“Tree health in near pristine, heavily invaded and restored riparian zones: the role of pests and fungal pathogens”.

by

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DECLARATION

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SUMMARY

Riparian zones represent an essential component of landscape biodiversity and are ecologically and socio-economically significant habitats. The riparian zones in South Africa are however threatened by numerous invasive alien plants (IAPs). These are dominated by several fast-growing Australian *Acacias* that are responsible for widespread replacement of native species. The impacts of IAPs on native ecosystems are widely acknowledged and have justified extensive eradication programmes such as the Working for Water (WfW) programme. To date, the WfW programme has only been partially successful in the eradication of IAPs in riparian zones in the short-term. However, studies that assess the success of this initiative seldom focus on the health of riparian tree communities. This under-representation of riparian tree health studies is largely because of a lack of knowledge of the diversity and abundance of pests and disease-causing agents associated with native plants in these systems.

The aim of this study was to examine the role that folivorous insects and fungal pathogens play in tree health of riparian tree communities that differ in invasion treatments. The predominant two native riparian tree species *Brabejum stellatifolium* and *Metrosideros angustifolia* and one alien tree species *Acacia mearnsii* were selected. Three perennial river systems within the South-Western Cape Province were selected as study areas. Within study areas, nine sites of three invasion treatments were chosen: three near pristine; three heavily invaded (predominantly *Acacia mearnsii*) and three restored sites (seven years after clearing of *A. mearnsii*). The following were done to accomplish the main aim: (i) foliage-active arthropod communities were collected from selected tree species and compared in terms of alpha- and beta-diversity within and among sites with different invasion treatments, (ii) the levels of damage caused by leaf pathogens and folivorous insects on focal tree species were determined and compared among sites (iii) leaf damage patterns were studied in context of native plant diversity and host abundance, (iv) the influence of changes in predatory arthropod numbers and community composition, as well as changes in total leaf nitrogen and phosphorus levels on the numbers of free-feeding folivorous insects (sap-sucking and leaf-chewing), in relation to the level of invasion were investigated, and (v) physiological parameters and nutrients in *Brabejum stellatifolium* leaves with varying levels of fungal infection and folivore damage were investigated.

Heavily invaded sites had low arthropod species richness and abundance as compared to restored and near pristine sites, which were more similar. Beta-diversity was also influenced by invasion treatment. However few significant differences in arthropod community composition could be detected between restored and near pristine sites, and this pattern was similar for all tree species and arthropod taxonomic groups. Arthropod richness and community composition are therefore potential indicators of ecosystem health after restoration. Comparison of sites within each invasion treatment revealed that rivers have distinct signatures in terms of their arthropod communities.

A study of different damage levels caused by leaf pathogens and folivorous insects showed that on native trees, folivore and fungal pathogen damage was significantly higher at restored and heavily invaded sites than at near pristine sites. For *A. mearnsii,* damage caused by both folivores and pathogens increased at heavily invaded sites, but returned to near pristine site levels at restored sites. Differences in native plant diversity did not explain these patterns, as restored sites had similar diversity levels to the near pristine sites. Surprisingly, as native host abundance increased, respective damage caused by fungal pathogens and folivores decreased. For *A. mearnsii*, increased host abundance was significantly correlated to increased folivore and pathogen damage. Insects and diseases not only reduced leaf area but also affected balances of physiological processes, thereby possibly delaying their recovery in a restoration scenario. Photosynthetic rate and other physiological parameters generally decreased with increasing damage severity caused by weevils and fungal pathogens in the canopy leaves of mature trees.

Folivorous insect densities associated with tree species showed different responses to changes in predator densities and plant nutritional status. *Brabejum stellatifolium* and *Acacia mearnsii* had highest leaf nitrogen levels at restored and heavily invaded sites respectively, as compared to near pristine sites. Increased folivore abundance was only significantly and positively correlated to levels of leaf nitrogen content for *B. stellatifolium* and *A. mearnsii*. The results suggest that leaf nitrogen content is important for folivore food plant selection on *B. stellatifolium* and *A. mearnsii*, but not on *M. angustifolia*. There was no relationship between leaf damage and nitrogen levels or altered arthropod communities on *M. angustifolia* Therefore, individual plant species should be treated as separate entities when assessing plant health in a restoration scenario.

Combined, these results indicate that; (i) native plant species and arthropod communities recover after alien plant clearing. Recovered arthropod assemblages may indicate the recovery of crucial processes like pollination, decomposition, and seed dispersal thereby strengthening the significance of conservation of arthropods from maintenance of biodiversity and ecosystem function and structure, (ii) the responses of sites to plant invasion treatment are site-specific, and therefore important to consider rivers individually when conceptualising activities of restoration, (iii) the role of fungal pathogens and folivorous insects in riparian ecosystems is more important than previously understood and there is a need for more research to provide better insights on why certain fungal pathogens and folivorous insects become major pests and diseases once the IAPs are cleared, (iv) folivorous insect responses to plant nutritional quality and predator densities is plant species-specific. Hence, individual plant species should be treated as separate entities when assessing plant health in a restoration scenario, and (v) insect feeding on vegetative parts of the plant and leaf fungal pathogens infections are damaging to the host plants. This may pose a serious threat to riparian tree communities already stressed by invasive alien plants, thereby delaying their recovery in a restoration scenario. Results of this study have potential to serve as valuable tools in riparian ecosystem management in South Africa.

OPSOMMING

Oewergebiede verteenwoordig 'n noodsaaklike komponent van landskapbiodiversiteit en is vanuit 'n ekologiese en sosio-ekonomiese oogpunt belangrike habitats. Die oewergebiede van Suid-Afrika word egter deur verskeie indringer uitheemse plante (IUP). Hierdie word gedomineer deur verskeie vinnig groeiende Australiese akasias wat verantwoordelik is vir die wydverspreide vervanging van inheemse spesies. Die impak van IUP'e op inheemse ekosisteme word allerweë erken en het as regverdiging gedien vir uitgebreide uitroeiingsprogramme soos dié van Werk vir Water (*Working for Water (WfW)*). Tot op hede was die WfW-program slegs gedeeltelik suksesvol in die uitroei van IUP'e in oewergebiede in die kort termyn. Studies wat die sukses van hierdie inisiatief ondersoek, fokus egter selde op die gesondheid van oewerboom-gemeenskappe. Hierdie onderverteenwoordiging van oewerboom-gesondheid is grootliks as gevolg van 'n gebrek aan kennis oor die diversiteit en volopheid van plae en siekteveroorsakende agente wat met inheemse plante in hierdie stelsels geassosieer word.

Die doel van hierdie studie was om die rol van blaarvretende insekte en swampatogene in die boomgesondheid van oewerboom-gemeenskappe wat op grond van die behandeling van indringers verskil, te ondersoek. Die vernaamste twee inheemse boomspesies op oewers is *Brabejum stellatifolium* en *Metrosideros angustifolia*, en een uitheemse boomspesie, *Acacia mearnsii*, is gekies. Drie standhoudende rivierstelsels in die Suidwestelike Kaap Provinsie is as studiegebiede gekies. Binne die studiegebiede is nege liggings van drie verskillende behandelings van indringers gekies: drie feitlik ongerep; drie wat swaar ingedring is (hoofsaaklik *Acacia mearnsii*) en drie gerestoureerde liggings (sewe jaar na die verwydering van *A. mearnsii*). Die volgende is onderneem om die doel te bereik: (i) blaar-aktiewe geleedpotige gemeenskappe is uit geselekteerde boomspesies versamel en vergelyk in terme van alfa- en beta-diversiteit binne en tussen liggings met verskillende behandelings van indringers, (ii) die vlakke van skade wat deur blaarpatogene en blaarvretende insekte op die boomspesies waarop gefokus is, veroorsaak is, is bepaal en tussen liggings vergelyk, (iii) die patrone van blaarskade is in die konteks van inheemse plantdiversiteit en gasheervolopheid ondersoek, (iv) die invloed van veranderinge in die getal roofgeleedpotiges en gemeenskapsamestelling, asook veranderinge in die totale vlakke van stikstof en fosfor in die blare, op die getal vryvoedende blaarvretende insekte (die wat sap suig en wat blare kou) is ondersoek in verhouding tot die vlak van indringing, en (v) fisiologiese parameters en voedingstowwe in *Brabejum stellatifolium* blare met verskillende vlakke van swambesmetting en blaarskade is ondersoek.

Swaar ingedringde liggings het lae rykheid en volopheid van geleedpotige spesies gehad in vergelyking met gerestoureerde en feitlik ongerepte liggings, wat meer eenders was. Beta-diversiteit is ook deur die behandeling van indringers beïnvloed. Minder beduidende verskille in geleedpotige gemeenskapsamestelling kon egter tussen die gerestoureerde en feitlik ongerepte liggings waargeneem word, en hierdie patroon was eenders vir al die boomspesies en taksonomiese groepe van geleedpotiges. Die rykheid en gemeenskapsamestelling van geleedpotiges is dus potensiële aanwysers van ekosisteemgesondheid ná restourasie. Die vergelyking van liggings binne elke verskillende behandeling van indringers het getoon dat riviere duidelike 'handtekeninge' het in terme van hulle geleedpotige gemeenskappe.

'n Studie van verskillende vlakke van skade wat deur blaarpatogene en blaarvretende insekte veroorsaak is, het getoon dat blaar- en patogeenskade op inheemse bome beduidend hoër was op die gerestoureerde en swaar ingedringde liggings as op die feitlike ongerepte liggings. Vir *A. mearnsii* het die skade wat deur beide blaarvreters en patogene veroorsaak is, noemenswaardig op die hoogs ingedringde liggings vermeerder, maar dit het omgekeer na feitlik ongerepte vlakke op die gerestoureerde liggings. Verskille in inheemse plantdiversiteit het nie hierdie patrone verklaar nie, aangesien gerestoureerde liggings soortgelyke diversiteitsvlakke gehad het as die feitlik ongerepte liggings. Wat verbasend was, was dat soos inheemse gasheer volopheid toegeneem het, die skade wat deur swampatogene en blaarvreters veroorsaak het, onderskeidelik verminder het. Vir *A. mearnsii* was 'n toename in gasheervolopheid beduidend gekorreleer met 'n toename in blaar- en patogeenskade. Insekte en siektes het nie net blaaroppervlak verminder nie, maar het ook die balanse van fisiologiese prosesse beïnvloed en het daardeur moontlik hul herstel in 'n restourasie-scenario vertraag. Die tempo van fotosintese en ander fisiologiese parameters het oor die algemeen verminder met 'n verhoging in die erns van die skade wat deur kalanders en swampatogene in die blaardak van volwasse bome veroorsaak is.

