Assessing the effects of invasive and native leaf litter decomposition dynamics in agricultural water impoundments

By

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Abstract

Leaf litter contributes to the functioning of aquatic ecosystems through allochthonous inputs of carbon, nitrogen and other elements. In many freshwater ecosystems, leaf litter inputs are among the most important cross-ecosystem nutrient contributions. However, native plant communities are under threat from invasive plant species, with largely unexplored consequences for recipient aquatic ecosystems. Broadly, ecological impacts of invasive alien species can be unpredictable and simultaneously span multiple habitat types and taxonomic groups. Invasive alien plants can have particularly severe ecological impacts, and plant inputs into aquatic environments can alter abiotic and biotic aquatic dynamics. Lakes and reservoir ecosystems are regarded as heterotrophic detritus-based habitats which are dependent upon allochthonous organic matter for the majority of energy inputs.

Allochthonous detritus is extremely important for the trophic dynamics of the microbial organisms, macroinvertebrates and benthic plants in lakes and reservoirs. In the present study, leaf litter nutrient inputs, decomposition and colonisation associated with four plant species was examined using a combination of mesocosm and field experimental approaches. Native sycamore fig *Ficus sycomorus* L., and silver cluster–leaf *Terminalia sericea* Burch. ex DC. decomposition dynamics were compared to invasive tickberry *Lantana camara* L and guava *Psidium guajava* L., whereby phosphate, nitrate, nitrite, silicate and ammonium releases were quantified over time. Leaf inputs significantly reduced pH, with reductions most marked by invasive *L. camara*. Conductivity was heightened by all leaf input treatments, excepting native *T. sericea*. Leaf inputs significantly affected all nutrient levels monitored in the water over time, except for silicate. In particular, leaf litter from invasive *L. camara* drove significantly increased nutrient concentrations compared to other native plant species, whilst effects of invasive *P. guajava* were less statistically clear.

The end weights of the leaf litter demonstrated decomposition differences among the species types, following a decreasing order of *P. guajava* > *T. sericea* > *F. sycomorus* > *L. camara*, further suggesting high organic inputs from invasive *L. camara*. Furthermore, *ex-situ* larval mosquito colonisation of with the above-mentioned native and invasive species leaves were assessed. Larval mosquito abundances differed significantly accordingly to leaf treatment, whilst no mosquitoes colonised leaf-free controls. Leaf litter from the invasive *L. camara*, invasive *P. guajava* and native *F. sycomorus* drove significant increases in mosquito abundances relative to native *T. sericea*. *In situ* macroinvertebrate colonisation, and quantify decomposition rates, of four species of native and invasive terrestrial plants was also assessed. Leaf treatments had a significant, group-specific effect on abundances and composition among focal macroinvertebrates. Invasive leaves reduced Physidae and Oligochaeta abundances, yet Ostracoda were significantly more abundant in the presence of invasive *P. guajava*. Chironomidae relative abundances increased under invasive *L. camara* treatments, whilst differences among leaf treatment effects on Coenogrionidae abundances were not statistically clear. In turn, macroinvertebrate diversity did not differ significantly among plant treatment groups, but the contributing taxa varied. The decomposition rate of the leaf litter demonstrated differences among the species types, following a decreasing order of *L. camara* > *F. sycomorus* > *T. sericea* > *P. guajava*. The study results highlight that differential leaf litter decomposition rates of invasive and native plant types play a significant role in nutrient release, thereby potentially supporting increased aquatic ecosystem productivity.

The study highlights that shifting terrestrial plant communities following invasion may alter aquatic nutrient availability and how insect communities may utilise such resources. In addition, the study highlights that even semi-aquatic organisms such as mosquitos are affected by differing leaf-litter inputs and this may have broader societal implications through vectoring of mosquito-borne disease. While the study showed that invasive leaf litter generally decomposes faster than native litter, the overall findings suggest that plant species-specific, rather than invasive versus native, considerations were important for colonization and nutrient release dynamics. As such, future studies should continue to assess characteristics of other dominant native and invasive plant species within the context of leaf litter allochthonous inputs into recipient aquatic ecosystems. Larger datasets will hopefully prove useful in developing a predictive framework for how riparian plant community shifts will impact on aquatic ecosystem functioning.

Key words: allochthonous input; leaf litter decomposition; native plant species; invasive plant species; colonisation, nutrient dynamics, macroinvertebrates; container-breeding mosquitoes; plant-vector interactions; Levubu.

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findings, conclusions or recommendations expressed in this material are those of the authors, and the NRF does not accept any liability in this regard.

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Preface

Each data chapter in this thesis is a stand-alone and has been published or is under review in international peer reviewed journal. As such, there may be some repetitions in the introductory sections of the chapters. The research reported in this thesis was done in conjunction with other scientists that are listed as co-authors of the mentioned articles:

Chapter 3: Mutshekwa T, Cuthbert RN, Wasserman RJ, Murungweni FM and Dalu T. Nutrient release dynamics associated with native and invasive 1 leaf litter decomposition: a mesocosm experiment. *Hydrobiologia*. **Under review** [*Contribution of T Mutshekwa: Concept, methods, analysis, interpretation, writing of article*]

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Declaration

I declare that "Assessing the effects of invasive and native leaf litter decomposition dynamics in agricultural water impoundments" is my own work. All other sources, used or quoted, have been indicated and acknowledged by means of complete references. This thesis has not been submitted for a degree at another university.

THENDO MUTSHEKWA

Signature:

February 2020

Chapter 1: Introduction

1.1 Background

To date, numerous studies (Coulis et al., 2016) into the role of leaf decomposition as a nutrient resource in aquatic environments (Ghimire et al., 2017). Studies on leaf litter (Zhang et al., 2008; Bruder et al, 2014; Riggs et al., 2015) show that the decomposition of leaves in aquatic environments can be regulated by various factors related to plant type, type of decomposers, and general abiotic conditions. Litter decomposition is considered an important ecological process that controls nutrient cycles (Ghimire et al., 2017). Most of the life on earth needs energy from carbon fixed by photosynthesis and litter decomposition is regarded as a key step in the carbon cycle. Terrestrial plant species produce large amounts of plant material that eventually wind up in streams supporting aquatic ecosystems (Ghimire et al., 2017).

Leaf litter decomposition plays an important role in determining nutrient accumulation, and the rate of nutrient release in forms available for uptake by plants. Litter decomposition is a key process in aquatic environments as it controls primary production and nutrient availability (Allison et al., 2013). The decomposition process does not involve only in the mass loss of litter decomposition, it also involves changes in the nutrient content and the release of nutrients (Solly et al., 2014). It is established that potential decomposition rates of plant leaves depend greatly on the chemical composition of the plants considered (Allison et al., 2013). The chemical composition of organic matter is reliant, among various.s factors, such as the climate region where plants grow (Solly et al., 2014). Invertebrates also contribute to leaf litter breakdown. The main role of invertebrates in the process of litter decomposition is breaking down the litter physically but not all invertebrates that feed on litter contribute to its break down (Alba et al., 2015). The most dominant invertebrates in streams are Diptera, Trichoptera, Odonata, Ephemeroptera, and Plecoptera (Wallace et al., 2015). Thus, the decomposition of litter influences the invertebrate diversity and colonisation.

South Africa is considered one of the most diverse countries in the world (Raimondo et al., 2009). South Africa is detailed to have more than 22 000 native plant species from almost 230 different families and containing 10% of the world's flowering plant species, and also act as a contributor to the global ecological scene (Raimondo et al., 2009). Over 200 introduced plant species are regarded as invasive plant species, covering over 20 million hectares of South African's land (Mostert et al., 2017). Invasive plant species such as *Solanum mauritianum* (bugweed), *Parthenium hysterophorus* (famine weed), *Lantana camara (*big-sage), *Eichhornia crassipes* (water hyacinth), and *Campuloclinium macrocephalum* (pom-pom weed*)* are listed as some of the biggest threats to the South African plant and animal diversity (Raimondo et al., 2009). In addition, SA is a water scarce country due to an increase in the destruction of river catchments through urbanisation, damming of rivers, water pollution etc. Despite this we still have high levels of aquatic biodiversity that are reliant on intact ecosystems. Increasing rates of habitat destruction in South Africa, owing to high development pressures on the country's rich environmental resources, have cast a new spotlight on biodiversity research.

1.2 Problem statement

To date, studies on leaf litter decomposition in South Africa have been limited, and, the relationship between decomposition rate, chemical composition, nutrient release and invertebrate colonisation received little attention in the region. The lack of research on leaf litter decomposition dynamics presents a problem in that we have huge knowledge gaps pertaining to arguably one of the most fundamental processes driving freshwater productivity. Therefore, this study seeks to address the gap and improve the understanding of leaf litter decomposition, nutrient release dynamics and invertebrate colonisation, by studying such dynamics within the context of changing plant communities. One of the major global concerns associated with changing environments is the biological invasions phenomena. Within the context of leaf-litter decomposition, shifting plant communities associated with invasive plant species are completely unexplored in the South African context.

1.2 Research aim and objectives

This study aims to investigate leaf litter decomposition dynamics associated with key dominant native and invasive plant species that are found along waterways in the Levubu catchment area (Limpopo, South Africa). The study more specifically addressed leaf litter dynamics around the invasive *Lantana camara* and *Psidium guajava* and native *Ficus sycomorus* and *Termin*alia *sericea* tree species. This research aim was associated with the following research objectives:

- Quantify the rate of invasive *L. camara* and *P. guajava* and native *F. sycomorus* and *T. sericea* leaf litter decomposition over time to determine differences between invasive and native
- Assess macroinvertebrate colonisation over time among two invasive *L. camara* and *P. guajava* and native *F. sycomorus* and *T. sericea* terrestrial plants leaf litter

- Understand nutrient (i.e. phosphate, nitrate, nitrite, silicate, ammonium) dynamics release rate associated with the leaf litter decomposition process over time for invasive *L. camara* and *P. guajava* and native *F. sycomorus* and *T. sericea*

1.4 Hypotheses

- Native *F. sycomorus* and *T. sericea* will decompose faster compared to that of invasive *L. camara* and *P. guajava*. This might have implications for aquatic food webs as nutrients will remain locked up in the leaves and leaves will also accumulate in rivers having serious implications on habitat and flow regimes
- Nutrient (i.e. phosphate, nitrate, nitrite, silicate, ammonium) release rates would be higher in native *F. sycomorus* and *T. sericea* plant leaves than invasive *L. camara* and *P. guajava* plant leaves. This might have implications for aquatic plant species as more nutrients will be released and available for utilisation
- Native *F. sycomorus* and *T. sericea* will support a more abundant and diverse invertebrate community compared to invasive *L. camara* and *P. guajava*. This might have implications for invertebrate colonisation of aquatic ecosystems as invertebrates will establish themselves in aquatic environments due to the specificity of organic matter.

1.5 Justification and significance of the study

The study of leaf litter decomposition in aquatic environments is important, not only because a great deal of the energy captured by photosynthesis is discharged by this process, but also because nutrients are mineralised or immobilised during the process (Keuskamp et al., 2015). This study will confine itself to assessing and understanding of leaf litter decomposition and its effects on aquatic invertebrates. Colonisation of invertebrates and litter decomposition play a role in assuring natural sustainability for all associated life forms, but it is greatly affected by human activities. Thus, this study will ultimately add to the knowledge base and contribute to the education of society on the value of the ecosystem processes and their associated tangible and intangible services.

Given the largely absent research on the decomposition of plant leaves in Limpopo and South Africa in general, this research is needed. This research is important for several reasons:

1. Improve the understanding of leaf litter decomposition, effects on aquatic invertebrate colonisation rates among invasive and native plant material, and nutrient cycling.
2. This study will be a significant attempt in extending existing knowledge about South African invasive and native plant species by studying their decomposition and implications on food webs.

Chapter 2: Literature Review

2.1 Introduction

Leaf litter decomposition is important for understanding nutrients cycle and primary production in the environments. International investigations, while extremely insightful, often offer limited local relevance. Since accumulation of plant leaves over the surface of the earth differs depending on the vegetation and the dominant elements of the vegetation (Freschet et al., 2013), local studies are extremely important. This chapter aims to explore the literature on the decomposition of invasive and native plant leaves and its effects on aquatic invertebrate altogether with nutrients release rates.

2.2 Leaf litter decomposition

The decomposition of leaf litter has long been studied as a fundamentally important ecosystem process (Freschet et al., 2013). Leaf litter contains carbon and hydrogen as well as several other elements such as oxygen, nitrogen, and phosphorus (Ander/son et al., 1973). The major input into the soil is from plants, in the form of aboveground litter i.e. leaf litter, twigs and stems, and/or belowground material i.e. root litter, exudates and mycorrhizal hyphae (Freschet et al., 2013). Litter decomposition and soil organic matter formation are driven by bacteria and fungi (Lehmann and Kleber, 2015). Their activity, and controls on soil community composition, influence N release and/or storage in the soil (Mooshammer et al., 2014). In addition to bacterial and fungal controls, soil mesofauna may influence microbial composition and community activity through top-down controls (López-Mondéjar et al., 2018).

