (GILL), spiracle (SPIR), plastron (PLAS), tegument (TEGU), plants/alga material (HB), detritus (CPOM), detritus (FPOM), animal (PREY), free-living (FR_L), permanent attachment (P_ATTA), Temporal attachment (T_ATTA), slow velocity (S_VEL; <0.1 m/s), medium velocity (M_VEL; 0.1–0.3 m/s) and fast velocity (F_VEL; 0.3–0.6 m/s).

Taxa	BS 1	BS 2	BS 3	BS 4	CY L	FL A	OVA	ST R	BU R	CL IM	CR AW	SK AT	SP RAW	SW IM	GI LL	TE GU	SP IR	PL AS
Aeshnidae	0	3	5	5	5	3	0	0	3	5	5	0	0	3	5	1	0	0
Ancylidae	0	5	0	0	0	3	5	0	1	1	3	0	0	1	5	1	0	0
Athericidae	0	3	5	0	5	0	0	1	5	1	3	0	3	0	3	3	5	0
Baetidae	3	5	1	0	5	1	0	0	0	0	3	0	0	5	5	1	0	0
Belostomatidae	0	1	5	3	0	3	5	0	0	3	3	0	0	5	0	0	5	5
Caenidae	3	5	0	0	5	3	0	0	0	0	3	0	0	5	5	1	0	0
Ceratopogonidae	0	3	5	0	5	1	0	0	3	0	3	0	1	1	5	1	0	0
Chironomidae	3	5	1	1	5	0	0	0	5	0	3	0	3	1	3	5	0	0

Chlorocyphidae	0	0	5	0	5	3	0	0	0	5	5	0	0	3	5	1	0	0
Coenagrionidae	0	1	5	5	5	0	0	0	0	5	5	0	0	3	5	1	0	0
Corduliidae	0	1	5	3	0	3	5	0	0	5	5	0	0	5	5	1	0	0
Corixidae	0	5	1	0	5	0	0	0	0	3	0	0	0	5	0	0	5	5
Crambidae	0	0	5	3	5	3	0	0	1	1	5	0	0	1	5	3	1	0
Dytiscidae	1	5	3	1	3	3	3	3	0	5	5	0	0	5	3	3	5	5
Ecnomidae	1	5	3	0	5	0	0	0	0	0	5	0	0	3	1	5	0	0
Elmidae	3	5	0	0	3	3	3	0	0	1	5	0	0	0	5	1	5	0
Ephydridae	0	5	3	0	5	0	0	0	5	0	0	0	3	3	0	1	5	5
Gerridae	0	5	3	0	5	5	0	0	0	0	0	5	0	0	0	0	5	5
Glossosomatidae	5	3	0	0	5	0	0	0	0	0	5	0	0	0	3	5	0	0
Gomphidae	0	1	5	4	5	3	0	0	5	1	5	0	0	3	5	1	0	0
Gyrinidae	0	3	5	0	3	1	3	0	1	5	5	0	0	5	5	3	5	0
Helodidae	1	5	0	0	5	0	0	0	0	5	0	0	0	0	5	3	1	0
Heptageniidae	0	1	5	0	0	5	0	0	0	0	3	0	3	1	5	1	0	0
Hydraenidae	5	0	0	0	3	0	1	0	0	5	5	0	0	1	3	3	5	5
Hydrophilidae	1	3	5	0	5	0	0	0	0	5	5	0	0	5	3	1	5	5
Hydropsychidae	0	1	5	0	5	3	0	0	0	0	5	0	0	1	5	1	0	0
Leptoceridae	0	3	5	0	5	0	0	0	0	3	0	0	3	5	5	1	0	0
Leptophlebiidae	0	5	3	0	5	1	0	1	3	0	3	0	0	5	5	1	0	0

Lestidae	0	1	5	5	5	3	0	0	5	5	5	0	0	3	5	2	0	0
Libellulidae	0	3	5	2	5	3	0	0	0	1	5	0	0	3	5	1	0	0
Naucoridae	0	5	3	0	1	3	5	0	0	3	3	0	0	5	0	0	5	5
Nepidae	0	0	5	3	3	5	0	0	0	3	5	0	0	5	0	0	5	5
Oligochaeta	0	0	5	5	5	0	0	0	5	0	3	0	0	0	0	5	0	0
Perlidae	0	0	5	0	0	5	0	0	0	0	5	0	0	3	5	0	0	0
Philopotamidae	0	3	5	0	5	0	0	0	0	0	5	0	0	3	1	5	0	0
Physidae	0	5	0	0	0	0	5	0	0	1	3	0	0	1	5	5	0	0
Pisuliidae	0	0	0	5	5	0	0	0	0	0	5	0	0	0	5	1	0	0
Planaria	1	5	1	0	0	5	0	0	0	0	0	0	5	3	0	5	0	0
Pleidae	5	0	0	0	0	0	5	0	0	3	0	0	0	5	0	0	5	5
Potamonautidae	0	1	5	4	0	5	1	0	5	0	5	0	0	1	5	0	0	0
Psephenidae	0	5	0	0	5	0	5	0	0	0	5	0	0	0	5	0	0	0
Sericostomatidae	3	5	0	0	5	0	0	0	3	0	5	0	0	0	5	1	0	0
Simuliidae	3	5	0	0	5	0	0	0	0	0	3	0	0	1	3	5	1	0
Synlestidae	0	1	5	5	5	0	0	3	0	3	5	0	0	3	5	1	0	0
Tabanidae	0	1	5	3	5	0	0	0	3	1	3	0	1	1	0	3	5	5
Tipulidae	0	3	1	3	5	0	0	0	5	0	1	0	1	0	3	3	5	0
Trichorythidae	0	3	5	0	0	5	0	0	0	0	5	0	0	3	5	1	0	0
Veliidae	5	1	0	0	5	0	5	0	0	0	3	5	0	0	0	0	5	5

Continued….