Die digtheid van blaarvretende insekte wat met boomspesies verband hou, het verskillende reaksies op veranderinge in roofdierdigthede en plant voedingstatus getoon. *Brabejum stellatifolium* en *Acacia mearnsii* het die hoogste blaarstikstofvlakke gehad in die gerestoureerde en swaar ingedringde liggings onderskeidelik, in vergelyking met die liggings wat feitlik ongerep was. Verhoogde blaarvretervolopheid was slegs vir *B. stellatifolium* en *A. mearnsii* beduidend en positief gekorreleer met vlakke van stikstofinhoud in die blare. Die resultate stel voor dat blaar stikstofinhoud belangrik is vir blaarvretende voedselplantseleksie op *B. stellatifolium* en *A. mearnsii*, maar nie op *M. angustifolia* nie. Daar was geen verhouding tussen blaarskade en stikstofvlakke of veranderde geleedpotige gemeenskappe op

M. angustifolia nie. Individuele plantspesies moet dus as aparte entiteite beskou word wanneer plantgesondheid in ’n restourasie-scenario geassesseer word.

Saam toon hierdie resultate dat: (i) inheemse plantspesies en geleedpotige gemeenskappe herstel ná uitheemse plante opgeruim is. Groepe herstelde geleedpotiges dui moontlik op die herstel van belangrike prosesse soos bestuiwing, ontbinding, en saadverspreiding, wat dus dui op hoe belangrik die bewaring van geleedpotiges is vir die behoud van biodiversiteit en ekosisteemfunksie en -struktuur, (ii) die reaksies van liggings op die behandeling van plant indringers is liggingspesifiek en dit is dus belangrik om riviere individueel te oorweeg wanneer restourasie-aktiwiteite konseptualiseer word, (iii) die rol van swampatogene en blaarvretende insekte in oewer-ekosisteme is belangriker as voorheen begryp en dit is nodig dat meer navorsing onderneem word om beter insigte te verkry in hoekom sekere swampatogene en blaarvretende insekte vername plae en siektes word wanneer IUP’e opgeruim word, (iv) blaarvretende insekresponse op die voedingskwaliteit van plante en roofdierdigtheid is plantspesie spesifiek. Dus moet individuele plantspesies as afsonderlike entiteite behandel word wanneer plantgesondheid in ’n restourasie-scenario geassesseer word, en (v) insekvoeding op vegetatiewe plantdele en blaar swampatogeniese infeksies beskadig die gasheerplante. Dit kan ’n ernstige bedreiging inhou vir oewerboomgemeenskappe wat reeds onder stres is as gevolg van indringer uitheemse plante en wat dus hulle herstel in ’n restourasie-scenario kan vertraag. Die resultate van hierdie studie het die potensiaal om as belangrike gereedskap in oewer-ekosisteembestuur in Suid-Afrika te dien.

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LIST OF ABBREVIATIONS

ANOVA	Analysis Of Variance
A_{max}	Photosynthetic rate
CFR	Cape Floristic Region
CO_2	Carbon dioxide
DWAF	Department of Water Affairs and Forestry
DW	Dried Weight
E	Transpiration rate
EC	European Commission
FW	Fresh Weight
GLMs	Generalised Linear Models
g_s	Stomatal conductance
HI	Heavily Invaded
IAP	Invasive Alien Plant
LAD	Leaf Area Damaged
N	Nitrogen
nMDS	non-metric Multi-Dimensional Scaling
P	Phosphorus
NP	Near Pristine
NPQ	Non-Photochemical Quenching
PERMANOVA	Permutational Multivariate Analysis of Variance
PERMDISP	Permutational test for Multivariate Dispersion
R	Restored
SA	South Africa
SE	Standard Error
Tleaf	Leaf Temperature
WfW	Working for Water

CHAPTER 1

General Introduction

This chapter introduces the background and key concepts of this dissertation. It first defines riparian zones, and presents a review of literature on functions of riparian zones and disturbances associated with them. It summarises the existing knowledge and highlights the gaps concerning the impacts of invasive alien plants (in particular *Acacia mearnsii*) on riparian structure and function, arthropod numbers and fungal pathogens. It also describes methods of controlling Invasive Alien Plants (IAPs) and the impacts of their removal on ecosystems. The impacts of fungal pathogens and folivores on host trees are also discussed here. Finally this chapter presents information on the chosen tree species and study sites that form the focus of this research.

1.1. Study Motivation

Riparian zones in South African Fynbos play many essential roles in the ecosystem structure and functioning of the Fynbos biome (Richardson et al., 2007). However, the vegetation of riparian zones in the Western Cape Province, where Fynbos is predominant, has largely been degraded due to the dramatic invasion of alien invasive plants, in particular *Acacia mearnsii* (Richardson et al., 1992; Dye and Jarmain, 2004; Reinecke et al., 2008). The identification of the various riparian areas where the impact was severe led to initiation of IAPs systematic removal by the national Working for Water (WfW) programme. Unfortunately, the process of removing alien trees disturbs river systems and may also alter riparian habitats (Samways et al., 2011). The WfW programme has only been partially successful in eradication of IAPs (Beater et al., 2008; De Lange and van Wilgen, 2010; van Wilgen et al., 2012) and studies that assess the success of this initiative seldom focus specifically on tree health. This paucity of studies on riparian tree health is largely due to the limited knowledge regarding the identity, diversity and abundance of pests such as insects and disease-causing agents, like fungi, that negatively impact on trees. This dissertation provides insights into how different management practices of riparian habitats may impact tree health.

1.1.1. Defining Tree health

Tree health can be interpreted at different scales. For example, tree health can be studied at the scale of a forest or an individual tree. In general, tree health is defined as the study of all factors (biotic and abiotic) that affect the well-being, physiological and biochemical processes of an individual tree. These factors can express themselves through different symptoms and damage types (Boa, 2003). At the scale of a forest, tree health refers to the health of an entire forest system as influenced by many interacting biotic and abiotic factors (Hanula et al., 2002; Castello and Teale, 2011). Biotic factors include living organisms such as insects, fungal pathogens, and wildlife, while abiotic factors include non-living drivers such as climatic variables (e.g. wind, water, temperature) or soil type or texture (Boa, 2003). Biotic factors are an integral part of an ecosystem; however, they can have undesirable ecological effects such as loss or reduction of important ecosystem functions and may lead to considerable economic losses (Lee et al., 2011). For example, root-rot fungi, (e.g. *Phytophthora cinnamomi* and *Armillaria luteobubalina*) can severely hamper tree health at forest level by causing tree mortality (Shearer and Smith, 2000), while insects like weevils are capable of killing they seedling the feed on (Hanula et al., 2002).

Tree health problems may be categorized into: pathogen induced diseases, insect folivores and other damage-causing animals, abiotic linked disorders and/ or other miscellaneous problems with unknown origins (e.g. declines and diebacks). Importantly, these tree health problems can usually be described qualitatively and such qualitative descriptions can be used to compare the severity of the symptoms, the damage done or crown condition between different individuals.

1.1.2. Defining riparian zones

Defining and explaining the spatial extent of riparian zones is challenging, as the boundaries differ depending on the nature of the study (Tickner et al., 2001; Blanchard, 2007). The most commonly used definition is that of Naiman and Décamps, (1997), that define riparian zones as the boundary between land (land that generally consist of trees, shrubs and grasses) and a river or stream. Gregory et al., (1991) defined riparian areas as "three dimensional transitional zones of direct interaction between terrestrial and aquatic ecosystems, extending from the edge of water bodies to the edge of upland systems”.

The size of the riparian zone ranges from narrow strips to multifaceted systems along large rivers. A common attribute of the narrow strips is that their geomorphic features are virtually completely embedded into the riparian forest, while multifaceted systems are characterised by physically diverse floodplains (Gregory, 1991; Naiman and Décamps, 1997). Based on hydrology, geomorphology and the structure of the vegetation, riparian zones can be effectively distinguished from terrestrial ecosystems (Arizpe et al., 2008). Patterns in vegetation distribution in stream floodplains can be inconsistent as a result of heterogeneity introduced by sedimentary processes (van Coller et al., 2000). Although riparian zones occupy a small part of the terrestrial area of a catchment, their relative impact on the surrounding landscape is high (Gregory et al., 1991). They offer various ecosystem services such as serving as land water interface mediators and integrators, acting as sites for water storage, and cycling of nutrients and organic matter (Arizpe et al., 2008). Therefore, riparian zones are crucial ecosystems within catchments.

1.1.3. Functions of riparian zones

Riparian zones comprise a complex mixture of biological communities, and environments. These zones are biodiversity hotspots of local and global importance (Naiman et al., 2005 ; Richardson and Danehy, 2007). They provide and maintain ecosystem health and ecosystem services (Ewel et al., 2001) for example by including nutrient cycling, maintaining water quality and quantity, and stream bank stabilisation (Corbacho et al., 2003; Naiman et al., 2005). Riparian zones play an essential part in the removal and holding of inorganic particles, and organic matter. Soil surface roughness is increased by riparian vegetation, which, in turn, decreases the velocity of flowing water and therefore promote sedimentation (Bardgett et al., 2001; Blanchard, 2007). These therefore have a significant buffer capacity for contaminants carried from upstream or from the adjoining terrestrial landscapes (Arizpe et al., 2008). They also regulate the conduction of solar energy into the aquatic ecosystem (Pusey and Arthington, 2003) and provide corridors for the movement of biota (Naiman and Décamps, 1997; Corbacho et al., 2003; Esler et al., 2008). Riparian zones offer various, plentiful food resources for both aquatic and terrestrial communities (Naiman and Décamps, 1997). Along with their ecological and economic significance, riparian zones are particularly sensitive to disturbances and therefore act as indicators of environmental change (Naiman and Décamps, 1997; Tickner et al., 2001).