The formation process of soil organic matter is primarily a biological one and it is composed of living plant, animal and microbial biomass, dead roots and other plant residues in various stages of decay. The amount and accumulation of litter are controlled by the composition and amounts of the plant residues, climatic conditions, soil texture, and soil chemical and physical properties (López-Mondéjar et al., 2018). Decomposition of litter can be considered as a two-stage process; first, the litter i.e. plants leaves are broken down by detritivores into little pieces which can be chemically reduced (Lefebvre and Gallet, 2017), and thereafter, through the activities of microorganisms. According to Rahman et al. (2013), decomposition processes represent a major flux of nutrients in most terrestrial ecosystems and quantifying rates of litter mass loss and the associated changes in nutrients bound in the litter are important aspects of evaluating ecosystem function.

Leaf litter decomposition is a continuous process involving biotic and abiotic factors. Litter decomposition is driven by three processes: fragmentation, leaching, and catabolism (Coulis et al., 2016). During fragmentation process, litter is reduced into smaller fragments that move down the soil profile, where they can be isolated as the soil organic matter light fraction. Leaching is the loss of water-soluble substrates from the litter layer into the soil and is responsible for fluxes of dissolved organic N from the standing litter to the mineral soil (Ong et al., 2017). Leaching depends on the concentration of soluble compounds in the litter and, thus, occurs largely in the early phase of litter decay (Coulis et al., 2016). Catabolism is the process by which saprotrophic organisms, mostly bacteria, and fungi, whose populations are regulated by microfauna, use the dead litter constituents for their growth and activity (Kaur and Debnath, 2015).

Bacterial and fungal decomposers are responsible for more than 95% of the biotic part of organic matter decomposition (Lladó et al., 2017). Decomposers rely on water and sunlight as a medium for transport of substrate (Lladó et al., 2017). Decomposers produced exoenzymes that are able to diffuse through the water films to the substrate in order for the breakdown of macromolecules into smaller pieces for ingestion. Different kinds of litter require different types of enzymes to break them down (Lladó et al., 2017).

In aquatic environments, leaf litter from terrestrial environment enters the aquatic and decompose. The litter lose mass rapidly due to leaching followed by colonisation of microorganisms such as bacteria and fungi which break down the litter and changes the physical and chemical characteristics i.e. microbial conditioning making the litter palatable for aquatic invertebrates. Aquatic invertebrates fragment the remaining leaf litter to fine particulate organic matter (Bärlocher 1985, Webster and Benfield 1986).

2.2.1 The importance of leaf litter decomposition

Leaf litter decomposition is regarded as important, not only because the energy captured during photosynthesis process is released during decomposition, but also because nutrients are mineralised during the process. The process of litter decomposition has a large influence on the food-web, diversity, and productivity of the photosynthetic plants. Furthermore, decomposition strongly influences soil formation and organic-matter accumulation.

Litter decomposition is essential in different processes and decomposer such as invertebrates, microorganisms, bacteria, and fungi depend on organic matter as their source of food leading to its break down into the simplest components. Water, carbon dioxide, and nutrients are released when organic matter has decomposed. Released nutrients are then made available to be utilised by plants to grow. Without decomposition process, nutrients in the plants will not be released, instead, they will remain in their tissue, resulting in less nutrients available for plants in the soil (Freschet et al., 2013). Decomposition rates may be used as a good indicator of functional status of streams (Gessner and Chauvet (2002).

2.2.2 Factors affecting the rate of leaf litter decomposition

According to Rinkes et al. (2014) particle size has a signification impact to decomposition, when the particle is smaller the surface area is greater, thus high rate of decomposition (Rinkes et al., 2014). The particle size also determines the amount of water in the soil which also impact litter decomposition directly and indirectly (Rinkes et al., 2014). Directly, in dry soil the amount of water is less that is required by decomposers as decomposers need water to survive, thus the decomposition rate decrease and indirectly, wet soil results to slow break down of litter because the water occupies the holes in the soil, depriving the microbes of oxygen. Decomposition is fast normally in the soil that undergoes both dry and wet periods. Around 60 to 80 inches of the water-holding capacity is needed for decomposition of litter (Rinkes et al., 2014).

Finn et al. (2015) highlighted that chemical composition i.e. N has the impact on the decomposition of litter. Plant material with high C:N ratios or lignin usually decompose slowly when N is added and when C: N ratios or lignin is low the decomposition tends to be faster (Finn et al., 2015). The C:N ratio of organic matter has a great influence on the rate of decomposition. The ideal C:N ratio in organic matter of the range of 20-25 is required for maximum decomposition since a positive soil condition is created to balance the processes of mineralization and immobilization. A wide C:N ratio results into slower decomposition of organic matter. Lignin is more impervious to organic matter decomposition compared to cellulose and it protects cellulose while reducing the process of decomposition. Wang et al. (2018) demonstrated that decomposable carbon in rape straw with low lignin incorporated in the soil can be broke down faster than residuals with high lignin content.

Temperature can also influence the rate of decomposition i.e. the rate of decomposition is faster at a temperature range of 30°C to 40°C (Ward et al., 2015). Litter breaks down faster in the summer, and in warm weather than winter and areas with cold weather. The pH affects how nutrients are bound to soil particles. If the pH in the soil is very low, nutrients tend to be inaccessible to plants because they are not dissolved in the soil water. Directly, soil pH affects the density, kind and the activity of bacteria and actinomycetes that play a role in the process of decomposition and the rate of decomposition of organic matter. The soil that is neutral has a more decomposition rate than acidic soils and actinomycetes (Ward et al., 2015).

2.2.3 Estimating the rate of leaf litter decomposition

Several methods have been used to determine the decomposition rate of leaf litter. All of them have been detailed to have problems and they serve mostly as indices of decomposition rate. Litterbag or mesh bag method is recommended by many scientists as a reliable method and other methods are regarded as potential alternatives.

2.2.4 Litterbag method or mesh bag technique

The litterbag method involves weighing the material in mesh bags, nutrient content, mass loss and the chemistry of carbon over a period. Microclimate within the mesh bag changes when the litterbag slows the death rates and can slow the rates of the colonisation of fungi and growth

(Chapman et al., 2013). To undertake this method a known amount of dry leaves is enclosed in bags with appropriate mesh sizes and placed on the chosen plots. Many bags are installed at the start of the experiment and sampled periodically over time. The decomposition rate is then determined from the remaining mass of decomposed organic matter in the bags. Nevertheless, this method has some limitations which include, the duration of the experiment and the size of the bag (Kurz-Besson et al., 2005). The size of the mesh bag is often chosen to optimize access by all organisms to the litter while minimizing excessive particle loss. Small mesh side will exclude certain organisms and high particle loss. Recommended size of the mesh bag in most studies is 5 mm if the objective is to allow access by macrofauna (Woordward et al., 2012; Boyero et al., 2015). Nytex or Plastic mesh bags are usually filled with only one species or mix of species when more realistic experiment is desired. Small woody debris can also be filled in the mesh bags (Robertson and Paul, 2000). Leaf litter of a known quantity is then placed in bags and deployed in the environment or slow moving pools within streams. At various time intervals, bags are retrieved, cleaned to remove debris, oven-dried and weighed to determine dry mass loss. Mass loss from leaf litter act as a crucial pathway for nutrient return to the environment. A portion of these nutrients will then be available to be reused by the plants.

2.3 Invasive and native plants

Invasive plants species are defined as species that are not native to a specific location and have an ability to spread over a large area outside their natural distribution, threatening biological diversity (Mooney and Hobbs, 2000). Invasive plant species are commonly known for their economic or environmental harm (Jos, 2013). According to Blackburn et al. (2011) the process of species invasion into a new environment involves the introduction, establishment and ultimately the spread of the species (Fig 2.1).

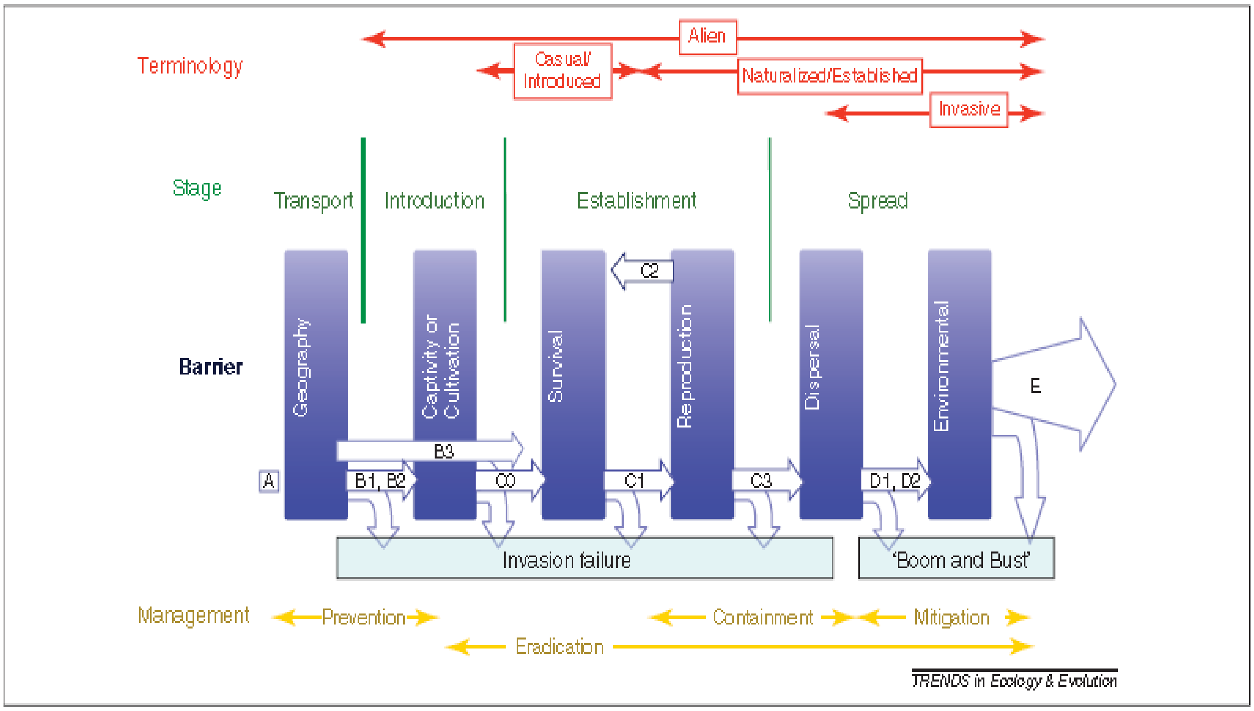

Fig. 2.1. Proposed invasion process extracted from Blackburn et al. (2011).

Native plant species are indigenous or naturally belonging to a particular geographic area and/or environment (Alba et al., 2015). Many of the indigenous species found in South Africa are endemic and highly localised. Other species are widespread and important in their ecosystems given their large contributions for ecological processes. Native plant species are worth protected given the direct and indirect ecosystem services they provide, many of which are of huge importance for society.

2.3.1 Invasive plant species in South Africa

Numerous invasive plant species have been introduced in South Africa for various purposes, which include crop production, dune stabilization, timber, construction, and ornamentals. Some of these plant species have naturalised, while others have become invasive in South Africa (Gaertner et al. 2016). Gaertner et al (2016) indicated that about 8000 shrubs, 750 trees and, 8000 herbaceous species have been introduced in South Africa. Invasive plant species occupy more than 20 million hactares of South Africa (Wynberg, 2003). Invaded lands in South African include forest, fynbos, savannah and grassland biomes (Henderson, 2007). The most prevalent invasive plant species over the biomes of South Africa are the *Fabaceae* family, which are *Acacia mearnsii*, *Acacia saligna* and *Acacia cyclops* (Henderson, 2007). There are, however, many more species that are becoming increasingly problematic.

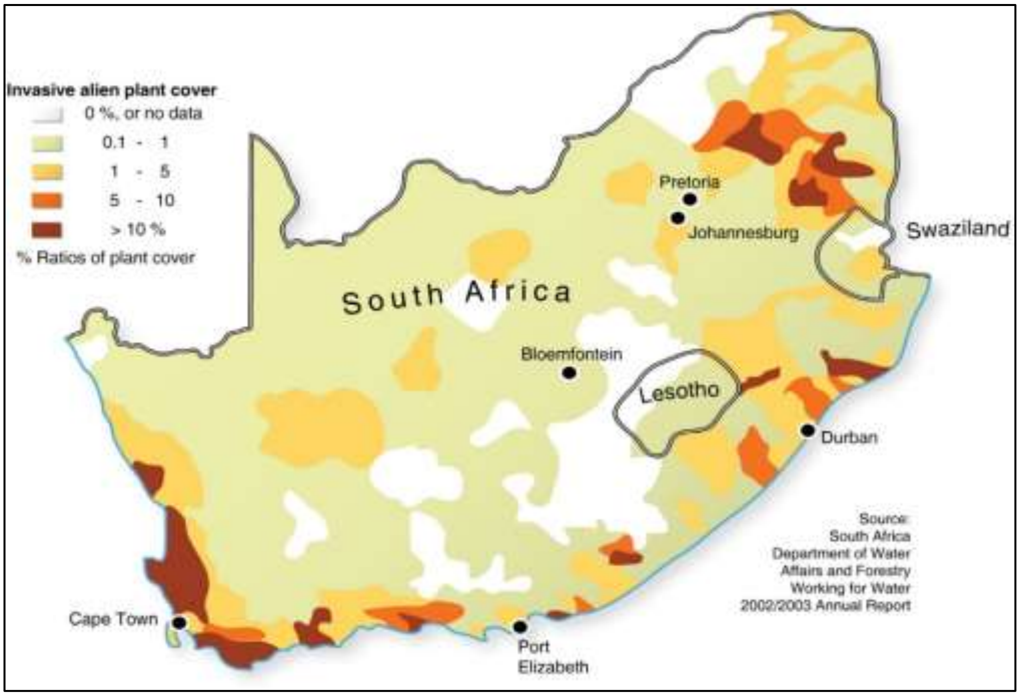

Fig. 2.2. Invasive alien plant cover in South Africa (Source, SA Department of Water Affairs and Forestry, 2003).