Taxa	FR_L	T_ATTA	P_ATTA	COL_G	FIL_F	SCRA	SHRE	PRED	FPOM	CPOM	HB	PREY	NS	NP	PD	S_VEL	M_VEL	F_VEL
Aeshnidae	5	3	0	0	0	1	0	5	1	0	0	5	0	0	3	0	3	3
Ancylidae	0	3	5	0	3	0	0	0	5	1	3	0	0	0	3	3	0	3
Athericidae	5	0	0	0	0	3	1	3	1	3	1	3	0	3	3	0	3	3
Baetidae	5	0	0	5	1	3	1	0	5	1	3	0	0	3	0	0	3	3
Belostomatidae	5	0	0	0	0	0	0	5	0	0	0	5	3	0	0	0	0	3
Caenidae	5	3	0	5	0	3	0	0	5	0	5	0	3	3	0	3	3	0
Ceratopogonidae	5	3	0	1	0	3	0	3	1	0	1	5	3	0	0	0	0	3
Chironomidae	5	0	0	5	0	3	1	3	5	1	3	3	3	0	0	0	3	3
Chlorocyphidae	5	0	0	0	0	0	0	5	0	0	0	5	3	0	0	0	3	0
Coenagrionidae	5	3	0	0	0	0	0	5	0	0	0	5	3	0	0	3	0	0
Corduliidae	5	0	0	0	1	1	0	5	1	0	1	5	0	3	0	3	0	0
Corixidae	5	0	0	5	0	0	0	3	0	0	5	0	0	3	0	3	3	0
Crambidae	5	3	3	0	0	5	3	0	3	1	3	0	3	0	0	0	3	0
Dytiscidae	5	3	3	0	0	0	0	5	0	0	0	5	3	0	3	3	3	0
Ecnomidae	5	3	3	0	3	0	0	3	3	3	1	3	3	0	0	3	0	0
Elmidae	5	2	2	5	1	0	0	0	5	3	1	0	3	3	0	0	0	3

Ephydridae	5	0	0	5	0	1	3	1	3	3	3	1	3	0	0	3	0	0
Gerridae	5	0	0	0	0	0	0	5	0	0	0	5	3	3	0	3	3	0
Glossosomatidae	5	3	3	3	0	5	0	0	3	0	5	0	0	5	0	0	0	3
Gomphidae	5	0	0	0	1	1	0	5	1	0	1	5	0	3	3	0	0	3
Gyrinidae	5	0	0	0	0	0	0	5	0	1	0	5	3	0	3	0	3	3
Helodidae	5	2	0	0	0	0	0	5	1	3	0	3	3	0	0	0	0	3
Heptageniidae	5	3	0	5	0	3	0	0	3	0	5	0	3	0	0	3	0	0
Hydraenidae	5	0	0	5	0	3	0	5	1	3	1	5	0	0	3	3	0	0
Hydrophilidae	5	0	0	5	0	0	3	5	0	3	3	5	3	0	0	0	3	0
Hydropsychidae	0	0	5	0	5	0	1	3	5	3	3	1	0	3	0	0	0	5
Leptoceridae	5	0	0	3	0	3	5	0	3	3	1	0	3	0	3	0	3	0
Leptophlebiidae	5	0	0	3	1	3	2	0	5	1	1	0	3	0	3	3	0	3
Lestidae	3	1	5	0	0	0	0	5	0	0	0	5	3	0	3	5	0	0
Libellulidae	5	0	0	0	1	1	0	5	1	0	1	5	3	0	0	0	3	0
Naucoridae	5	0	0	0	0	0	0	5	0	0	0	5	3	0	3	3	0	0
Nepidae	5	0	0	0	0	0	0	5	0	0	0	5	0	0	3	3	0	0
Oligochaeta	5	3	0	3	3	1	0	0	5	1	0	0	3	3	0	3	3	0
Perlidae	5	0	0	0	0	5	0	0	0	0	5	0	3	3	0	0	3	3
Philopotamidae	3	1	5	0	5	0	0	0	5	0	3	3	0	3	3	0	0	5
Physidae	0	1	5	0	0	5	0	0	3	1	3	0	3	0	0	3	0	0

Pisuliidae	5	0	0	0	0	0	5	0	0	0	5	0	0	3	3	0	3	3
Planaria	5	0	0	0	0	0	0	5	0	0	0	5	0	3	0	0	0	3
Pleidae	5	0	0	0	0	0	0	5	0	0	0	5	3	0	0	3	0	0
Potamonautidae	5	0	0	3	0	3	1	0	0	1	3	3	3	0	0	3	3	0
Psephenidae	0	3	5	0	0	5	0	0	0	0	5	0	0	0	5	0	0	3
Sericostomatidae	5	3	0	0	0	0	5	0	0	0	5	0	0	3	0	0	0	3
Simuliidae	0	3	5	0	5	1	0	0	5	1	1	0	0	3	3	0	3	3
Synlestidae	5	0	0	0	1	1	0	5	1	0	1	5	0	3	0	3	0	0
Tabanidae	5	0	0	0	0	1	3	5	0	1	3	5	5	0	0	0	0	3
Tipulidae	5	0	0	1	0	1	3	5	5	1	1	3	3	0	0	0	3	0
Trichorythidae	5	3	0	5	0	0	3	0	5	3	2	0	0	3	0	0	0	5
Veliidae	5	0	0	0	0	0	0	5	0	0	0	5	0	0	3	3	0	3