1.2. Disturbances of riparian zones

Disturbance is a vital, often essential, component of many natural ecosystems, and plays an important role in plant evolution and community dynamics (Le Maitre and Midgley, 1992; Moreno et al., 2011). Riparian systems are especially susceptible to frequent and extreme natural disturbances, specifically flood surges and fire (Stromberg et al., 2009). These disturbances strongly influence riparian systems. They cause changes in soil moisture, light and temperature regimes of the ecosystem, geomorphology, soil micro-organisms composition, soil chemistry and fire regime, which impact the structure, capacity and dynamics of these systems

(Naiman and Décamps, 1997; van Coller et al., 2000; Naiman et al., 2005; Reinecke et al., 2007; Richardson et al., 2007; Blanchard, 2007; Slabbert et al., 2014).

Invasive alien species are considered major disturbances to riparian zones worldwide (Richardson et al., 2007; Esler et al., 2008; Holmes et al., 2008) with South African river systems amongst the most well-studied (e.g. Galatowitsch and Richardson, 2005; Richardson et al., 2007; Blanchard and Holmes, 2008; Reinecke et al., 2008). Many are heavily invaded by woody plants, particularly Australian Acacias. The most invasive of these is *Acacia mearnsii* (DeWild) (commonly known as black wattle) (Richardson et al., 1992; Reinecke et al., 2008).

1.3. Biological invasion of riparian systems and with *Acacia mearnsii* in particular

Invasive alien plants threaten natural resources and the social and economic systems dependant on riparian zones (Rowntree, 1991; Galatowitsch and Richardson, 2005; Richardson et al., 2007; Blanchard and Holmes, 2008; Johnson et al., 2009). *Acacia mearnsii*, a member of the family Fabaceae, is a pervasive alien plant species and occurs in most modified river ecosystems of South Africa (Invasive Species Specialist group, 2013). This species is also considered to be the most invasive species in the mist belt of the eastern highlands of Zimbabwe (Nyoka, 2003). Hence, *A. mearnsii* has been the subject of many research projects and its impacts in natural riparian ecosystems have been widely documented (Le Maitre et al., 2011). This species is native to Australia and Tasmania and was introduced to South Africa in 1864 (Nyoka, 2003) for shade, windbreaks, fuel wood and later for the tannin content of its bark (Dye and Jarmain, 2004; Le Maitre et al., 2011). Based on spatial data analysis, researchers estimate more than 2.5 million hectares of *Acacia mearnsii* occur throughout South Africa, particularly along rivers (Galatowitsch and Richardson, 2005). The invasiveness of this species is mostly ascribed to its ability to produce large quantities of seeds (which lie dormant and germinate following bush fires) and the development of a large canopy that shades other vegetation (Costermans, 1994). The species has all three attributes of a successful invader: short juvenile phase, good annual seed crop, and small seed size (Rejmanek, 1995).

1.4. Impacts of Invasive Alien Plants (with special focus on *A. mearnsii*)

Invasive alien plants (IAPs) are a global concern as they have detrimental effects on forestry, agriculture, water resources ecosystem services and human health (Mooney and Hobbs, 2000; Hood and Naiman, 2000; Richardson and van Wilgen, 2004; Richardson et al., 2007;

Pretorius et al., 2008; Gaertner et al., 2009; Hellmann et al., 2011). Their impacts on riparian zones have been reported to intensify over time (Holmes and Cowling, 1997). Some are well-known to negatively affect ecosystem function and structure with severe consequences for indigenous and endemic populations (including rare and endangered species) and community dynamics (Mack and D'Antonio, 1998; Ehrenfeld, 2004; Marchante et al., 2010; Le Maitre et al., 2011). Impacts of IAPs on biodiversity can be large; for example, they threaten 57% of endangered native plant species in the United States (Wilcove et al., 1998).

Globally, the impacts of invasive organisms on economies are also significant, in the region of tens of billions of US$ each year (Pimentel et al., 2005). The European Commission has spent over €132 million on eradication and control of IAPs in the past 15 years (Scalera 2009). In South Africa, government expenditure between 1995 and 2000 on the control and eradication of invasive plants was over R100 million (van Wilgen, et al., 2001). Consequently, various attempts locally and globally have been made to address the effect of invasive species on ecosystem structure and function (Ehrenfeld, 2003; Levine et al., 2003; Gaertner et al., 2009; Hellmann et al., 2011), and on the interactions between plants and their associated organisms (Weisser and Siemann, 2004; White et al., 2006).

Acacia mearnsii, like other invasive plants, negatively impact on indigenous plants and animal biodiversity through the loss of suitable habitat and competition with native species (Rowntree, 1991; Richardson et al., 1992; Stock et al., 1995; Holmes and Cowling, 1997; Yelenik et al., 2004; Marachante et al., 2008; Reinecke et al., 2008; Le Maitre et al., 2011). Similar negative impacts have been reported for riparian invaders globally (e.g. *Arundo donax*; Rundel and Gustafson, 2005 and *Tamarax* spp.; Shafroth and Briggs, 2008). Table **1.1** summarises some key characteristics altered by IAPs (especially *A. mearnsii*) that increase the susceptibility of riparian zones to fungal and insect attack. Insufficient attention has been given to the impacts of IAPs and their management on tree health, particularly the role of pest insects and fungal pathogens. Pest insects and fungi are integral parts of ecosystems, but under conditions that lead to tree stress, they may produce undesirable effects.

Acacia mearnsii acts a transformer species, when it grows extensively along floodplains. It obstructs natural water flow thereby increasing the risk of flooding to adjacent lands (Le Maitre et al., 2000). As the aboveground biomass dries in the hot, dry summer months that characterise Mediterranean-type climates, *A. mearnsii* replaces moisture-rich riparian corridors that usually form natural fire barriers with an unnatural fire hazard (van Wilgen and Richardson, 1985). *Acacia mearnsii* infestation is also responsible for altered microclimates, soil chemistry, litter decomposition, and soil physical properties (e.g. Musil and Midgley, 1990; Witkowski, 1991; Stock et al., 1995; Yelenik et al., 2004, 2007; Cilliers et al., 2005; Jovanovic et al., 2009), changes in river geomorphology (e.g. Macdonald and Richardson, 1986; Rowntree, 1991) and diminished capacity for rivers to provide enough water from water loss due to high water consumption by *A. mearnsii* (Dye and Jarmain, 2004).

While invasive plants have detrimental effects on the environment, economy and ecosystems, the presence of these plants may also bring benefits (van Wilgen et al., 2001). For example, the South African government, through its control and prevention of spread of *A. mearnsii* (amongst other invasive species) has employed 32,935 people in labour-intensive clearing, follow up control and restoration projects aimed at bringing invasions of alien plants under control (DWAF, 2004).The direct benefit of *A. mearnsii* are through sales of charcoal, pulp and poles (DWAF, 2004).

Table **1.1**: Riparian ecosystem characteristics that may affect performance, survival and interactions of folivores and predators and their interactions with plants in riparian zones. The bolded characteristics are studied in this dissertation.

	Riparian zone type		
Characteristics	Near pristine	Heavily invaded (by *A. mearnsii*)	Restored
Plant population	• High diversity and abundance of native species. Mixed stands are well-known to be less susceptible to insect or fungal disease outbreaks, a phenomenon termed 'associational resistance' (Andow, 1991). • Diverse native plant communities may support higher quantities of predators as influenced by structural qualities of tree species (Alonso and Herrera, 1996). Increased plant structural diversity has been show crucial for web-building by predators (Halaj et al., 2000)	• High abundance of invasive alien trees, altering ecosystem properties (Vitousek, 1990). • Reduced native tree composition due to the loss of suitable habitat through competition with alien species (Richardson et al., 2000; Williamson and Fitter, 1996; Lockwood et al., 2005). • These losses of native tree species diversity and plant structural diversity may modify interactions, like mutualism, predation, folivory, or infectious disease (Chapin et al., 2000)	• Reduced alien plant populations, lower cover than native stands (Blanchard and Holmes, 2008). • Low diversity of native species that may be more prone to pathogen and insect attack (Chapin et al., 2000).
Host- spacing	• Closely-spaced hosts: Little hunt exertion is required for folivores to reach potential host, compared to sparse or host mixed with non-hosts where folivores are able to escape the attention of predators while searching for hosts (Schowalter and Lohman, 1999).	• Dense stand of IAPs: Native trees may be hidden from view, or their chemical cues might be veiled by the scent of IAPs, consequently, folivores invest more time searching, which is both energetically costly and increases exposure to predators (Schowalter and Lohman, 1999).	• Sparse hosts: influences the capacity of insects to see host signals, to reach a potential host within restricted time and energy reserves, (Kareiva, 1983; Schowalter, 1985). This also means folivores may suffer from greater exposure to predators (Schowalter and Lohman, 1999).