Invasive plant species negatively impact native plant communities by out-competing species or changing abiotic and biotic conditions in their introduced range (Richardson et al., 2007). Invasive plant species are a significant concern around waterbodies as they can easily spread rapidly using the water and the riparian zone as pathways. Riparian zones are among the natural habitats more prone to be invaded by invasive plants species (Richardson et al., 2007). Riparian zones receive high propagule i.e. seeds or spore pressure of invasive plants, their abiotic conditions are benign for plant life, and their biotic resistance from native vegetation is released by natural disasters such as floods and anthropic disturbances i.e. hydrological changes (Castro and Alonso Fernández, 2017). River systems and/or riparian zones are especially vulnerable to biological invasion because waterways tend to function as invasion corridors and are efficient transport vectors for plant propagule (Van Oorschot et al., 2017).

2.4 Aquatic invertebrates

Invertebrates are a diverse group occupying marine, freshwater, and terrestrial habitats. Populations and communities of aquatic invertebrates make incredible models for essential ecological studies since they are available in almost every habitat on the planet, including the Antarctic continent (Hill et al., 2015). The significance of their ecological role involves influencing ecosystem processes and functions such as nutrient cycling, primary production, and decomposition (Graça, 2001). Invertebrates are considered as one of the largest groups of organisms on earth. Over one billion invertebrates have been estimated to exist and less than million has been identified (Adis, 1997).

Aquatic invertebrates are a diverse group of organisms that live in water such as lakes, ditches, ponds, streams, lakes, and puddles and even marine environments (Corti and Datry, 2016). Freshwater aquatic invertebrates occur in lentic waters, at the bottom of lakes, open water, and along vegetated margins, or in flowing or lotic waters (Corti and Datry, 2016). These organisms invertebrates are often found under stones, woody materials, leaf litters, and snags. Terrestrial invertebrates include *Phylum Arthropoda* (insects, spiders, and crustaceans), and *Phylum Annelida* (worms and leeches). Aquatic invertebrates feed on tree leaves, wood, algae, and detritus. While many groups are wholly aquatic, certain aquatic invertebrates are only reliant on water for art of their life-cycle. In particular, many insects have aerial and terrestrial adult life-stages, with the egg, larval or nymphal stages only being reliant on water (Merritt et al., 2008). Many aquatic invertebrates are regarded as good indicators of ecosystem health, and form a part of aquatic food webs, with some even being important in terrestrial food webs (Merritt et al., 2008). While these organisms often take on the role of intermediary trophic links between primary producers (e.g. algae) and higher trophic levels, many representatives can themselves sit higher in the food web (Merritt et al., 2008). Aquatic invertebrate communities, therefore, vary in their diversity, density, and abundance among aquatic ecosystems and can even drive systems through different ecological states (Merritt et al., 2008).

2.4.1 Influence of invertebrates on leaf litter decomposition

Organic matter decomposition is largely driven by microbial activity, but invertebrates also play a significant role (Ulyshen and Wagner, 2013). Invertebrates are important in the environments for organic matter decomposition which then leads to the creation of organic layer and release of nutrients. Released nutrients are then available for uptake by living plants and play are important during the formation of soil (Lavelle et al., 2006). In aquatic ecosystem when leaves enter the streams, their nitrogen content generally increases (Abelho *et al*., 2005). Moreover, leaves start to undergo loss of mass at a rate proportional to invertebrate colonisation production (Suberkropp and Chauvet, 1995).

Various mechanisms have been detailed that are likely to influence the decomposition process which includes enzymatic digestion, substrate alteration, biotic interactions and nitrogen fertilisation. The effects of individual invertebrate taxa or functional groups can be accelerative or inhibitory, but the net cumulative effect of all invertebrates is almost certainly accelerative, at least during the early stages of decomposition (Ulyshen and Wagner, 2013). Some taxa appear to be particularly influential with respect to promoting decomposition. These include large organic matter-boring beetles and termites, especially fungus-farming macrotermitines (Ulyshen and Wagner, 2013). The presence or absence of these species may be more consequential than species richness or other community metrics (Ulyshen and Wagner, 2013).

In aquatic environments, invertebrates are one of the key variables influencing nutrients mineralization. Organic matter that enters streams is responsible to invertebrate fragmentation. physical abrasion and, microbial degradation (Ulyshen and Wagner, 2013). Aquatic invertebrates feeding on leaves are known as shredders and their densities tend to be correlated with the spatial and temporal accumulation of organic matter in streams. When invertebrates feed on organic matter they incorporate some nutrients in secondary production, accelerate leaf fragmentation (Graça, 2001). Invertebrates have therefore the potential to accelerate decomposition (Graça, 2001).

2.4.2 The relative importance of aquatic invertebrates in the incorporation of leaf energy into food webs

The importance of invertebrates in the incorporation of leaf energy into foods webs is an ongoing debate (Graca, 2001). The density of invertebrates is controlled by the quality and quantity of leaf litter. Therefore, invertebrates can be considered as less important in energy transference in decomposed leaves based on streams, while in other cases they may be the key elements of energy transference (Graca, 2001).

2.5 Plant nutrients

Nutrients are important to the growth and health of plants. The nutrients in plants are divided into three categories (primary, secondary, and micronutrients) (Franzluebbers and Hons., 1996). Each plant nutrient has its own important role to perform in the development and growth of plants. Primary plant nutrients which are nitrogen (N), phosphorus (P), and potassium (K) are the most valuable nutrients in plants (Marklein et al., 2016).

As organic matter decomposes, nutrients are released and nutrient release from decomposing organic matter is a critical pathway for nutrient flux/cycle in an ecosystem (Marklein et al., 2016). Nutrients may be released from organic matter by mineralization or leaching and nutrients which are released are available for plants to utilise for growth (Marklein et al., 2016). These nutrients affect primary production since these nutrients become readily available for plant uptake and are not lost from the environment. Organic matter quality affects not only the rates of mass loss but also the patterns and rates of nutrient release (Marklein et al., 2016). Organic matter quality is characterised by chemical composition and the ration of chemical composition influence organic matter indirectly because they act as determinate of the availability of nutrients to decomposers of organic matter and decomposability of organic matter (Li et al., 2015). Nutrients are a good indicator of decomposition rate because they affect organic matter decomposition both as an internal chemical component of litter tissue and as an external part of the decomposition environment (Li et al., 2015).

2.6 Nutrient cycles

Berg and McClaugherty (2008), define the nutrient cycles as a process that occurs primarily in the edaphic environment and involves litter accumulation and subsequent decomposition (Berg and, 2008), followed by the transfer of nutrients to the soil for the uptake by plants. Within the context of aquatic systems, the nitrogen cycle process take place when plant litter is introduced intro the stream from terrestrial ecosystems. Nutrient-containing leaves are usually the dominant litter fall, with riparian plants donating nutrients into aquatic habitats in this way.

Plant leaf litter is considered the most direct pathway for nutrient return into the soil and uptake by living things, especially phosphorus and nitrogen (Berg and McClaugherty, 2008). In aquatic habitats, once the plant litter has settled into the bottom of the stream/water, the decomposition process takes place which is influenced by the fungus, bacteria, invertebrates and/or physical and chemical properties. During this process the nutrients are released into the soil below and form part of the cation exchange capacity of the soil. Once nutrients have entered the soil and the decomposition process is accomplished the nutrients, they become available for reabsorption by plants through their roots (Berg and McClaugherty, 2008).

2.7 Key riparian invasive and native plant species in the study area

Plant communities in riparian zones are usually somewhat distinct from those of other terrestrial environments (Strain and Bazzaz et al., 1983). In many regions, certain species are predictably found near water, with their plant matter contributing disproportionately to allochthonous input into nearby aquatic habitats. Similarly, as part of invasion processes, riparian zones are often particularly vulnerable to certain invasive species. As a result, changes in riparian plant communities are common following plant invasions (Richardson et al., 2007; D'Antonio, 2000). The Levubu catchment area of the Limpopo province is no exception, with riparian zones being compromised by invasive species. Given the aims of the study, it was therefore necessary to identify key and locally relevant native and invasive riparian plant species that would give an indication of the potential implications of shifting plant communities for leaf litter decomposition dynamics in recipient aquatic ecosystems.

Field visits to various sites revealed that there were consistent patterns in species found in riparian zones in the study area. Native sycamore fig *Ficus sycomorus* and silver cluster–leaf *Terminalia sericea* were commonly encountered along natural and man-made lentic and lotic watercourses. Both species are widespread in southern Africa and typically grow in the riparian zones and wetlands (Pothasin et al., 2014; Venter and Venter, 2015; Sunil et al., 2016). Non-native species commonly encountered were tickberry *Lantana camara* and guava *Psidium guajava*. Both of these species are native to tropical central and southern America and are categorised as fully invasive in South Africa (Henderson, 2007; Gaertner et al. 2016). In the region these invasive species are well-known to have a substantial negative impact on native plant species through competition and replacement (Richardson and van Wilgen, 2004; Vardien et al., 2012; Urquía et al., 2019). Here, I explore the leaf-litter breakdown and nutrient release associated with these four species using an *ex-situ approach* (Chapter 3). I then explore how these processes may facilitate colonization by important semi-aquatic insect taxa Culicidae (Chapter 4). Finally, using *in situ* approach I assess aquatic invertebrate colonisation dynamics in the field (Chapter 5).

Chapter 3: Nutrient Release Dynamics Associated with Key Native and Invasive Leaf Litter Decomposition: a Mesocosm Experiment

3.1 Introduction

Plant litter decomposition is a key process in organic matter and nutrient recycling, supporting primary production in many terrestrial and aquatic ecosystems (Vogt et al., 2015). Various studies (e.g. Aber and Melillo, 1982; Zhang et al., 2008; Bruder et al., 2014; Riggs et al., 2015) have demonstrated that plant litter decomposition is driven by abiotic and biotic processes. The breakdown by these abiotic and biotic processes facilitates nutrient release and subsequent bioavailability. Leaf litter is broken down by microarthropods, invertebrates and microorganisms. However, climatic factors such as temperature and rainfall are the strongest determining factors on litter mass loss (Chen et al., 2019). Since litter decomposition involves several factors such as physical, chemical and biological breakdown processes (Krishna and Mohan, 2017), changes in plant species in a landscape through, for example, biological invasions may alter leaf litter decomposition dynamics. In turn, this may have implications for nutrient cycling processes in environments receiving such allochthonous inputs, and have emergent effects on aquatic community structuring.

The plant litter quality also influences the decomposition process, as it decreases over time due to the loss of carbon and soluble compounds (Dilly et al., 2001; Krishna and Mohan, 2017). The nature and quality of plant litter is fundamentally driven by the plant community (Krishna and Mohan, 2017), with implications for nutrient dynamics within ecosystems and the adjacent receiving environments. Plant species are highly variable with regard to physical structure of leaves, and exhibit heterogeneity and specificity in chemical compound presence (Fernández et al., 2016). Globally, natural plant communities are increasingly changing given numerous pressures associated with human activities such as deforestation, soil erosion and species augmentation. One major way in which plant communities are being altered is through alien plant species invasions (Wolfe and Klironomos, 2001). While invasive plant species nutrient resource acquisition dynamics are prevalent in the literature, for instance Drenovsky et al. (2008) and Leishman et al. (2010), studies on their nutrient return dynamics are lacking (Hasanuzzaman and Hossain, 2014). This is a major gap in the literature and hinders our understanding of how nutrients are cycled in landscapes increasingly dominated by invasive species.

Terrestrial plant litter which ends up in aquatic environments act as a key pathway for nutrient release and supply to aquatic biota. The leaf litter decomposition process is comprised of three phases: (i) leaching of components from the leaf litter; (ii) conditioning by microorganisms, and; (iii) fragmentation and consumption (Webster and Benfield, 1986). The biological degradation of litter is mostly influenced by microbial decomposers such as fungi and bacteria, which have significantly lower carbon (C) to nitrogen (N) ratios (McGroddy et al., 2004). Brinson (1977) indicated that the loss of dissolved organic matter from fallen leaf litter is an important indicator of nutrient release from these organic inputs. The leaf litter chemical composition, together with habitat characteristics and environmental abiotic and biotic factors, control the process of decomposition which drives nutrient release from the plant litter (McGroddy et al., 2004). Here, I aim to assess such nutrient release dynamics associated with leaf litter from key native and invasive plant species, using an *ex situ* mesocosms approach.

The aim of the present study was to examine nutrient release (i.e. phosphate, nitrate, nitrite, silicate, ammonium) dynamics associated with the leaf litter decomposition process over time for two native (fig *Ficus sycomorus*, silver cluster–leaf *Terminalia sericea*) and two invasive (lantana/tickberry *Lantana camara*, guava *Psidium guajava*) terrestrial plants. *Lantana camara* and *Psidium guajava* are native to tropical central and southern America and are known to have a substantial negative impact on native plant species through competition and replacement (Richardson and van Wilgen, 2004; Vardien et al., 2012; Urquía et al., 2019). Both species are recognised as invasive in South Africa (Henderson, 2007; Gaertner et al. 2016). These invasive species were selected because they have been reported to spread fast within the riparian zones, in turn threatening the abundance and diversity of native plant species and community stability of aquatic ecosystems. Ramaswanu and Sukumar (2014) indicated that highest *L. camara* abundances are found closer to aquatic ecosystems. Dominant native plant species were selected for comparison. Native *F. sycomorus* and *T. sericea* are both native to southern Africa and can naturally grow in the riparian zones and wetlands (Pothasin et al., 2014; Venter and Venter, 2015; Sunil et al., 2016). It was hypothesised that: (i) nutrient (i.e. phosphate, nitrate, nitrite, silicate, ammonium) release rates would be higher in native plant leaves than invasive plant leaves, and; (ii) native fig *F. sycomorus* and silver cluster–leaf *T. sericea* will decompose faster compared to invasive tickberry *L. camara* and guava *P. guajava*, with native communities less effective at breaking down litter from exogenous species. These decomposition processes are important to understand, as such differences in decomposition of leaf litter may have implications for aquatic food webs and community structuring.

3.2 Materials and methods

3.2.1 Experimental design

Plant leaves of invasive i.e. *L. camara* and *P. guajava* and native i.e. *F. sycomorus*, and *T. sericea* were collected in November 2018 by hand from trees along Mvudi River riparian zone (22° 58.967'S 30° 26.840'E) in Thohoyandou, Limpopo province, South Africa, before being air dried at room temperature (range 27–30 °C). Only fresh plant leaves were selected, given many plants reabsorb nutrients prior to senescence (Staaf, 1982). After drying, approximately 3 g of the dried material for each species was weighed and added into 5 L white polyethylene buckets, filled with 3.9 L of borehole + river water (70:30 ratio), all filtered through 63 µm mesh cloth. The initial mean water temperature was 27.2 ± 0.2 °C (SD), conductivity 169.3 ± 4.0 µS cm^{-1}, total dissolved solids 85.5 ± 2.6 mg L^{-1} and pH 6.6 ± 0.1. Buckets were regularly topped up to 3.9 L with filtered (GF/F filter 0.02 µm, Ø 47 mm) borehole water. The twenty–five 5 L buckets (i.e. 5 replicates × 4 species, + 5 controls) were placed outside in a partially shaded area in the University of Venda campus in a randomised array.

3.2.2 Sampling and analyses

Water parameters (i.e. conductivity (µS cm^{-1}), total dissolved solids (mg L^{-1}), pH and temperature (°C)) were measured every 7 days for 6 weeks using a portable handheld multi–parameter probe (PCTestr 35, Eutech/Oakton Instruments). Approximately 50 mL of water samples were collected from each treatment and replicated weekly for nutrient (ammonium, nitrite, nitrate, phosphates, silicate) analyses. The nutrients were analysed at NRF SAEON Elwandle Node Coastal Biogeochemistry Laboratory using an Auto-Analyser model AA3 segmented flow colourimetry (SEAL Analytical). Phosphates were analysed using the calorimetric method and then read at 880 nm. The test range was 0–50 µg L^{-1}. Silicate was analysed using the reduction of silico-molybdate in acidic solution to molybdenum blue by ascorbic acid (Grasshoff et al., 1983). The test range was 0–41 µmol L^{-1}. Nitrate and nitrite were analysed based on a procedure where nitrate is reduced to nitrite by a copper-cadmium redactor column (Armstrong et al., 1967). This method is based on the nitrate determination in Standard Methods and in the dissolved inorganic nitrogen standards for automatic nitrate measurements. The test range was 0–50 µmol L^{-1}. Ammonium was based on the Berthelot reaction at 660 nm. The test range was 0–10 µmol L^{-1}.

3.2.3 Statistical analyses

The effects of leaf *treatment* (5 levels, including controls) and observation *week* (6 levels), and their interaction, on key water parameters (pH, conductivity and temperature) were examined using linear mixed effects models (Bates et al., 2015). Individual containers were included as a random effect (slope and intercept) to account for repeated measures over the experimental period. Leaf *treatment* and observation *week* effects on key nutrients (phosphate, nitrate, nitrite, silicate and ammonium) were analysed similarly, following omission of missing records ($n = 5$). Type III analyses of variance with Satterthwaite's method were used to infer effect sizes and significance levels of main effects (Kuznetsova *et al*., 2017). End–weights of leaves following the experimental period were compared using one–way analysis of variance according to leaf species treatment (4 levels). For all models, *post–hoc* Tukey comparisons were performed *via* estimated marginal means where effects were significant (Lenth, 2018). Normality and homoscedasticity of residuals was checked using diagnostic plots (Zuur et al., 2010), with $\log_{10}$ transformations applied where necessary to meet model assumptions. All statistical analyses were performed using R v3.4.2 (R Development Core Team, 2018).

3.3 Results

Water temperature did not differ significantly among leaf treatments yet differed significantly over the monitoring period owing to daily temperature undulations (Table 1). This highlights that all water quality differences observed were independent of potentially confounding temperature effects. Leaf *treatment* and observation *week* had a significant effect on the pH and conductivity of water, with leaf effects on conductivity also differing significantly over time owing to a significant interaction term (Table 1). All leaf treatments drove significantly reduced pH compared to controls (all $P < 0.001$), whilst *L. camara* also caused significantly reduced pH compared to *P. guajava* ($P = 0.04$). Compared to week 1, pH levels were significantly reduced in the early monitoring period (weeks 2, 3; both $P < 0.001$), yet neutralised over time (weeks 4, 5, 6; all $P > 0.05$; Fig 1a). *F. sycomorus, L. camara* and *P. guajava* always significantly increased conductivity relative to controls (all $P < 0.05$), whilst *T. sericia* treatments had no significant effect over the monitoring period (all $P > 0.05$). In turn, *P. guajava* leaf treatments displayed significantly reduced conductivity relative to *F. sycomorus* and *L. camara* (all $P < 0.001$). Conductivity increased significantly over time relative to week 1 (all $P < 0.001$; Fig 1b).

Table 3.1. Linear mixed effects model results considering key water parameters as a function of leaf *treatment* and *week*, and their interaction. F–values are discerned with Type III sums of squares *via* Satterthwaite's method. Significant *P*–values are emboldened.

Parameter	Predictor	F–value	*P*–value
Temperature	Treatment	1.52	0.23
	Week	9155.33	**< 0.001**
	Treatment: Week	0.70	0.82
pH	Treatment	42.03	**< 0.001**
	Week	92.66	**< 0.001**
	Treatment: Week	1.15	0.31
Conductivity	Treatment	69.99	**< 0.001**
	Week	170.40	**< 0.001**
	Treatment: Week	2.70	**< 0.001**
Phosphate	Treatment	30.51	**< 0.001**
	Week	15.22	**< 0.001**
	Treatment: Week	1.00	0.47
Nitrite	Treatment	23.33	**< 0.001**
	Week	13.93	**< 0.001**
	Treatment: Week	1.96	**0.01**
Nitrate	Treatment	18.18	**< 0.001**
	Week	32.94	**< 0.001**
	Treatment: Week	4.91	**< 0.001**
Silicate	Treatment	1.82	0.13
	Week	31.80	**< 0.001**
	Treatment: Week	0.67	0.85
Ammonium	Treatment	7.39	**0.001**
	Week	11.11	**< 0.001**
	Treatment: Week	2.47	**0.002**

Leaf *treatment* had a significant effect on all nutrient levels monitored in the water, except for silicate, and nutrient levels always differed significantly according to monitoring *week* (Table 1). Leaf inputs always significantly increased phosphate relative to controls (all $P < 0.001$). Further, leaf litter from invasive *L. camara* drove significantly increased phosphate compared to all other plant species overall (all $P < 0.05$; Fig. 1c). Excepting week one, invasive *L. camara* also significantly increased nitrite levels relative to control groups (all $P < 0.01$), whilst effects of other plant inputs were not statistically clear (all $P > 0.05$). Nitrite levels following *L. camara* treatments were significantly higher than all other plant species overall over the monitoring period (all $P < 0.001$; Fig. 1d). Contrastingly, leaf litter generally drove reductions in nitrate; yet, these reductions tended to become less pronounced compared to controls over time. At week 1, all plant inputs significantly reduced nitrate compared to controls (all $P < 0.01$), whilst only *T. sericea* treatments were significantly reduced at week 6 ($P = 0.02$). Nitrate levels also tended to be elevated following treatment with invasive *L. camara* and *P. guajava* compared to native *F. sycomorus* and *T. sericea* (Fig. 1e), with nitrate levels following treatment with invasive *L. camara* significantly higher than native plants (both $P < 0.01$). Leaf inputs also tended to increase silicate levels overall, however this effect was not statistically clear, with silicate levels only differing significantly over time (Table 1; Fig. 1f). A significant interaction term indicated that ammonium levels responded differently over time according to leaf treatments, with increases relative to controls becoming less marked over the monitoring period (week 1, all $P < 0.05$ (excepting *P. guajava*); week 6, all $P > 0.05$). In particular, invasive *L. camara* significantly increased ammonium levels during the early monitoring stages relative to all other treatment groups (week 2: all $P < 0.01$; Fig. 1g).

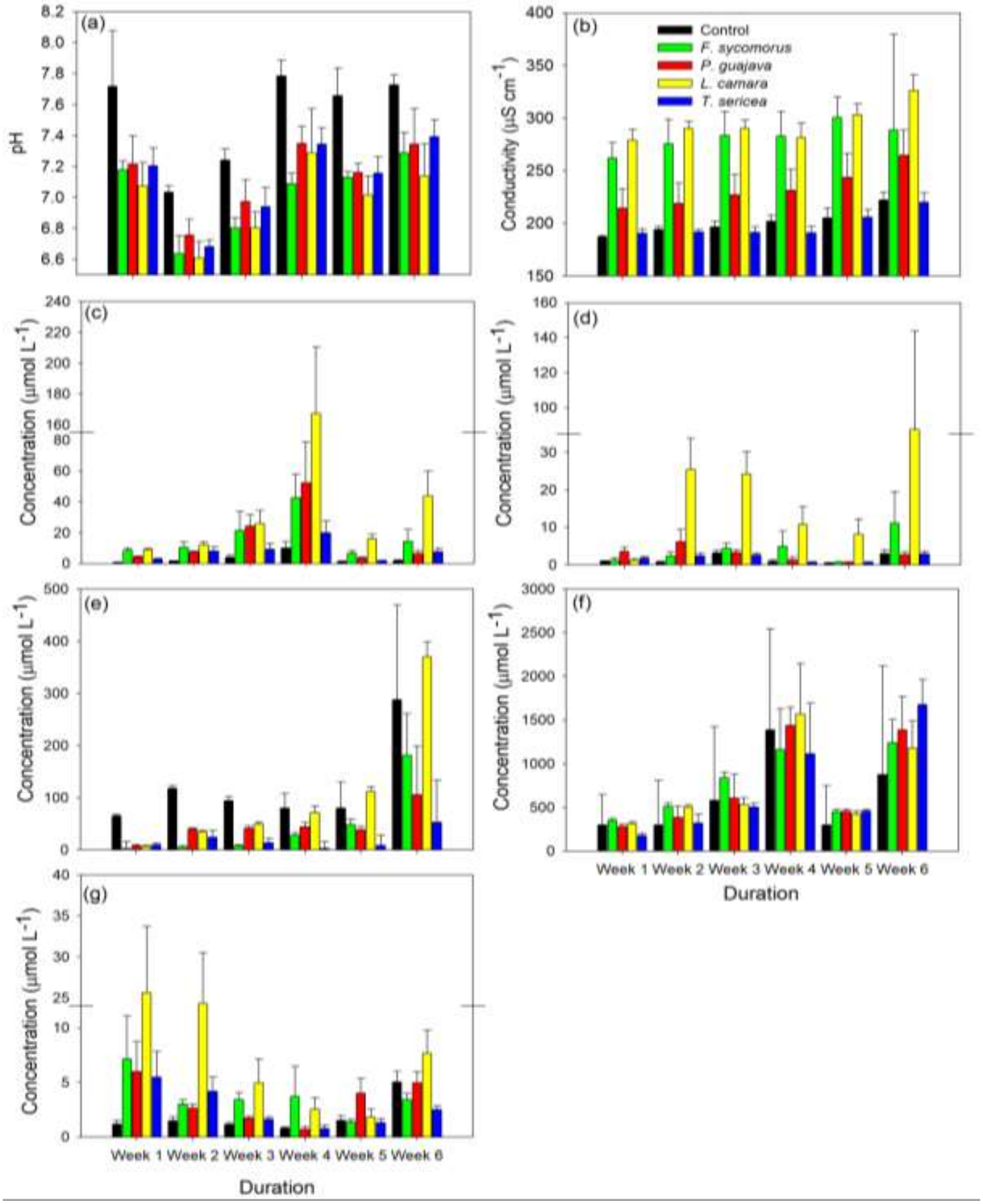

Fig. 3.1. Nutrient release concentrations (±standard deviation) of invasive *L. camara* (yellow) and *P. guajava* (red), and native *T. sericea* (blue) and *F. sycomorus* (green), over a six-week period: (a) pH, (b) conductivity, (c) phosphates, (d) nitrite, (e) nitrate, (f) silicate and (g) ammonium.

Leaf weights at the end of the experiment differed significantly according to species ($F_{3,16} = 26.41$, $P < 0.001$) in comparison with the initial weights. Invasive *L. camara* and native *F. sycomorus* weights were reduced most, and significantly compared to native *T. sericea* and invasive *P. guajava* (all $P < 0.001$; Fig. 2).