Microclimate	Diverse light, moisture and temperature regime. This can affect visual orientation of some insect species toward necessary resources (Stireman et al., 2005).	• More uniform shading: altered light penetration through canopy and temperature regimes. This can lead to increased abundance and virulence of pathogenic viruses, bacteria, fungi. (Mattson and Haack, 1987).	• Intermediate between invaded and native conditions (Chen et al., 1999). Open canopies are liable to greater penetration of light, precipitation and wind. These factors influence the development of folivore and predator populations through survival and performance (e.g., progeny production may increase with longer warm periods) (Stireman et al., 2005).
Plant quality	Palatability of plants diminished due to interplant competition (Shaukat et al., 2009) Competition can also reduce the water and protein constituent of foliage (Vavra et al., 2007).	• Some invasive plants (e.g. *Nasturtium officinale* introduced in California) are poisonous to larvae of insects that lay their eggs on them, hence preventing development to maturity (Graves and Shapiro, 2003). For larvae with restricted capacity for movement in the early stages of development, this situation might lead to complete mortality for eggs deposited on such a plant (Graves and Shapiro, 2003).	• Exotic *Acacia* spp. have been shown to enrich the riparian ecosystems with nutrients (like nitrogen and phosphorus) (Naudè, 2012). Evidence to date suggests that increased soil nutrients often lead to increased levels of leaf nitrogen and phosphorus content in plant tissues (Wolf, 2008). Insect outbreaks are triggered by supply of nitrogen in their diet Ayres and Lombardero, 2000).
Disturbance	• Resilient to flood disturbance and fire.	• Altered soil erosion regime: Thick stands of tall IAPs in the catchment lessen runoff and hence the erosive power of stream, which can change the system towards one of sediment deposition (Holmes et al., 2005). • Increased fire frequency due to high flammable biomass (Keeley, 2006).	• Felling, fire, creation of wounds on trees. • Prone to soil erosion: Soil erosion affects microorganism population, composition density, litter accumulation and arthropods (Palacios-Vargas et al., 2007).

Consequences of disturbance on pathogen and insect populations	• Stable levels of folivorous insects and fungal pathogens (Tilman, 1999), since they have adapted to specific plants by evolving mechanisms to detoxify or escape their defences.	• Reduction in the number of folivores due to loss of native plant densities (Traveset and Richardson, 2006; Litt et al., 2014). • Predispose surviving trees to attack by insects since stressed native plants (as a result of completion brought by IAPs) lessen production of defensive compounds against folivores (Agrawal et al., 2000).	• Injured (while clearing IAPs) native trees, exposed root mats and root plates: injuries become habitats of insects like bark beetles and root-feeding beetles (Paine and Baker, 1993; Bouget, 2003). • There is no balance between individual plant defences and ecological regulating factors (Mauricio et al., 1997). For instance, wound repair and substitution of lost foliage or root tissues to meet metabolic demands oblige redirection of carbohydrates and nitrogen from production of compounds like phenol and alkaloids (Agrawal et al., 2000). • Altered abundances of predators that regulate folivorous insect abundances due to increased light intensity (Oboyski, 1995). Increased developmental rates of insects due to increased temperatures as result of open canopies (Walker, 1999).

1.5. Impacts of IAPs on invertebrate numbers and fungal pathogens

Despite the substantial impacts that invasive plants can have on local plant and animal communities, the impact of plant invasions on arthropod communities and related drivers have rarely been studied (van Hengstum et al., 2014; Litt et al., 2014). This is surprising since invertebrates contribute the bulk of global species diversity, regulating many processes fundamental to structure and function in most biomes throughout the world (Greenwood et al., 2004; Magoba and Samways, 2011). Previous studies report significant changes in arthropod abundance as well as in species richness following plant invasions (e.g. Greenwood et al., 2004; Harris et al., 2004; Magoba and Samways, 2011). For example, in the Cape floristic Region (CFR) of South Africa, it has been shown that the abundance and composition in native ant communities are altered by IAPs, with implications for seed dispersal of native plants (French and Major, 2001). However, under some conditions, plant invasions increase local plant species richness and also invertebrate richness and abundance, for example, at the edge of the invasive range (Sax and Gaines, 2003; Ries and Sisk, 2004). In numerous systems, invaders have had a significantly negative impact on flower visitation (via pollinator abundance) and regenerative achievement of native co-occurring plants (Morales and Traveset, 2009)

Several factors may explain differences in the impacts of plant invaders on arthropod communities (Belnap et al., 2005; Wilkie et al., 2007). A frequent observation of plant invaders is that they form dense patches and become dominant members of the community (Sakai et al., 2001; Powell et al., 2011). This leads to changes in a broader set of habitat attributes (Niemela and Mattson, 1996, de Souza and de Souza Modena, 2004; Lassau et al., 2005) like water availability, microclimate and resource quality (Levine et al. 2003; Kimmins, 2004). Under such conditions, plant-herbivore interactions may be affected (Weisser and Siemann, 2004; White et al., 2006). The 'resource concentration hypothesis' predicts that specialist herbivores become more abundant, because they are more likely to find host plants[1] in dense patches and remain there (Rand and Louda, 2004; Blitzer et al., 2012). In addition, IAPs through their chemical and structural complexity can affect flight, host-finding, orientation,

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[1] 'Host plant/tree' is generally defined as plant species that a specific organism (e.g. specific folivore or fungus) must consume to develop properly (Malcolm et al., 2013). In this study this term is used loosely to *include any plant species that harbours fungal pathogens and folivorous insects (not necessarily specific) regardless of the presence of specific disease symptoms of folivory signs.*

life-cycles (Haribal and Renwick, 2001) and oviposition behaviour (Harvey and Fortuna, 2012) in insects. Dense stands of IAPs also alter micro-climatic variables (Folgarait et al., 1995; Facelli et al., 1999).

Climatic variables, especially temperature, have strong direct influences on reproduction, survival and geographical distribution of arthropods (Bale et al., 2002). A rise in temperature may either increase or decrease insect development rates and, depending on the optimum developmental temperature range of the insect species, could directly impact the degree of tree damage (Bale et al., 2002). Change in soil water regimes have been shown to adversely affect arthropods especially micro-arthropods and hibernating insects (Block, 1996) by decreasing their density and richness (O'Lear and Blair, 1999). Water availability is another habitat attribute that may be influenced by plant invaders. Elevated rates of total evaporation have been observed from dense infestations of IAPs occurring in riparian zones (Dye and Jarmain, 2004).

Another threat associated with plant invasion is the spread of fungal disease (Pimentel et al., 2005). The study of the spread of fungi represents an essential component of biodiversity, not only because of their evolutionary, socio-economic and ecological importance, but also because of fungi diversity (Desprez-Loustau et al., 2007). However, until recently, few studies have explored the effect of IAPs on the prevalence of fungal pathogens (see Harvey and Fortuna, 2012). Increases in alien plant population size and density may be advantageous for the development of fungal disease epidemics (Burdon and Chilvers, 1982, Burdon, 1993). In addition, the low genetic diversity of an invading plant population may also increase the probability of such plants being attacked by a pathogen and subsequently increase the rate of disease development and spread (Burdon, 1993). The spread of fungal pathogens as influenced by IAPs deserve additional consideration because comprehensive understanding of advances in fungal biology can provide insights that are applicable to issues of biological invasions.

As discussed with arthropod communities, IAPs may alter habitat attributes and these altered attributes may explain the effects of plant invaders on tree fungal diseases. There is rich literature describing increased soil nutrients due to IAPs (Blank and Young, 2002; Ehrenfeld, 2003; Dassonville et al., 2008). In turn, increased soil nutrients lead to increased pathogen fungal activities and this could directly impact on the degree of tree damage (Wolf, 2008). Apart from altered soil nutrients, IAPs alter soil water content (Dye and Jarmain, 2004) through their high water consumption, a significant effect in water-stressed ecosystems such as those with Mediterranean-type climates. Direct impacts of dry spells on fungal pathogens are usually negative (Desprez-Loustau et al., 2006; Wolf, 2008). Old and Stone (2005) suggested that occurrence of drought can potentially reduce host tree recovery and resilience from pests and fungal pathogen attack, disposing trees to stem borer and canker fungi. A recent study in the Western Cape Province by Jacobsen et al., (2012) noted that a fungus (*Pythium* sp.) infecting root vascular tissue of plants caused severe plant stress, while the ultimate cause of death of infected plants appeared to be extreme water stress. It is thus possible that fungal pathogens could greatly impact tree health in systems that are water dependant and heavily disturbed such as riparian ecosystems.

1.6. Impacts of fungal pathogens and folivorous insects on host trees

Folivorous insects and fungal pathogens impact plant species composition and ecosystem function negatively or positively (Ayres and Lombardero, 2000). For example, in Australia and South Africa, various disease symptoms have been recorded on the ecologically and economically important black wattle (*Acacia mearnsii*, native to Australia and invasive in South Africa) (Roux and Wingfield, 1997; Old et al., 2003). Fungi that damage the stems of trees (e.g. canker fungi) reduce form and commercial value of logs as well as cause tree mortality (Old et al., 1986; Old and Davison, 2000) and could reduce the competitive ability and fitness of the individual in an ecosystem. Similar to fungal pathogens, some of the insects that consume seeds or damage smaller seedlings have potential to kill their host plants beyond recovery (Clark and Clark, 1985) thereby strongly impacting on host populations. The importance of folivores for tree regeneration and local diversity in tree communities has therefore attracted much attention (e.g. Welter 1989; Trumble et al., 1993; Thomson et al., 2003; King and Caylor, 2010).

The effects of folivorous insects on physiological processes (e.g. photosynthesis, stomatal conductance and hydraulic conductivity) are evident as they often directly remove photosynthetic surface area, hence affecting net primary production (Cyr and Pace, 1993; Luque et al., 1999). For instance, the low leaf damage by caterpillars (>5%) on *Pastinaca sativa*, was enough to reduce photosynthesis by 20% in the remaining foliage (Zangerl et al., 2002; Nabity et al., 2009). The mechanisms decreasing photosynthesis in undamaged leaf tissues are complex, ranging from self-inflicted diminishment in metabolic processes to disorders in transport of fluid or nutrient. Folivorous insects can affect photosynthesis by:

- Severing leaf vasculature (Welter, 1989; Heng-Moss et al., 2006) and obstructing fluid flow with cell fragments that ultimately alter photosynthesis (Delaney and Higley, 2006).

- Changing sink demand by insect folivory which can affect photosynthesis (Trumble et al., 1993; Retuerto et al., 2004; Dorchin et al., 2006). For instance, feeding activity of a leaf-mining moth that lives enclosed inside the leaf tissue of apple trees is known to reduce their biophysical properties (e.g. carbon assimilation rates) by decreasing transpiration (Pincebourd et al., 2006).