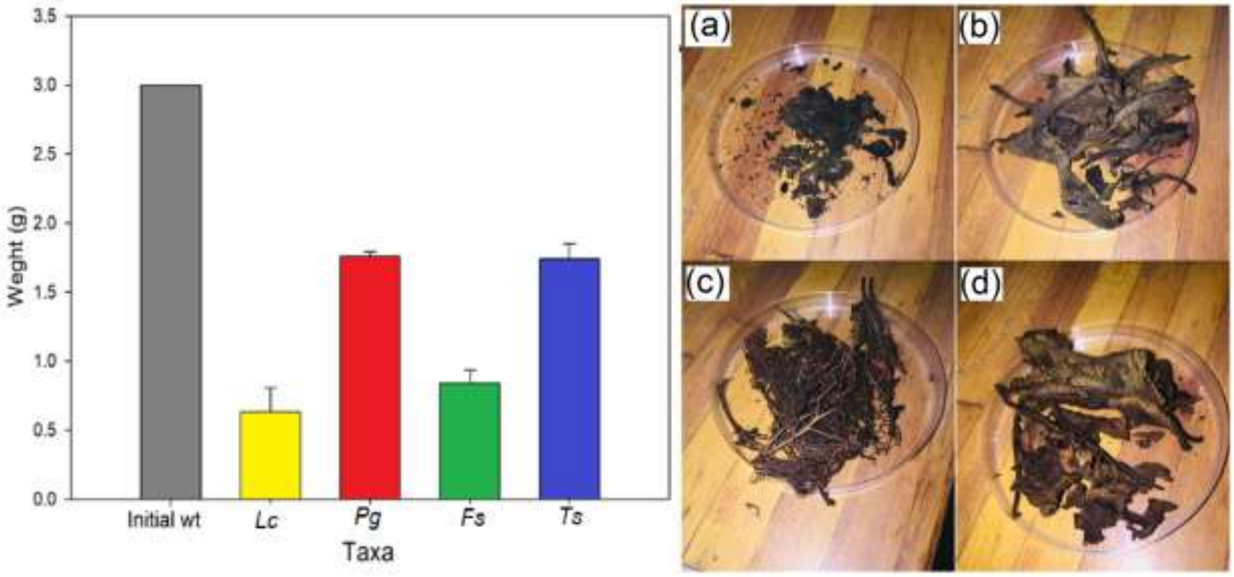

Fig. 3.2. Mean values (±SD) of end–weights of invasive (a) *L. camara* (*Lc*, yellow) and (d) *P. guajava* (*Pg*, red) and native (c) *F. sycomorus* (*Fs*, green) and (b) *T. sericea* (*Ts*, blue). Initial weights for all species were 3.0 g of dried leaf material.

3.4 Discussion

The study demonstrated that interspecific differences in leaf litter decomposition are important for allochthonous nutrient dynamics within aquatic systems. Leaf litter from the invasive *L. camara* drove greater nutrient concentrations than all other species. *P guajava*, however, was largely similar to the native *F. sycamorus* with regards to nutrient release, while the native *T.*

sericea nutrient release was generally the slowest. Thus, nutrients in the invasive litter became readily available in water quickly which, in turn, could be taken up by aquatic biota. In relation to leaf litter decay, our results indicated that invasive *L. camara* and native *F. sycomorus* decomposed faster than invasive *P. guajava* and native *T. sericea.*

Both hypotheses were rejected, with differences in nutrient release and weights inconsistent across invader and native groupings. It was first hypothesised that native plant inputs would release higher nutrient concentration than invasive species, yet invasive *L. camara* drove higher nutrient concentrations than native *F. sycomorus* and *T. sericea*. *L. camara* had higher nutrient release concentrations in every nutrient than native *F. sycomorus* and *T. sericea*. Effects of native *P. guajava* on nutrient concentrations were less pronounced, however. It was secondly hypothesised that native leaf types will decompose faster than invasive. However, invasive *L. camara* and native *F. sycomorus* decomposed faster than invasive *P. guajava* and native *T. sericea*, although *L. camara* generally decomposed faster compared to all leaf litter types. Hence, this hypothesis is also rejected, with interspecific differences between grouped invasive and native species emergent. These differences are likely due to variations in leaf structure which facilitate more rapid decomposition, with this decomposition rate correlating tightly with nutrient release dynamics.

The outcomes of this study suggest that certain invasive litter inputs into aquatic ecosystems may contribute to dynamic interactions among invasion, microbial, invertebrate and microorganism colonisation, and nutrient cycling. Nutrient–rich leaf litter such as *L. camara* decomposes faster due to low concentrations of defensive compounds such as lignin (Reich et al., 1997). Accordingly, organic inputs into ecosystems such as small streams by specific invasive leaf litter types influence nutrient status, potentially contributing to eutrophication and negatively affecting aquatic ecosystems through, for example, sudden increases in primary productivity. Similarly, Ong et al. (2017) highlighted that litter leaching process of decomposition plays a vital role in nutrient release into the environment. Leaf litter decomposition had a significant effect on nutrient release throughout the experiment, however the timings of release between species varied over time. As such, experimental duration may also substantially alter observations of nutrient release.

Considering decomposition alone, *L. camara* and *F. sycomorus* leaves had the most significant weight losses compared to *T. sericea* and *P. guajava*. The pattern of leaf litter decomposition generally follows two phases i.e. leaching and microbial conditioning (Petersen and Cummins, 1974; Webster and Benfield, 1986; Allan and Castillo, 2007). The initial stage could have resulted in a rapid loss of mass during the first 24–48 hours of decomposition possibly due to leaching of the soluble compounds from the leaf, such as phenolics, carbohydrates and amino acids. In the present study, the decay of leaf litter was first observed during the first 24–48 hours of the experiment, with broken leaves accumulating and changing the colour of the water (T. Mutshekwa, *personal observation*). Nevertheless, the context-dependency of such nutrient release between species requires further elucidation, considering the influence of other driving abiotic factors such as temperature.

In conclusion, it was found that nutrient inputs proved to be a good decomposition indicator of nutrient input in our study, giving that high nutrient levels were often measured in *L. camara* and *F. sycomorus* and these species also decomposed faster than *P. guajava* and *T. sericea*. Further, the former two species drove the most marked changes to pH and conductivity, synonymous with greater decomposition rates. Whilst nutrient level inputs from one invasive plant was the highest of all four species investigated, this effect was not generalisable owing to differences at the species level, with little difference between select native ad invasive species. Further research is needed to investigate whether the decomposition of other native and invasive plant leaf litter not assessed in this study exhibit generalities in terms of decomposition and nutrient dynamics. Moreover, the effects of physical leaf characteristics on decomposition and nutrient dynamics should be investigated to further understandings of the nutrient release process.

Chapter 4: Leaf Inputs From Invasive and Native Plants Drive Differential Mosquito Abundances

4.1 Introduction

The ecosystem-level impacts of invasive alien species can be multifaceted, with introductions often driving unexpected consequences in novel ranges. For example, invasive alien species can alter recipient ecosystems through greater provisioning of habitat, increased resource availability and direct interference with consumers (e.g. Green et al., 2011; Kobak et al., 2016; Sheppard et al., 2018). As plants are often the most important energy sources in terrestrial ecosystems, invasive alien plants can have especially marked ecological consequences (Sax et al., 2005), and these effects can span multiple habitat types. In particular, aquatic ecosystems may be heavily impacted by changes to terrestrial vegetation composition. Indeed, it is known that the species-level identity of plant parts being input into waterbodies can significantly alter invertebrate community dynamics in aquatic habitats, where mosquitoes are often numerically dominant and benefit directly from leaf litter as a resource (Leonard and Juliano, 1995; Yanoviak, 1999; Reiskind et al., 2009). Furthermore, invasive terrestrial plants can provide increased shelter or breeding sites for adult mosquitoes, with potential benefits for population-level fitness and the transmission of mosquito-borne disease (Rajnikant et al., 1992; Webb et al., 2012; Stone et al., 2018). Mosquitoes are known to be highly responsive to plant cues when ovipositing (Shaalan and Canyon, 2018), and an increasing body of evidence suggests that some invasive alien plant species may enhance mosquito demographics and exacerbate disease risk (Reiskind et al., 2010; Mack and Smith, 2011). However, understanding differential colonisation responses of mosquitoes among plant species has remained elusive.

The aim of the present study was to examine the effects of leaf litter inputs from two invasive and two native terrestrial plant species on larval mosquito abundances (*Culex* spp. and *Aedes* spp. combined): the invasive tickberry *Lantana camara*, invasive guava *Psidium guajava*, native sycamore fig *Ficus sycomorus* and native silver cluster-leaf *Terminalia sericea*. The *L. camara* species complex is native to tropical central and southern America, and is known to impact ecosystems through excessive resource additions, alterations to fire regimes and increased erosion (Richardson and van Wilgen, 2004; Vardien et al., 2012). *Psidium guajava* is also native to central and southern America and has substantial negative impacts on native species through competition and replacement (Urquía et al., 2019). Both species are recognised as invasive in South Africa and known to colonise close to waterbodies, whilst the two native plants serve as representative native plants.

4.2 Materials and methods

Plant leaves of invasive i.e. *L. camara* and *P. guajava* and native i.e. *F. sycomorus*, and *T. sericea* and were collected in November 2018 by hand from trees along Muvudi River riparian zone (22° 58.967'S 30° 26.840'E) in Thohoyandou, Limpopo province, South Africa, before being air dried at room temperature. Only fresh plant leaves were selected, given many plants reabsorb nutrients prior to senescence (e.g. Staaf, 1982). After drying, approximately 3 g of the dried material for each species was weighed and added into 5 L white polyethylene buckets, filled with 3.9 L of filtered (filter size 63 µm) borehole + river water (70:30 ratio). The initial mean water temperature was 27.2 ± 0.2 °C (SD), conductivity was 169.3 ± 4.0 µS cm^{-1}, total dissolved solids were 85.5 ± 2.6 mg L^{-1} and pH was 6.6 ± 0.1. Weekly, the buckets were topped up to 3.9 L with filtered borehole water. The twenty-five 5 L buckets (i.e. 5 replicates × 4 species, + 5 controls) were placed outside in a partially shaded area in the University of Venda campus in a randomised array, and mosquitoes could freely colonise any bucket. Mosquito larvae counting was initiated after three weeks to allow for colonisation, with first instar stages omitted from counts due to low visual detectability. During each sampling event, container contents were strained, and larval mosquitoes counted before being returned to their respective containers. Container-breeding mosquito larvae (*Culex* spp. and *Aedes* spp.) were subsequently enumerated weekly over 4 weeks.

Generalised linear mixed models (GLMMs) assuming a Poisson error distribution and log link were used to analyse larval mosquito counts over the experimental period (Brooks et al., 2017) according to the *treatment* and *time* terms, and their interaction. Bucket *ID* was included as a random effect to account for repeated measures of each bucket over time, with *time* included as a within-subject variable. Resulting models were tested for zero inflation and overdispersion *via* simulation comparisons (Hartig, 2017). Analysis of deviance with Type II sums of squares was used to infer significance levels of main effects (Fox and Weisberg, 2011), with estimated marginal means used *post-hoc* for Tukey comparisons (Lenth, 2018). In all analyses, significance was inferred at $P < 0.05$. All statistical analyses were performed using the R environment (v3.5.1; R Development Core Team, 2018).

4.3 Results

No larval mosquitoes were recovered from control groups, and thus this treatment level was removed from further analyses. Contrastingly, mosquitoes colonised all containers treated with leaf litter, however abundances in native *T. sericea* were considerably reduced overall (Fig. 1). The interaction term between treatment and week was not statistically clear (likelihood ratio test: $\chi^2 = 6.66$, df = 9, $P = 0.67$), and was thus removed from the final model. Accordingly, differences among leaf treatments in larval abundances were consistent across the monitoring period. Leaf treatments significantly affected larval mosquito abundances (GLMM: $\chi^2 = 61.66$, df = 3, $P < 0.001$). Significantly fewer mosquitoes were present in containers treated with native *T. sericea* as compared to invasive tickberry *L. camara*, invasive guava *P. guajava* and native *F. sycomorus* (all $P < 0.001$); there were no significant differences among the latter three plant species (all $P > 0.05$). Larval mosquito abundances also differed significantly over time overall (GLMM: $\chi^2 = 20.23$, df = 3, $P < 0.001$), with abundances falling significantly between the first and fourth week of the monitoring period ($P < 0.001$) (Fig. 2).