- Initiating complex relations between defence processes and photosynthesis (Aldea et al., 2006b). For example, carbon and nitrogen can be diverted away from primary metabolism (Agrawal et al., 2000), during the induction of an arsenal of defence compounds (Kessler and Baldwin, 2002). Numerous defence compounds adjust the photochemical reactions of the leaf. For instance, leaf colour changes after folivore and fungal pathogen damage (Herde et al., 1999; Aldea et al., 2006b) and the generation of reactive oxygen species (Bown et al., 2002) may diminish photosystem II quantum functioning proficiency and biophysical properties (e.g. carbon assimilation rates) in damaged leaves (Aldea et al., 2006b).

- Changing defence responses which cause downregulation of photosynthesis-related genes quantity (Giri et al., 2006; Howe and Jander, 2008). Transcription analysis done by Hui et al., (2003) and Giri et al., (2006) of plant-folivore interactions found that photosynthesis-related genes are downregulated after attack, thereby reducing the abundance of the gene coding for Rubisco activase.

In contrast to folivory, the effects of fungal pathogens on host physiological processes (e.g. photosynthesis) are more varied. To characterise common patterns and modes of action, Barón et al., (2012), divided fungi into three damage guilds, viz. endophytic fungi, vascular-wilt fungi and leaf-disease fungi. They documented that although endophytic fungi are often considered to induce increases in host plant photosynthetic rate; this is not always the case (Marks and Clay, 1996). Vascular-wilt fungi include a number of fungi species that grow mostly on xylem tissues of host plants, causing reduction of hydraulic conductance, through aspiration of tracheid tori (Guerard et al., 2000) or vascular plugging with resin and fungal mycelium spread in the sapwood area (Bramble and Host, 1940; Guerard et al., 2000). This leads to increased xylem tension and accelerates the rate and amount of cavitation in an attacked tree. Reductions in photosynthetic rate induced by wilt-disease fungi can be as large as 30-90% and is typically caused by a reduction in stomatal conductance (Nogués et al., 2002; Rotenberg et al., 2004). Leaf fungal pathogens largely:

- reduce CO_2 assimilation of host plants by reducing the photosynthesising leaf area (Scholes et al., 1994; Manter and Kavagh, 2003; Robert et al., 2005).
- induce decreases in photosynthesis in the remaining green-leaf areas, in some cases by up to 30-50% (Manter and Kavanagh, 2003; Scharte et al., 2005).
- induce changes in chloroplast ultra-structure that leads to decreased photosynthetic rates (Scholes and Rolfe, 1996).
- alter photochemistry and PSII function (Manter et al., 2007). For instance, the decline in the rate of photosynthesis and the corresponding increase in Non-Photochemical Quenching (NPQ) during infection by a pathogen (Chou et al., 2000).

1.7. Impacts of IAPs on plant traits

Plant traits play a critical role in shaping the interactions between plants and herbivores, such that understanding the influence of IAPs on plant traits is tantamount to understanding the functioning of invaded ecosystems. Plant nutritional content is one of the most important traits that may affect damage levels of folivorous insects and fungal pathogens (Price, 1991; Peeters, 2002a; reviewed in Cornelissen et al., 2008). Leaf nitrogen content can affect the growth rates of leaf-mining moth that lives enclosed inside the leaf tissue (Coley et al., 2006) and feeding efficiencies of coleopteran larvae (Kagata and Ohgushi, 2006). Leaf damage by folivorous insects has been found to associate with increases in soluble protein levels (Casotti and Bradley, 1991). There is a substantial pool of literature that indicates IAPs may alter soil properties and nutrient fluxes (Blank and Young, 2002; Chapuis-Lardy et al. 2006; Liao et al., 2008) in a variety of ways that fundamentally differ from indigenous plants. Many invasive alien plants have a common suite of traits like high specific leaf areas, growth rates, and leaf nutrient concentrations, relative to the indigenous plants, and these traits potentially alter ecosystem properties like increases litter quality, decomposition rates and nutrient cycling (Allison and Vitousek, 2004). Recently, van der Waal (2009) found that the invasion of *A. mearnsii* increased acidity and concentrations of nitrogen, potassium, sodium and manganese in soil.

1.8. Control of IAPs (*A. mearnsii* and other Australian Acacias) in South Africa

Because of the numerous impacts associated with *A. mearnsii* infestations on rivers, streams, and adjacent ecosystems, large-scale *A. mearnsii* and other IAP control efforts have been initiated throughout South Africa led by the extended public works programme, Working for Water (WfW). This programme, initiated in 1995, aims to remove water-demanding IAPs from waterways and catchment areas (van Wilgen et al., 1998, 2012), while also providing employment (van Wilgen et al., 1998). It is estimated that 1.7 million ha of land containing about 15 woody invaders will be cleared by 2015 (van Wilgen et al., 1998, 2012), in an attempt to reduce the impacts of these invaders. The most common means of removing *A. mearnsii* and other invasive Acacias from the Fynbos is clearing by felling and either removing the biomass or burning it (Mugasi et al., 2000). Follow-up treatments involve the use of herbicides to prevent sprouting of cut stumps, or to kill seedlings (van Wilgen et al., 2000). *Acacia mearnsii* removal efforts primarily cover areas along higher riparian terraces because of the ways by which it spreads and colonizes foothill sections of riparian zones. Research is still needed to investigate the ecophysiology of *A. mearnsii* to determine the most suitable time to apply herbicides to kill the roots of these plants. The biological control of *A. mearnsii* was initiated in 1973 (Campbell, 2000) but a conflict of interest with forestry and wood-harvesting initiatives, delayed their release until 1993 with the introduction of seed-feeding weevils species (Dennill et al., 1999) and the subsequent release of the midge *Dasineura rubiformis* in 2008 (Impson et al., 2008). These agents cause reductions in seed availability and do not damage the vegetative parts of *A. mearnsii* trees (Impson et al., 2011).

The removal of IAPs constitutes a further disturbance to riparian systems and may cause additional changes to water physical and chemical properties of riparian habitats (Bunn et al., 1999; Samways et al., 2011). The process of clearing of IAPs may play a significant role in either enhancing or delaying the recovery of invaded riparian plant communities. Clearing of IAPs may affect interactions between plants and herbivorous arthropods and fungal pathogens through several direct and indirect pathways, hence affecting plant health and vigour. Clearing vegetation results in gap formation, which alters the microenvironment including light, moisture, and temperature regimes and increases patchiness, and habitat heterogeneity (Carlton, 1996; Torchin and Mitchell, 2004). Disturbed gap patches can function as source-sink dynamics for insect population by affecting reproductive rates, abundance and diversity of predators and survival of fungi or insects (Bouget, 2003). For instance, the development of herbivore populations may specifically rely on temperature, and their effect on host plants will change quickly because of altered climatic conditions that stem from a reduction in canopy cover (Folgarait et al., 1995, Facelli et al., 1999).

Despite the overwhelming evidence of IAP impacts and a growing appreciation of ecosystem services, researchers and policy makers rarely directly address the connection between plant invasions and pressures of biotic stressors like fungal pathogens and folivorous insects on tree health (Purcell and Saunders 1999). Currently, nothing is known of the impacts of IAPs and their control on the health of riparian trees in relation to biotic stressors. It is this concern that prompted the present study. When restoring endangered ecosystems, controlling herbivore outbreaks, especially when a plant's life stage makes it vulnerable to such outbreaks, can increase population growth of those plants (Bevill et al., 1999) and therefore aid ecosystem recovery. Also, in some cases, where herbivores have been introduced for control of an invasive plant species, those herbivores may have ecological consequences on native flora and fauna (Louda and O'Brien, 2002).

1.9. Aims and Organisation of Dissertation

In this dissertation, the four data chapters examine the role of folivorous insects and plant fungal pathogens on the health of trees in riparian zones of the CFR, Western Cape Province, South Africa (Figure **1.1**). This research is relevant to restoration ecology of such systems where 84% of South African freshwater ecosystems are threatened, with 54% critically endangered, the latter having less than 20% of their original habitat remaining (Nel et al., 2009). The main hypothesis is that increased stress due to IAPs in river systems throughout South Africa has promoted fungal pathogens and folivory on native trees. Each research chapter has been written in the form of a stand-alone research publication.

Chapter 2: The main aim of this chapter was to characterize the differences in arthropod community composition between habitats with differing invasive alien tree management histories by focussing on two native and one invasive tree species.

Chapter 3: In this chapter the main objective was to test several currently debated hypotheses about the effect of plant community characteristics, including host density and plant species diversity, on patterns of damage caused by folivorous insects and fungal pathogens. It focuses on three woody tree species (two native and one invasive) in riparian systems with differing invasive alien tree treatments.

Chapter 4: Here differences in the abundance and richness of folivorous insects for three host trees (two native and one IAP) from riparian habitats with differing invasive tree treatments were determined. The differences in richness and abundance of folivorous insects detected were correlated to (i) levels of folivory, (ii) leaf nutrients (Nitrogen and Phosphorus) and (iii) predator abundance.

Chapter 5: Insect folivory and fungal pathogens have been shown not only to reduce leaf area but also to induce changes in plant metabolic functioning (e.g. photosynthetic rate) through various mechanisms. In this chapter the influence of selected fungal pathogens and folivorous insects on plant physiology and plant nutritional status was determined. Specifically I determined how gas-exchange parameters, leaf nutrients and leaf water content relate to damage severity.

Chapter 6: This chapter summarises the key findings of the dissertation, and highlights how this study contributes towards restoration ecology and research throughout CFR riparian ecosystems. Key recommendations for riparian management and control of IAPs are discussed and future research opportunities are identified.