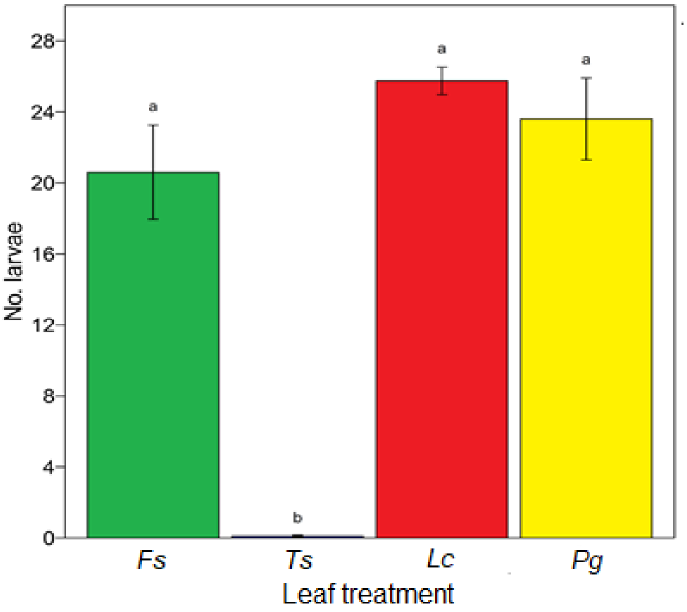

Fig. 4.1. Mean (± SE) larval mosquito abundances in 3.9 L containers under four plant leaf treatments (*Fs*, native *Ficus sycomorus* (green); *Ts*, native *Terminalia sericea* (blue); *Lc*, invasive *Lantana camara* (red);

Pg, invasive *Psidium guajava* (yellow)) over four week monitoring period. Letters denote significant differences.

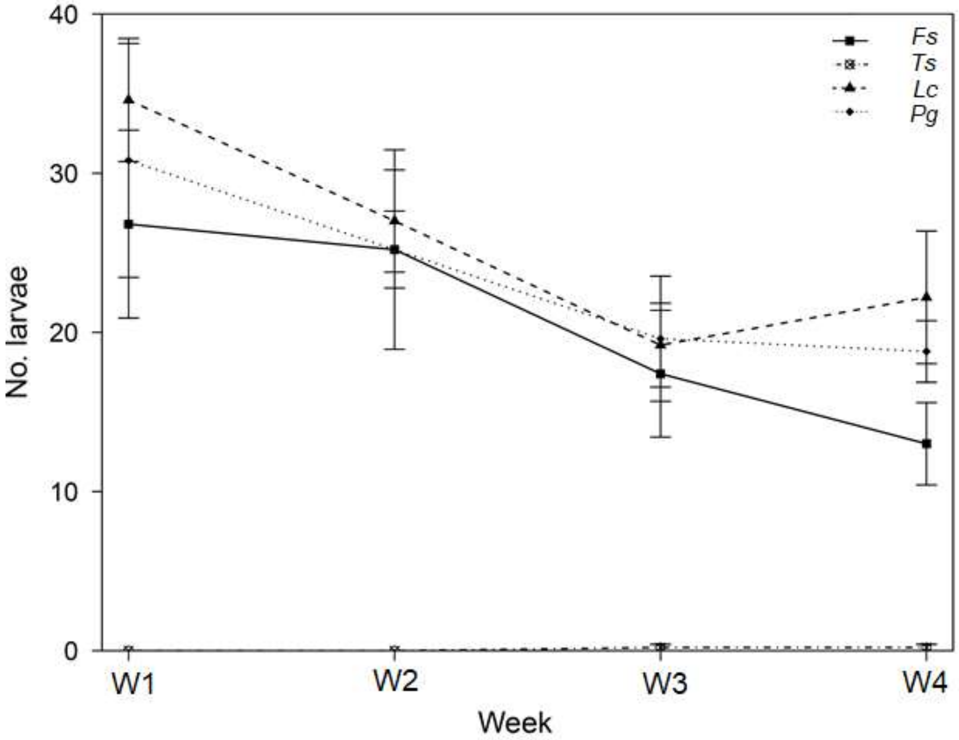

Fig. 4.2. Mean (± SE) weekly larval mosquito abundances in 3.9 L containers under four plant leaf treatments (*Fs*, native *Ficus sycomorus*; *Ts*, native *Terminalia sericea*; *Lc*, invasive *Lantana camara*; *Pg*, invasive *Psidium guajava*) over four week monitoring period. Locally-weighted scatterplot smoothing lines are presented (9/10 smoother span) to illustrate trends.

4.4 Discussion

The establishment and spread of invasive alien species can often have unexpected ecosystem-level impacts across multiple habitat types. In the present study, I demonstrate that leaf litter inputs among invasive and native terrestrial plants can differ markedly in their effects on larval mosquito abundances. Specifically, two highly invasive terrestrial plant species, *L. camara* and

P. guajava, and the native *F. sycomorus*, drove significantly elevated mosquito abundances compared to native *T. sericea*. Mosquitoes were entirely absent from leaf-free controls, owing to a lack of organic content. Accordingly, alterations to plant stands following invasions could worsen mosquito problems by driving increases in mosquito abundances, with possible implications for public health. Yet, this effect is likely species-specific Furthermore, plant incursions into previously unvegetated areas in proximity to water bodies could further promote mosquito proliferation.

The complete lack of colonisation of leaf-free controls in the present study is not surprising, given the strong general attractant effect of organic material on gravid mosquitoes (Hazard et al., 1967). Indeed, given the capacity for mosquitoes to exploit small, container-style habitats which are often present in high densities across terrestrial landscapes, there is frequently a high degree of selectivity for 'rewarding' habitats among available patches. Whilst I observed negligible colonisation of native *T. sericea* treated waters, a much stronger attractant effect was found towards native *F. sycomorus*. Accordingly, botanical effects appear to be explicitly linked to plant identity, with effects of invasion potentially dependent on background vegetative compositions.

Nevertheless, although not assessed in the present study, natural compounds from plants can have lethal effects on larval mosquitoes (Shaalan and Canyon, 2018), and so it cannot be ruled out that these effects altered abundances in specific treatments, rather than *via* selectivity. Indeed, whilst previous research has demonstrated attraction to *P. guajava* fruits by adult mosquitoes (Müller et al., 2010), compounds from *P. guajava* and *L. camara* have demonstrated mosquitocidal properties through lipophilic extracts (Mendes et al., 2017; Hari and Mathew, 2018). Yet, the specific compounds that drive colonisation differences require further investigation. In particular, to our knowledge, no studies have examined effects of compounds from *T. sericea* on mosquito abundances. Given the importance of the identity of plant inputs for the productivity of mosquito populations in aquatic habitats (Reiskind et al., 2009, 2010), quantifications of differential responses among invasive and native plant species is of high importance in the context of public health. Further research should therefore examine the direct effects of these and other plant inputs on additional mosquito life history traits, such as size, sex and fecundity, as well as direct lethal effects. In turn, this may identify specific compounds that determine colonisation.

Chapter 5: Macroinvertebrate Colonisation Associated with Native and Invasive Leaf Litter Decomposition

5.1 Introduction

Litter decomposition is a key ecosystem process that greatly influences the formation of soil organic matter, the release of nutrients for plants and microorganisms, and carbon dioxide (CO_2) fluxes in forest ecosystems (Jeyanny et al., 2015). Litter decomposition rate is controlled by intrinsic factors, for instance chemical and physical properties of leaf litter, and by extrinsic abiotic and biotic factors (Chen et al., 2019). Biotic factors include heterotrophic microorganisms (e.g. fungi and bacteria) and macroorganisms (e.g. macroinvertebrates) that break down leaves, with these processes also strongly influenced by environmental factors such as temperature (Chen et al., 2019). As such, the leaf litter decomposition process is comprised of three phases: (i) leaching of components from the leaf litter, followed by (ii) conditioning by microorganisms and finally, (iii) fragmentation and consumption by macroorganisms (Webster and Benfield, 1986).

Allochthonous plant material inputs into lakes and reservoirs are subject to the same processes (Ulyshen and Wagner, 2013). When terrestrial plant litter enters the aquatic system, it starts to decompose and rapidly loses mass due to the leaching process, with up to 30% lost within the first 24 hrs of decomposition (Petersen and Cummins, 1974). Secondly, microorganisms, such as fungi and bacteria, then colonise and start to decompose and alter the physico-chemical characteristics of the litter (i.e. microbial conditioning). Invertebrates colonise leaf material in lakes and reservoirs after a minimum level of microbial colonisation has taken place (Oliveira et al., 2014), as this process makes the litter more palatable for detritivores macroinvertebrates. Macroinvertebrates then fragment the remaining litter over time into fine particulate organic matter.

Aquatic invertebrates feeding on leaf litter are dominated by shredders and their densities tend to be correlated with the spatial and temporal accumulation of organic matter in lakes and reservoirs (Graça, 2001). Accordingly, community composition of above-ground plants may exert marked influence on aquatic invertebrate communities (Graça, 2001). The invertebrates that feed on organic matter incorporate derived nutrients in secondary production, further contributing to temporal leaf fragmentation processes (Graça, 2001). However, not all nutrients are directly incorporated into secondary productivity of macroinvertebrates, with much content being made available for other biotic and abiotic processes (Wallace et al., 2015). Leaf litter which accumulates in lakes and reservoirs generally increases the nitrogen content in waterbodies (Abelho et al., 2005; Hasanuzzam and Hossain, 2014). Moreover, leaves start to undergo loss of mass at a rate proportional to invertebrate colonisation (Suberkropp and Chauvet, 1995). Further, high decomposition rates have, in several studies, been positively correlated with high species richness (Dangles and Malmqvist, 2004). As such, community dynamics of colonising macroinvertebrates are important for nutrient cycling processes. Similarly, macroinvertebrate communities in lakes and reservoirs rely on input of terrestrial litter as an energy source. However, terrestrial landscapes are changing, with the plant communities with riparian zones being no exception. The invasion process is regarded as one of the major drivers of plant community shifts, whereby native plants may be outcompeted and subsequently replaced by non-native species (Wallace, 2012). While these terrestrial processes and their implications have been explored extensively (Ehrenfeld et al., 2003), the implications of cross-ecosystem subsidies from invasive species in riparian zones for aquatic macroinvertebrate communities lack quantification.

The aim of present study was to assess macroinvertebrate colonisation and quantify leaf litter decomposition rates over time among two native (fig *Ficus sycomorus*, silver cluster–leaf *Terminalia sericea*) and two invasive (lantana/tickberry *Lantana camara*, guava *Psidium guajava*) terrestrial plants in a small farm reservoir. *Lantana camara* and *Psidium guajava* are native to tropical central and southern America and are known to have a substantial negative impact on native plant species through competition and replacement (Richardson and van Wilgen, 2004; Vardien et al., 2012; Urquía et al., 2019). Both species are recognised as invasive in South Africa (Henderson, 2007; Gaertner et al. 2016). These invasive species were selected because they have been reported to spread fast within riparian zones, in turn threatening the abundance and diversity of native plant species and community stability of aquatic ecosystems. Ramaswanu and Sukumar (2014) indicated that the highest *L. camara* abundances are found in proximity to aquatic ecosystems. Dominant native plant species were selected for comparison. *Ficus sycomorus* and *Terminalia sericea* are both native to southern Africa and both species are often naturally found in riparian zones and wetlands (Henderson, 2007; Pothasin et al., 2014; Sunil et al., 2016). It was hypothesised that: (i) native *F. sycomorus* and *T. sericea* will support a more abundant and diverse invertebrate community compared to invasive *L. camara* and *P. guajava*; and (ii) native plant leaf litter will decompose faster compared to that from invasive plants.

5.2 Materials and methods

5.2.1 Study area

The study was conducted within the Levubu River catchment (-23.091403 S, 30.313697 E) which falls under Vhembe district, Limpopo, situated in a sub-tropical fruit farming area in the Makhado Municipality, South Africa. The catchment contributes to the Limpopo system, which flows into Mozambique. The Luvuvhu River and a portion of its tributaries, i.e. Mutale and Mutshindudi Rivers, rise in the Soutpansberg Mountains. The Luvuvhu River flows to about 200 km between various ranges of landscape before joining the Limpopo River that is near Kruger National Park. The climatic conditions vary considerably within the Levubu River catchment. The mean annual temperature ranges from approximately 18 °C to 35 °C, with an average of about 25.5 °C (SA Weather Service, 2018). Maximum temperatures are experienced in December, January, and February, and minimum temperatures occur on average in July. Rainfall is seasonal and occurs mainly during the summer months (i.e. October to March). In the Luvuvhu River catchment, the mean annual rainfall and evaporation is 608 mm and 1678 mm, respectively (SA Weather Service, 2018). Regional climate is strongly influenced by landscape topography (Angliss et al., 2001). The present study was carried out in winter (22nd of May to 19th of July 2019). One small reservoir which experienced little human interference was selected along the Luvuvhu River catchment middle reaches.

5.2.2 Experimental design

Our experiment was conducted *in situ* through the use of the meshed bag technique at a time of low rainfall to mitigate risk of disturbance from flooding. Fresh plant leaves of invasive, i.e. *L. camara* and *P. guajava*, and native, i.e. *F. sycomorus*, and *T. sericea*, were collected in

November 2018 by hand from trees along Mvudi River riparian zone (22°58.967’S 30°26.840’E) in Thohoyandou, Limpopo province, South Africa, before being air dried at room temperature (range 27–30 °C). After drying, 3.00 ± 0.1 g of the dried material for each species was weighed and added into each of 5 mm (5000 µm) coarse-mesh bags, with 100 bags used in total (i.e. 5 mm mesh size, × 4 species, × 5 temporal sampling events, × 5 replicates each). The coarse-mesh bags were sealed and attached with zip ties. According to species, each bag was tagged with different plastic colours (~0.25 cm^2) for identification during retrieval. All bags were deployed on Day 0, with bag retrieval taking place 14, 28, 42 and 56. The bags were randomly attached to ropes, tied with weights and attached to an abandoned boat for anchorage. Bags were randomised and separated to avoid spatial confounds. To quantify leaching, 20 additional bags (i.e. 5 mm mesh size, × 4 species, and × 5 replicates each) were deployed in buckets with filtered (63 µm mesh) reservoir water in the laboratory on Day 0 and removed on Day 2, to examine decomposition within 48 hrs with macroinvertebrates.