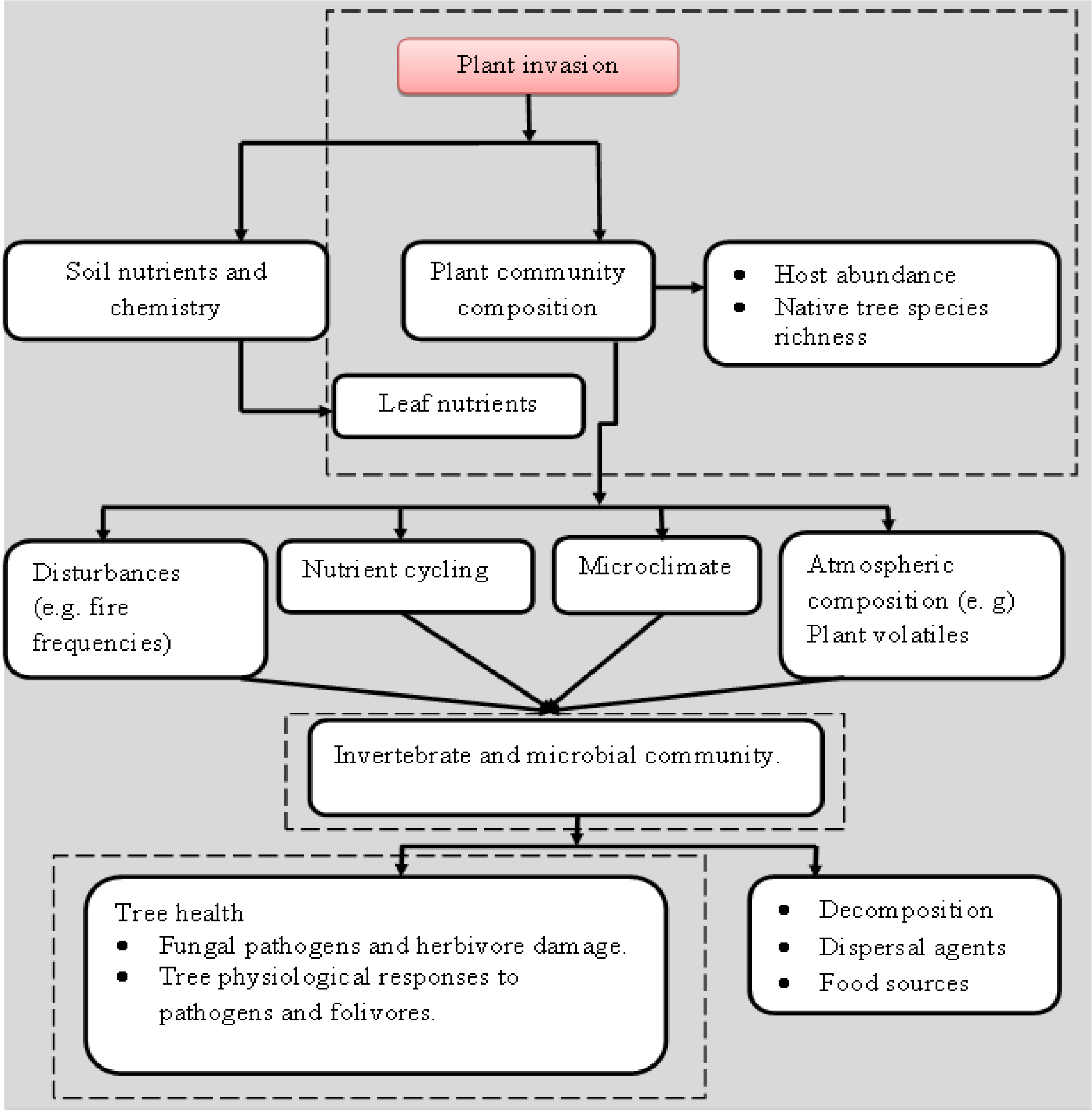


Figure **1.1**: Flow diagram showing various proposed hypothetical scenarios to explain impacts of IAPs on riparian systems. The outlined boxes indicate the focus of this dissertation. (See Table 1.1 for detailed descriptions of proposed hypothetical scenarios to explain susceptibility of riparian zones that differ in invasion treatment to utilisation by fungal pathogens and folivores).

1.10. Descriptions of tree species and sites selected for this study

1.10.1. Tree species

The predominant native riparian tree species, *Brabejum stellatifolium* (L.) (Proteaceae) and *Metrosideros angustifolia* (L.) (Myrtaceae) (Coates-Palgrave, 2002; Reinecke et al., 2007) were selected as target plants to study. These evergreen trees are widespread across Western Cape river systems (Prins et al., 2004; Galatowitsch and Richardson, 2005; Crous et al., 2011).

Brabejum stellatifolium

Metrosideros angustifolia

Brabejum stellatifolium resembles Australian Proteaceae more closely than it does South African members of the family because of its morphological characters like flower pairs and stylar pollen presentation (Hoot and Douglas, 1998). It is confined to the Fynbos biome of the Western Cape Province, South Africa. It prefers moist areas, and commonly occurs in riparian areas (Swift, 2008; Crous et al., 2011). Several interesting organisms are exclusively associated with *B. stellatifolium* (Procheş 2007). For example, a range of characteristic fungi have been described (Table **1.2**). Some of the arthropods associated with it are not conspicuous, but may prove to be ecologically important (Table **1.2**).

Metrosideros angusitifolia is capsular-fruited tree with seeds dispersed by air currents (Wright et al., 2001). Like *B. stellatifolium*, it occurs in riparian areas (Galatowitsch and Richardson, 2005; Crous et al., 2011). The genus *Metrosideros* is largely of Australasian-Pacific origin, reaching out as far as Hawaii. However *Metrosideros angustifolia* is an outlier, and indigenous to the Cape Floristic Region (Procheş et al., 2008). It has been suggested that *Metrosideros angustifolia* may not be of the genus *Metrosideros*, but represents the after-effect of a noticeably early dispersal occasion amid the tertiary, from the Australasian core area of distribution of Myrtaceae (Sytsma et al., 2004; Procheş et al., 2008). Nothing is known about the arthropods associated with this tree species, however, several canker-causing fungi have recently been discovered on trunks of this species (Chen et al., 2013) (Table **1.2**).

The two native trees listed above are known to be drought-tolerant (Swift, 2008 ; Crous et al., 2011). Sorkheh et al., (2011) discovered that during drought stress, the antioxidant response of *B. stellatifolium* limits cellular damage caused by reactive oxygen species and may be of key importance for the selection of drought-resistant rootstock. Discoveries like this can be used to predict the response of host plants to attack by natural enemies (e.g. pests and fungal pathogens) since they give an indication of how well defended plants are against natural enemies.

Acacia mearnsii

The health of these two native tree species was compared with evergreen invasive woody species *Acacia mearnsii* (Fabaceae), commonly known as black wattle. It is a fast growing leguminous (nitrogen fixing) tree. This species is more dependent on water than phyllodinous *Acacia* spp. and therefore often invades river systems (Morris et al., 2011). Like the two native species listed above, *Acacia mearnsii* is known to be drought-tolerant (Crous et al., 2011). This species outcompetes native plants for water, nitrogen and organic materials through mechanisms of high production of shallow and deep roots, and symbiotic N_2-fixation (Pretorius et al., 2008; Morris et al., 2011). Water dispersal and the development of extensive, steady seed banks empower *A. meanrsii* to disperse quickly in riparian systems. Germination of *A. mearnsii* seeds is usually prompted by disturbances, particularly fire (Galatowitsch and Richardson, 2005; Pretorius et al., 2008). It threatens native habitats by reducing native biodiversity and increasing water loss from riparian zones (Richardson et al., 1992; Galatowitch and Richardson, 2005). However, this species is also one of the most important forestry plantation species in South Africa and is therefore extensively planted. It is mostly utilised for the paper industry (Roux and Wingfield, 1997; Adair, 2004), but wood can also be used for various functions such as fencing, electric poles, etc. (Dye and Jarmain, 2004; Le Maitre et al., 2011).

Because of its invasiveness, a number of studies have investigated the organisms associated with it (Table **1.2**), particularly those that negatively influence its growth and reproduction. This includes the classical biological control agents, cecidomyiid fly (*Dasineura rubiformis*) and the seed weevil (*Melanterius maculatus*) (Impson and Moran, 2004; Impson, 2008). Not only were biocontrol agents of *A. mearnsii* investigated, but also the forestry industry studied organisms that may negatively affect quality of its wood (Table **1.2**). A detailed list of fungal pathogens from *A, mearnsii* is provided in Roux and Wingfield, (1997).

Table **1.2**: Pests and fungal pathogens associated with *B. stellatifolium* and *M. angustifolia* (native trees) and *A. mearnsii*.