5.2.3 Sampling

On each sampling event (days 14, 28, 42, 56) conductivity (µS cm^{-1}), total dissolved solids (mg L^{-1}), pH, temperature (°C), sodium chloride (ppm), oxidation reduction potential (mV) and resistivity (Ohms – Ω)) were measured using a portable handheld multi–parameter Cyberscan Series Waterproof Portable Meter (Eutech Instruments). Collections of deployed bags were made on June 5th (day 14), June 19th (day 28), July 5th (day 42) and July 19th (day 56) 2019 (i.e. *c*. 14-day increments). Twenty bags (i.e. × 4 species, and × 5 replicates each) were retrieved during each sampling day, with only 2 replicates each being recovered for day 56 (i.e. × 4 species, and × 3 replicates each were lost). Once removed from the water, the bags were immediately placed into polyethylene zip bags to prevent loss of macroinvertebrates and decomposed leaves. At the laboratory, the bags were emptied into trays with a small amount of distilled water, and the macroinvertebrates were enumerated as absolute and relative abundances (%) and each replicate was preserved in 50 mL containers with 70 % ethanol. Decomposed leaves were then rinsed with sterilised distilled water to remove sediments, oven-dried at 60 °C for 48 hrs and weighed.

5.2.4 Statistical analyses

The effects of leaf treatment and observation week, and their interaction, on the absolute abundances of each group of invertebrates were analysed using separate generalised linear models. A Poisson error distribution was used initially, with the resulting models checked for overdispersion and zero inflation *via* examination of fitted residuals against model simulations (Hartig, 2017). Where residuals were found to be overdispersed, a negative binomial error distribution was employed (Venables and Ripley, 2002). A simple zero inflation regression model was applied in instances of zero inflation (Zeileis and Hothorn, 2002). Binomial generalised linear mixed models were used to examine the effects of leaf treatment, macroinvertebrate group and observation week, and their interactions, on relative abundances. To account for repeated measures of invertebrates within each mesh bag, a replicate-level random effect was included (slope and intercept). The final sets of observations were excluded from analyses as 3 of the 5 replicates from the field could not be recovered, limiting the minimum number of replicates required for statistical investigation (see before). Type III analyses of deviance were used to infer effect sizes, whilst sequential likelihood ratio tests were used in the case of zero inflation models (Zeileis and Hothorn, 2002). *Post-hoc* pairwise comparisons were performed using Tukey tests *via* estimated marginal means (Lenth, 2018).

Richness within each experimental replicate over time was calculated using Menhinick's diversity index (D), which is the number of taxa divided by the square-rooted sample size ($D = s/\sqrt{N}$), where "s" corresponds to the number of species groups, and "N" the total number of organisms within a sample (Whittaker, 1977). Resulting indices were $\log_{10}$ transformed and analysed using linear models as a function of leaf treatment and observation week (as above), with residuals checked for normality and homoscedasticity *via* diagnostic plotting (Zuur et al., 2010). All statistical analyses were performed using R v3.4.2 (R Development Core Team, 2018).

Decomposition rates of each leaf type were estimated using the decomposition coefficient (k) resulting from the exponential decay model (Olson, 1963): $Y_t = Y_o . e^{-kt}$, where Y_o and Y_t are the final and initial weight of leaves (g), respectively, t is time in days (d), e is the natural logarithm and k is the decomposition rate coefficient. According to Petersen and Cummins (1974), based on the decomposition rate coefficient, leaves can be classified as "*fast*" ($k > 0.01$), "*medium*" ($k = 0.005–0.01$) and "*slow*" ($k < 0.005$). Differences in end leaf weights were analysed using linear models as a function of leaf *treatment* (see before).

5.3 Results

Table 1 summarises the mean values of environmental parameters within Levubu River farm reservoir for the study period. Conductivity values were high at day 14 (561.5 µS cm^{-1}), then decreased at day 28 (549.7 µS cm^{-1}) before increasing at day 42 (561.2µS cm^{-1}) and 56 (558.8 µS cm^{-1}). High total dissolved solids of 192.4 mg L^{-1} were measured at day 14 and tended to reduce over time, with low values of 176.6 mg L^{-1} at day 42. The pH was slightly alkaline ranging from 7.2 (day 56) to 7.9 (day 42) (Table 1). The water temperature and salinity varied slightly throughout the study period (Table 1). High oxidation reduction potential (ORP) of –36.2 mV was recorded at day 14 before decreasing to –50 mV at day 56.

Table 5.1. Descriptive environmental parameters measured within the Luvuvhu River farm reservoir

Parameters	**Units**	**Day 14**		**Day 28**		**Day 42**		**Day 56**	
		Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Conductivity	μS cm^{-1}	561.5	2.6	549.7	0.3	561.2	1.2	558.8	0.3
Total dissolved solids	mg L^{-1}	192.4	0.6	180.4	0	176.6	1.3	177.5	1.4
pH		7.4	0.1	7.7	0	7.9	0	7.2	0
Temperature	°C	16.8	0	16	0.1	15.2	0	16.1	0.1
Salinity	Ppm	279.8	4.9	265.4	2.1	269	1.4	269.3	0.7
Oxidation reduction potential	mV	-36.2	3.9	-43.6	0.3	-49.5	0.8	-50	0.1
Resistivity	Ω	2.7	0	2.8	0	2.8	0	2.8	0

Leaf treatment alone had a significant effect on absolute abundances of Oligochaeta and Ostracoda, but a statistically unclear effect on Chironomidae, Physidae and Coenagrionidae (Table 2). Further, excepting Coenagrionidae and Physidae, all species group abundances differed significantly over time (Fig. 1; Table 2). Chironomidae abundances fell significantly over time, whilst Oligochaeta abundances increased. Oligochaeta numbers were reduced in the presence of invasive *L. camara* and *P. guajava*, with significant reductions relative to native *T. sericea* (both $P < 0.01$), and by invasive *L. camara* compared to native *F. sycomorus* ($P < 0.01$). Ostracoda numbers also increased significantly over time, and were always significantly higher in the presence of invasive *P. guajava* (all $P < 0.05$). Whilst lacking statistical clarity, Physidae abundances were generally reduced under both invasive leaf litter treatments, whilst Coenagrionidae abundances were more similar.

Table 5.2. Model (generalised linear model, GLM; zero inflation regression model, ZI) results considering species groups as a function of plant treatment (Plant) and observation week (Week), and their interaction. Significant *P*-values are emboldened.

Species group	**Model (family)**	**Predictor**	**Df**	**χ^2-value**	***P*-value**
Chironomidae	GLM (negative binomial)	Treatment	3	2.06	0.56
		Week	1	31.30	**< 0.001**
		Treatment: Week	3	1.52	0.68
Oligochaeta	GLM (negative binomial)	Treatment	3	11.40	**0.01**
		Week	1	42.35	**< 0.001**
		Treatment: Week	3	4.31	0.23
Ostracoda	ZI (negative binomial)	Treatment	3	23.48	**< 0.001**
		Week	1	14.48	**< 0.001**
		Treatment: Week	3	7.31	0.06
Coenagrionidae	GLM (negative binomial)	Treatment	3	0.96	0.81
		Week	1	1.22	0.27
		Treatment: Week	3	1.14	0.77
Physidae	GLM (negative binomial)	Treatment	3	1.64	0.65
		Week	1	0.004	0.95
		Treatment: Week	3	0.68	0.88

Proportional abundances among macroinvertebrate groups differed significantly according to plant leaf treatment and observation week, owing to a significantly three-way interaction term (Table 3). Chironomid relative abundances tended to decrease over time (Fig.5.1) and were significantly greater under invasive *L. camara* treatments relative to native *T. sericea* ($P < 0.01$), whilst other plant pairs were similar (all $P > 0.05$). Conversely, Oligachaeta relative abundances were significantly greater following native *T. sericea* treatments compared to all other plant groups (all $P < 0.05$), which were more similar (all $P > 0.05$). Oligachaeta abundances generally became relatively greater over the monitoring period (Fig. 5.1). Ostracod relative abundances increased over time and were significantly increased following treatment with invasive *P. guajava* compared to all other plant litter types (all $P < 0.001$), which were, in turn, not significantly different (all $P > 0.05$) (Fig. 1). Relative abundance contributions from Coenogrionidae did not differ significantly among plant treatments (all $P > 0.05$) and were relatively unchanged temporally (Fig. 1). Invasive *P. guajava* exhibited significantly reduced relative abundances of Physidae compared to either native plants (both $P < 0.001$), with other plant input types more similar (all $P > 0.05$). This asymmetric response among macroinvertebrate groups is further reflected by a lack of statistically-clear difference in species group diversity among leaf treatments ($F_{3, 52} = 1.99$ $P = 0.13$), observation weeks ($F_{1, 52} = 0.36$, $P = 0.55$) or their interaction ($F_{3, 52} = 0.60$, $P = 0.62$).

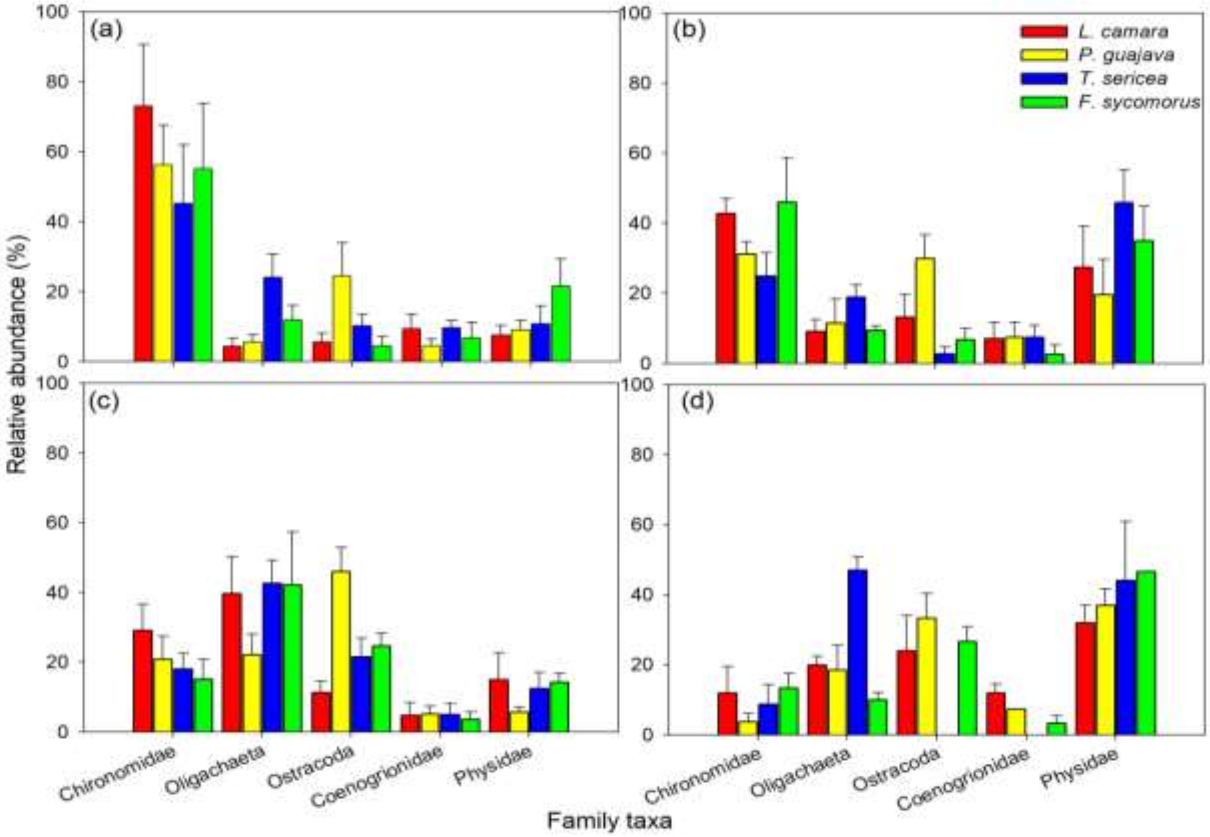

Fig. 5.1. Day 14 (a), 28 (b), 42 (c) and 56 (d) relative abundance (%) of macroinvertebrates colonisation (± SD) of invasive *L. camara* (red), invasive *P. guajava* (yellow), native *T. sericea* (blue) and native *F. sycomorus* (green)

Daily decay rates differed significantly among leaf species. Invasive *L. camara* had the highest decay rates (Fig. 5.2), which indicates faster decomposition ($k > 0.01$) in relation to other leaf types. Native *F. sycomorus* had slightly greater decays rates than native *T. sericea* and invasive *P. guajava*, with *L. camara* having the highest throughout sampling days. *T. sericea* and invasive *P. guajava* decomposed slower (both $k < 0.005$).

Table 5.3. Binomial generalised linear mixed model results considering relative macroinvertebrate abundances as a function of plant treatment (Plant), macroinvertebrate group (Group) and observation week (Week), and their two- and three-way interactions. Significant *P*-values are emboldened.

Predictor	**Df**	**χ^2-value**	***P*-value**
Plant	3	6.36	0.10
Group	4	331.39	**< 0.001**
Week	1	7.34	**< 0.001**
Plant: Group	12	65.36	**< 0.001**
Plant: Week	3	3.29	0.35
Group: Week	4	209.56	**< 0.001**
Plant: Group: Week	12	29.93	**0.003**

In terms of decomposition during the leaching process, leaf weights after 48 hrs differed significantly according to species ($F_{3, 12} = 12.72$, $P < 0.001$). Invasive *L. camara* and native *F. sycomorus* weights were reduced most, and significantly compared to native *T. sericea* and invasive *P. guajava* (all $P < 0.01$; Fig. 3).

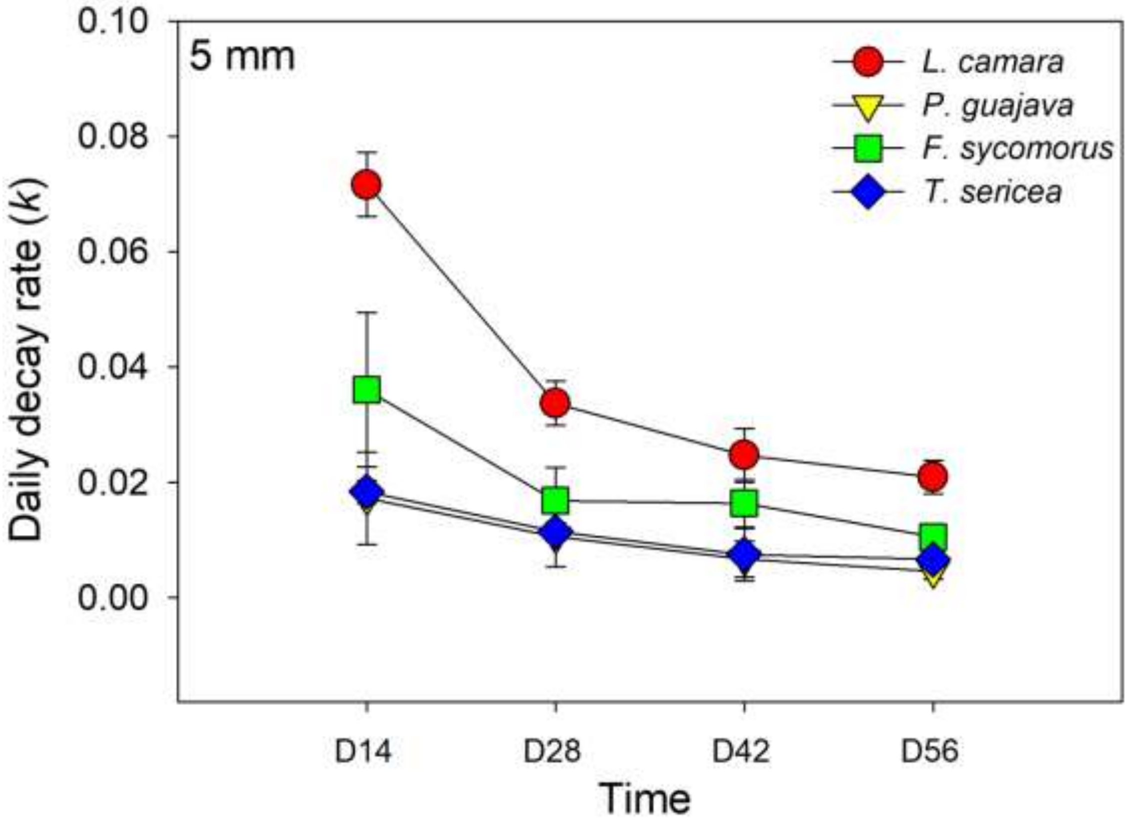

Fig. 5.2. Decomposition rates (*k*) (± SD) of invasive *L. camara* (red), invasive *P. guajava* (yellow), native *F. sycomorus* (green) and native *T. sericea* (blue).

5.4 Discussion

This study demonstrate that leaf litter decomposition can play an important role in determining the abundance of macroinvertebrates by assessing *in situ* reservoir macroinvertebrate colonisation on four leaf litter treatments. However, responses to leaf litter treatments differed depending on macroinvertebrate group, and generalities according to invasive and native treatment groups were not found. Similarly, decomposition rates did not differ dichotomously between collective invasive and native plants. Our results show that invasive *L. camara* and native *F. sycomorus* decomposed fastest, whilst invasive *P. guajava* and native *T. sericea* leached more slowly. In turn, these decomposition rates likely significantly alter nutrient release dynamics in aquatic environments, with implications for ecosystem structure and function.

Both hypotheses of this study were rejected, with abundance of macroinvertebrates and decomposition rates differing among leaf litter types over time at the species-level among invasive and native plants. It was first hypothesised that native *F. sycomorus* and *T. sericea* would support a more abundant invertebrate community compared to invasive *L. camara* and *P. guajava*, yet abundances tended to differ among macroinvertebrate groups according to specific leaf treatment over time. In turn, no significant effects among invasive and native plant inputs on overall diversity were found. Second, it was hypothesised that native leaf types would decompose faster than invasive. However, the hypothesis was not supported, with invasive *L. camara* and native *F. sycomorus* decomposing fastest, and invasive *P. guajava* and native *T. sericea* slowest.

Results from this study suggest that the rates of decomposition can contribute to macroinvertebrates colonisation. Differences in macroinvertebrate colonisation of experimental litter bags have been proposed as the mechanism underlying varying rates of leaf decomposition in response to litter quantity (Graça, 2001). A rapid decomposition in leaf litter using mesh bags can be caused by macroinvertebrates and other factors other such as temperature (Boulton and Boon 1991), however abiotic effects on decomposition were likely minor in our study given environment parameters did not differ considerably across sampling days or among treatments. Moreover, macroinvertebrates responses to leaf litter decomposition differed temporally in the present study, and effects manifested differently among taxonomic groups. Bärlocher (1982) indicated that leaf-eating macroinvertebrates ingest leaf areas rich in fungal cells or leaf matter with high nutritional quality. High feeding activity of a particular leaf type results in faster decomposition since feeding directly results in the breakdown of the litter (Barlocher, 1982). The rates of decomposition can be thus related to the quality of litter, which in turn relates to physico-chemical properties. Anecdotally, the highest rate of decomposition was found during the first sampling day or two of the study, followed by a gradual mass loss for the subsequent 54 days, which indicates two stages of decomposition: an initial stage and an advanced stage (Semwal et al., 2003). In the initial stage, a relatively large decrease in mass was likely observed due to the leaching of readily-soluble substances and nonlignified carbohydrates (Ibrahima et al., 2008). While in the advanced stage, the further decrease in mass loss may be attributed to the release of higher percentage of recalcitrant fractions like cellulose, lignin, and tannin of leaf litter (Nail et al., 2018). Hasanuzzam and Hossain (2014) highlighted that higher decomposition rates could be an indicator of higher litter quality.

The current study provides an understanding of decomposition and colonisation rates within a changing landscape. It is important to understand their colonisation provided that different macroinvertebrates tolerate different food sources and conditions of aquatic ecosystems and in turn, act as a food source for other organisms. Furthermore, given that the present study was not carried out in streams/rivers like previous studies (Reice, 1980; Leroy and Marks, 2006; Boreyo et al., 2011; Santonja at al., 2018), it offers insight into colonisation in reservoir systems, which may be differently affected by human activity. Such systems are increasingly common given that streams/rivers continue to be dammed, with numbers of off-stream dams increasing. South Africa, for example, currently has >50 000 small reservoirs and waterbodies that are not naturally connected to streams (e.g. irrigation ponds). Fornarelli and Antenucci (2011) indicated that small reservoirs encounter less disturbance from human activities compared to streams/rivers.

In conclusion, the abundance of macroinvertebrates and the rate of decomposition varied among leaf types over time. Decomposition rates of leaf litter appear not to be a good indicator of the abundance of macroinvertebrates which feed on the leaf since *L. camara* decomposed faster and generally had low abundances, while *F. sycomorus* also decomposed faster and had high abundances. Further, the leaf types assessed here support higher abundances of Chironomidae, Oligachaeta, Ostracoda and Physidae than Coenogrionidae, however the abundance of macroinvertebrates can be also determined by the size of the bag mesh. Whilst I did not find macroinvertebrate abundances to explicitly relate to invasive status, further research is also needed to investigate decomposition rates of other invasive and native plant species. Moreover, the effects of leaf structure on decomposition and colonisation of macroinvertebrates should be studied to expand the understanding of macroinvertebrate colonisation and the decomposition process.

Chapter 6: General Discussion

6.1 Summary and conclusions

The purpose of this study was to explore the effects of invasive and native leaf litter decomposition on nutrient (i.e. phosphate, nitrate, nitrite, silicate, ammonium) release rates and macroinvertebrates colonisation over time. The first study (chapter three) examined nutrient release dynamics every week for a period of 6 weeks and weight-ends after a period of 6 weeks associated with two native (fig *Ficus sycomorus*, silver cluster–leaf *Terminalia sericea*) and two invasive (lantana/tickberry *Lantana camara,* guava *Psidium guajava*) terrestrial plants in *ex situ* mesocosms. The intention was to evaluate the response of leaf litter decomposition of different plant types, and examine the rate at which nutrients are released from the leaves during the process of decomposition.

The results of this study indicated that the rate at which nutrients are released from the leaves vary with the type of the leaves. The influence of leaf decomposition on nutrient release dynamics varied between species. The degree at which nutrients were released and end-weights did no relate to the water parameters (i.e. conductivity (µS cm^{-1}), total dissolved solids (mg L^{-1}), pH and temperature (°C)). Hypotheses of this study were, therefore, rejected. The results showed that phosphorus and nitrate were highly leached in *L. camara* in comparison to other leaf types. However, the level of nitrate in other leaf type was not clear. The level of nitrate tended to be higher in all invasive leaf types compared to native types with invasive *L. camara* having the highest levels. The effects of leaf inputs on the release of silicate were also not clear throughout the monitoring period. In addition, leaf inputs affected the level of ammonium differently over time. However, invasive *L. camara* release the highest significant rate of ammonium than other leaf types. In terms of decomposition, invasive *L. camara* and native *F. sycomorus* decomposed faster than invasive *P. guajava* and native *T. sericea.*

The second study (Chapter 4) examined abundance of larval mosquitos using the same experimental design from the last study (Chapter 3). Water parameters (i.e. conductivity (µS cm^{-1}), total dissolved solids (mg L^{-1}), pH and temperature (°C)) were measured to evaluate the condition of the water. This study was conducted at the same time with the first study (Chapter three). However, the purpose of this study was to examine the effects of leaf litter inputs from two invasive and two native terrestrial plant species on larval mosquito abundances. *Culex* and *Aedes* spp. colonised the mesocosms, however leaf inputs have a significant effect on the colonisation dynamics, varying discretely across leaf litter species treatments. Larval mosquitoes were not encountered in control treatments, highlighting the importance of decaying vegetation as an attractant for oviposition by adult mosquitos. Differences in the abundance of larval mosquito were recorded among the leaf treatments throughout the monitoring period. The lowest abundance of larval mosquito was recorded in containers treated with *T. sericea* as compared to invasive tickberry *L. camara*, invasive guava *P. guajava* and native *F. sycomorus*. Presence of invasive *L. camara* and *P. guajava* therefore facilitated increased abundance of larva mosquito.

The last research section (i.e. Chapter 5) assessed the colonisation of macroinvertebrates and quantified decomposition rates over time (56 days) among the two native and two invasive plants in the field. The intention was to evaluate the respond of macroinvertebrates colonisation to different leaf types under the same aquatic environment over time and to determine if leaf litter of different plant types decomposes differently. This study differs to the first two studies

(Chapters 3 and 4) because this study was conducted in a small reservoir using mesh-bag technique i.e. 5 mm (5000 µm) coarse-mesh bags and end-weights. Leaching was also quantified during this study to understand the relative contributions of biological and non-biological leaf breakdown processes. The results of the study indicated that macroinvertebrates are present during all periods of decomposition. Furthermore, the abundance of macroinvertebrates varied among leaf types. The abundance of Macroinvertebrates i.e. Chironomid, Oligochaeta, Ostracod, Coenogrionidae and Physidae recorded differed according to leaf types (Fig. 5.1). Invasive *L. camara* and native *F. sycomorus* decomposed fastest, whilst invasive *P. guajava* and native *T. sericea* leached more slowly.

6.2 Recommendation for further research

- This study examined leaf litter decomposition from two invasive and two native leaf types for a period of 56 days. Many studies have been conducted for longer and shorter periods. Further research into a temporal pattern of leaf litter decomposition using mesh-bags technique may useful to determine the appropriate length of time using mesh-bags to measure leaf litter decomposition.
- This study measured macroinvertebrates colonisation within two invasive and two native terrestrial plants with the use of 5 mm(500um) mesh-bags size. Many studies use mesh-bags smaller than 5 mm(500um) to study macroinvertebrates associated with leaf litter decomposition. Further research into macroinvertebrates associated with leaf litter using larger mesh-bags may be useful to assess large macroinvertebrates which are unable to penetrate through 5mm mesh-bags.

- Microbial contributions need to be explored within context of native and invasive species to enhance the understanding of the relationship between microbes and plant species and the role microbial play role in terms of decomposition, nutrient release, plant growth and development.
- Results of this study show that *T. sericea* support low abundance of larval mosquitos. Further research into potential larvicidal effects of key native plant species are worth exploring (*T. sericea*).
- This study needs to be repeated using more native and invasive species to determine if unifying themes emerge to aid in predictive capacity for nutrient cycling dynamics in changing landscapes.

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