	Organisms		
Host plant	Fungal pathogens	Arthropods	References
Brabejum stellatifolium	*Anthostomella brabeji*		Lee and Crous (2003)
	Phyllostica owaniana		Swart et al., (1998)
	Periconiella velutina		Arzanlou et al., (2007)
	Harknessia capensis		Lee et al., (2004)
		Aceria rusti	Procheş (2007)
		Setapion provinciale and *Setapion quantillum*	Procheş (2007)
Metrosideros angustifolia	Canker disease: *Diversimorbus metrosiderotis*		Chen et al., (2013)
	Holocryphia capensis		Chen et al., (2013)
	Holocryphia mzansi		Chen et al., (2013)
Acacia mearnsii	Root disease: *Lasiodiplodia theobromae*		Laughton (1937)
	Butt disease: *Phytophthora nicotianae*		Zeijlemaker (1971)
	Leaf rust: *Uromycladium alpinum*		Morris and Wingfield (1988)
	Wilt disease:		

Ceratocystis fimbriata		Morris et al., (1993)
Ceratocystis albifundus		Wingfield et al., (1996); Heath et al., (2009)
Sphaeropsis sp.		Roux and Wingfield (1997)
Leaf spot:		
Stigmina verruculosa Syd		Doidge et al., (1953)
Root and heart rot:		
Thanatephorus cucumeris,		Doidge et al., (1953)
Thanatephorus hirsuta		Doidge et. al., (1950)
Thanatephorus meyerii		Doidge et al., (1953)
Thanatephorus roseola		Doidge et al., (1953)
Trichoderma spp.		Roux and Wingfield (1997)
Tryblidopycnis pinastri Höhn		Roux and Wingfield (1997)
Verticilium spp.		Roux and Wingfield (1997)
	Vectors of wilt-fungal pathogens (Coleoptera: Nitidulidae) *Brachypeplus depressus,* *Carpophilus bisignatus,* *Carpophilus hemipterus*	Heath et al., (2009)
	Cutworms, *Agrotis* spp.	Sherry (1971)
	Termites	Sherry (1971)
	Wattle semi-looper,	Sherry (1971)

	Achaea spp.	
	Biocontrol agents:	
	Melanterius maculatus	Dennill et al., (1999)
	Dasineura rubiformis	Impson et al., (2008)

1.10.2. Study sites

This study was conducted in mountain stream and foothill sections of several riparian systems within the south-western Cape Province of South Africa (Fig. **1.2**). The meteorological information and invasion treatment of each site is provided in table **A1.1** in appendix A1. Site choice was focused around the accompanying criteria: the existence of the selected suite of two native and one invasive plant species; good knowledge of certain important habitat parameters such as treatment of invasion and clearing; and for their relatively close proximity to Stellenbosch University. The sampling design called for three catchments, consisting of near pristine (used as reference sites), heavily invaded (dominated by *A. mearnsii* for at least 10 years) and restored (cleared of *A. mearnsii* for at least 7 years before this study) riparian sites. The area is characterised by seasonal climate with cool rainy winters (June-August) and hot dry summers (December-February) (Deacon et al., 1992), typical of Mediterranean ecosystems. In winter, temperatures commonly drop below freezing on mountain peaks, but the lowlands are generally frost free. The riparian ecosystems are largely covered by Fynbos vegetation (dominated by Ericaceae, Proteaceae and Restionaceae) (Mucina and Rutherford, 2006) including a variety of tree taxa that often locally form forest pockets (Goldblatt and Manning, 2000) (Fig. **1.3**).

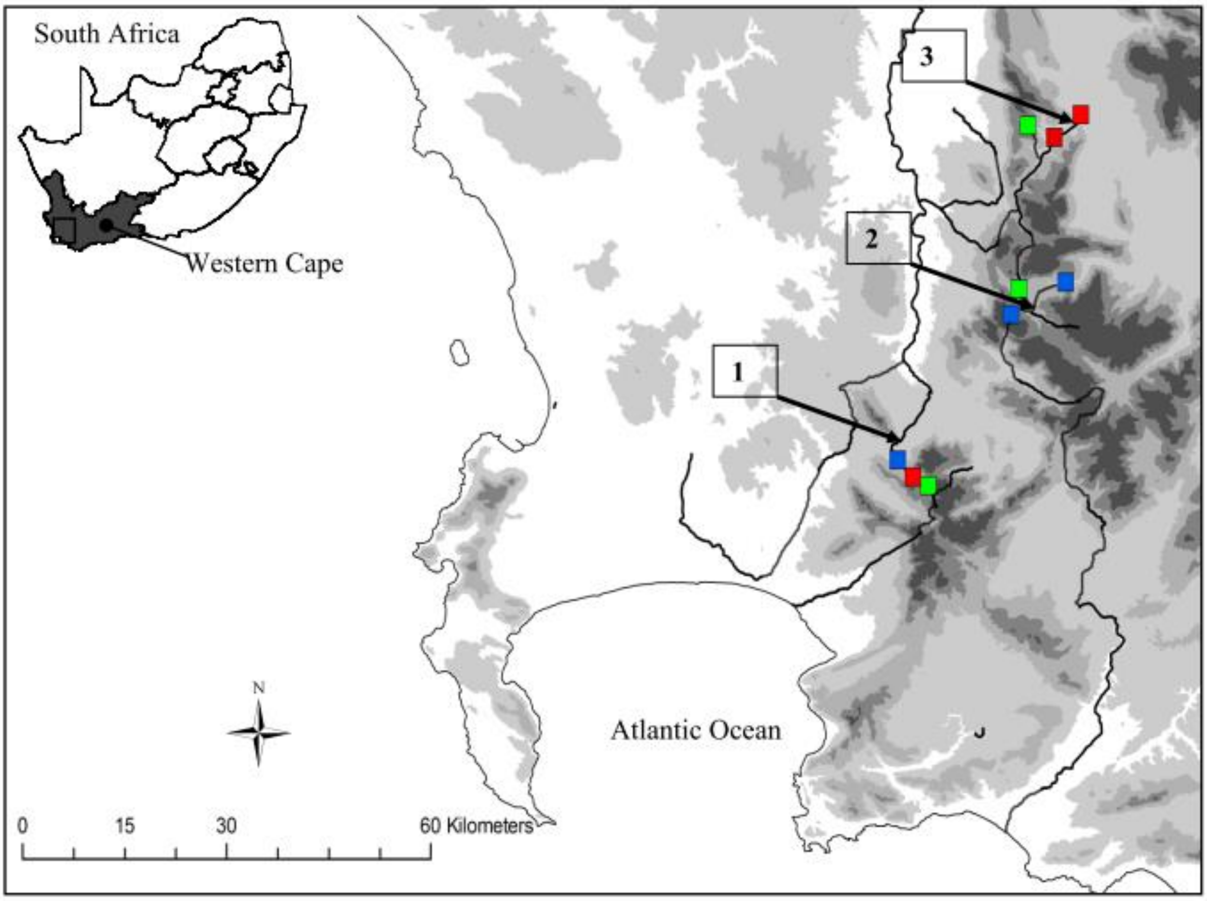
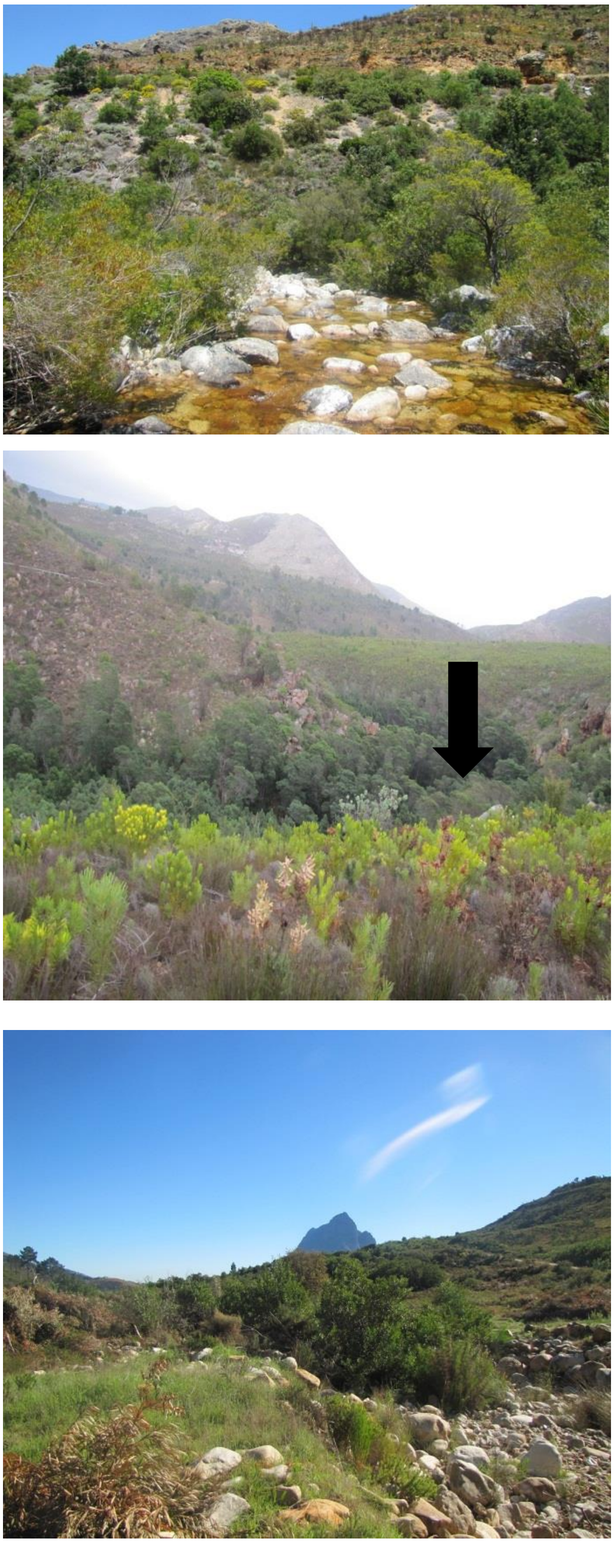


Figure **1.2**: Location of the three Western Cape rivers: 1= Dwars, 2 = Molenaars, and 3 = Wit and the nine sites (green: near pristine, red: heavily invaded and blue: restored) used in this study.



Near Pristine (NP)

Heavily Invaded (HI)

Restored (R)

Figure **1.3**: Images of representative sites that differ in invasion treatment (black arrow signifies heavy infestation of *A. mearnsii*).

Dwars River

The Dwars river system is a perennial tributary of the Berg River and originates in the Dwarsberg mountains from where it flows in a northerly direction before joining the Olifants River.

Geology: The upper reaches of the catchment flow mainly through Table Mountain Group formations, which yield relatively low volumes of sediments (Beck, 1960) resulting in shallow soils with significant rock cover in terrestrial areas.

Vegetation and major landuses: The catchment is dominated by agricultural activities consisting of vineyards and orchards which expand across much of the central and gently rising slopes of the Dwars River Valley. Natural vegetation occurs along the steeply rising mountainous areas along the northern edge of the catchment. The vegetation is categorised as a mixture of Boland Granite Fynbos and Kogelberg Sandstone Fynbos (Rebelo et al., 2006).

Important ecological linkages and Biodiversity assets: The Dwars River is barely functional as a riparian corridor and provides little to no buffering of the stream from adjacent land use activities throughout its reaches. With improved management, such as clearing of invasive *Eucalyptus* spp., this catchment could improve water quality passing into the Berg River.

Wit River

The Wit River is a perennial tributary of the Breede River and originates in the Hawequas mountain range in Bainskloof Pass.

Geology: The geology of the Wit River catchment comprises basically of the Peninsula foundation supporting Hawequas Sandstone Fynbos vegetation type (Rebelo et al., 2006). The bedrock is deep in numerous spots with cobble/boulder-bottomed pools which are likewise interspersed with longer riffle/run segments (Brown et al., 2004).

Vegetation and major land-uses: Major land-uses are predominantly natural, given the steepness of the valley slopes. However there are some agricultural activities like vineyards and fishing. The vegetation is predominantly mountain Fynbos (*M. angustifolia*, *B. stellatifolium*, *Brachyleana neriifolia*, *Erica caffra* and *Elegia capensis*) with remnants of indigenous forest in some of the wetter kloofs

(Campbell, 1985). Alien trees, including *A. mearnsii*, *Hakea* spp. and pine have invaded sections of the river (Blanchard, 2007).

Important ecological linkages and Biodiversity assets: Ecological networks along river systems, connecting Limietberg Nature Reserve with the Du Toitskloof mountain range are viewed as critical since they permit natural movement of species, e.g. searching for food, reproducing, migration and dispersal. Such corridors are however generally degraded by IAPs throughout the river. Although sections of a system may be relatively well conserved (e.g. the mountain stream and foothill reaches of the Wit River), there is widespread degradation in the valley bottom reaches, and thus habitat connectivity has been lost. The catchment also provides important ecological and hydrological linkages between the Breede River and Berg River. This may be an important natural passage of invertebrate adults between systems and along rivers (Job and Driver, 2006).

Molenaars River

It flows along a steep gradient, within a laterally confined valley, for some 5 km before combining with the Krom River. It is quite different in character from all the river reaches upstream, and more characteristic of a foothill river in the south-western Cape (King, 1988). Its geomorphology, the river reach at the confluence of the Molenaars and Elands Rivers may be classified as segment boundary, using the classification hierarchy of Wadeson and Rowntree (1998). It exhibits physical features characteristic of mountain streams in the south-western Cape (acidic, black waters, naturally low in nutrients) (Job and Driver, 2006).

Geology: Much of the catchment consists of predominantly quartzitic sandstone from the Table Mountain Group (Parsons and Wentzel, 2007).

Vegetation and major landuses: The catchment is dominated by protea farming and trout farming. Indigenous riparian vegetation is present, but some of the areas where trout farming occurs, have become infested with alien plants, in particular the black wattle *Acacia mearnsii* (Boucher, 2002). Elements of indigenous riparian vegetation have remained in pockets along the river, and sparsely between the dense alien infestations. These include trees such as wild almond *Brabejum stellatifolium*, *Metrosideros angustifolia*, Cape willow *Salix mucronata*, red alder *Cunonia capensis*, as well as the palmiet reed *Prionium serratum* and restios e.g. broom reed *Elegia capensis* and the sedge *Isolepis* spp (Samways et al., 2011).

Important ecological linkages and Biodiversity assets: The sub-catchment provides important ecological and hydrological linkages between the Breede River valley and the Berg/Hottentots mountain regions. This may be an important natural passage of invertebrate adults between systems and along rivers. Some faunal studies have been undertaken, and the river supports a high diversity of sensitive invertebrate taxa, many of which are endemic to the south-western Cape (King, 1981, 1983; Ractliffe and Brown, 1994; Samways et al, 2011). Ractliffe and Brown (1994) reported 129 invertebrate taxa from the Molenaars River.

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Appendix **1**

Table **A1.1**: Site-specific information, including the major geomorphological characteristics, site treatment and mean annual rainfall. (All the streams are perennial).

Site	Geology	Treatment of Invasion	History clearance	Fire History	Mean annual rainfall (mm)	Longitudinal zone	Coordinates
Near pristine							
Upper Dwars (UD)	Sandstone /granite	None	None	No proof of latest fire	578	Mountain Headwater stream	33°57'05.54"S; 18°58'39.22"E **Elevation**: 446 (m)
Bains Kloof (BK)	Sandstone	None	None	No proof of latest fire	888	Mountain Headwater stream	33°34'08.49"S; 19°08'19.03"E **Elevation**: 303m
Du toits Kloof (DK)	Sandstone	None	None	No proof of latest fire	1468	Foothill	33°43'47.41"S; 19°06'37.06"E **Elevation**: 472m
Heavily invaded							
Lower Wit (LW)	Sandstone	None	None	2012	833	Foothill	33°32'19.56"S; 19°10'51.77"E **Elevation**: 243m
Mid Wit (MW)	Sandstone	None	No clear evidence	2012	833	Foothill	33°34'06.33"S; 19°08'47.52"E **Elevation**: 283m
Mid Dwars (MD)	Sandstone/ granite	None	No clear evidence	No proof of latest fire	578	Mountain stream	33°56'53.36"S; 19°58'11.25"E **Elevation**: 400m
Restored							
Upper Molenaars (UM)	Sandstone	>7 years ago (*A. mearnsii*)	Initial treatment: 2002-2003. 2 follow-up	2012	889	Upper Foothill	33°42'38.56"S; 19°11'49.24"E **Elevation**: 335m

			treatments. (Fell and remove)				
Du Toit (DT)	Sandstone	>7 years ago (*A. mearnsii*)	Initial treatment 2002 (Fell and remove)	No proof of recent fire	1477	Upper Foothill	33°43'34.21"S; 19°06'01.02"E **Elevation**: 544m
Lower Dwars (LD)	Sandstone	>8 years ago(*A. mearnsii* and *A. longifolia*)	Initial treatment: 2002. 3 follow-up treatments. (Fell and remove)	No proof of recent fire.	578	Mountain stream	33°56'45.74"S; 18°57'57.51"E **Elevation**: 385m

CHAPTER 2

Restoration of Cape Floristic Region riparian systems affected by invasive alien plants leads to a recovery in arthropod alpha- and beta-diversity.

ABSTRACT

The Cape Floristic Region (CFR) of South Africa is a global biodiversity hotspot threatened by invasive alien plants (IAPs). Systematic removal of IAPs was initiated to remove water-demanding IAPs from waterways and catchment areas while also providing employment. This study assesses the effect of IAP management practises on CFR riparian arthropod diversity. Foliage-active arthropod communities were collected from two native tree species, *Brabejum stellatifolium* and *Metrosideros angustifolia* and from the invasive alien *Acacia mearnsii*. Their associated arthropod communities were compared of alpha- (species richness) and beta-diversity within and between areas with different invasion treatments (invasion treatments: near pristine, heavily invaded by *Acacia mearnsii* and seven years after clearing of *A. mearnsii*). Arthropod alpha-diversity at near pristine sites was higher than it was at restored sites, and was lowest at heavily invaded sites. This was true for most arthropod taxonomic groups associated with all three tree species and suggests a general recovery trend in arthropod alpha-diversity after IAP clearing. Overall, arthropod species turnover among sites (β1 diversity) was significantly influenced by invasion treatment with communities at near pristine sites being higher than those at restored and heavily invaded sites. However, this was not evident at the level of individual tree species. Although arthropod community composition (i.e. beta-diversity between invasion treatments, β2) was significantly influenced by invasion treatment, only few significant differences in arthropod community composition could be detected between restored and near pristine sites. This was true for all tree species and arthropod taxonomic groups. Assemblage composition on each tree species generally differed between riparian sites with similar management histories indicating a strong natural changeover of arthropod communities across the landscape. These results suggest that both arthropod alpha-diversity and arthropod beta-diversity can recover after habitat restoration efforts, given sufficient time. Both arthropod richness and arthropod community composition have the potential to be used as measures of restoration success. The results further indicate that rivers have distinct signatures in their terrestrial arthropod communities and restoration efforts should therefore include as many river systems as possible.

Keywords: Cape Floristic Region, Riparian zones, Invasive alien plant management, Invertebrates, Arthropod responses.

2.1. INTRODUCTION

Terrestrial arthropod populations and communities are commonly associated with characteristic vegetation types, and the loss of suitable plant habitat can lead to declines in arthropod populations (Kremen et al., 1993; Herrera and Dudley, 2003; Longcore, 2003). Among the primary threats to arthropod diversity of many ecosystems, are the introductions of invasive species (Tallamy, 2004; Magoba and Samways, 2012). For some countries, invasive species are one of the most challenging environmental threats of the 21st century (Stohlgren et al., 2006). Dense stands of Invasive Alien Plants (IAPs) are a growing threat to ecosystem functioning and native biodiversity (Vitousek et al., 1997; Sala et al., 2000; Le Maitre et al., 2004; Clavero and Garciá-Berthou, 2005; van Wilgen et al., 2008). They lead to changes in vegetation structure, composition and host quality and therefore affect arthropod assemblages (Bock et al., 1986; Beerling and Dawah, 1993; Joern, 2005). For example, Slobodchikoff and Doven (1977) showed that increased cover of the non-native grass *Ammophilia arenaria* disrupted the structure of sand dune arthropod communities in California. Similarly, abundance and composition in native ant communities has been altered by IAPs in the Cape Floristic Region (CFR) of South Africa, (French and Major, 2001), with implications for the seed dispersal of native plants (Mokotjomela and Hoffmann, 2013).

Little is known of the effect of IAPs on arthropod assemblages associated with riparian trees in the CFR, a region heavily impacted by IAPs. Riparian ecosystems are among the most endangered CFR habitats, with less than 20% of their original extent still intact (Nel, et al., 2007). Riparian vegetation is used for resting, feeding, reproduction and refuge by both aquatic and terrestrial arthropods, and provides a critical resource base for vertebrates (Gray, 1993; Doyle, 1990). One of the most notorious invasive species in the CFR is *Acacia mearnsii*, which the Working for Water (WfW) invasive plant clearing programme has designated as a top priority for removal (van Wilgen et al., 2008; 2012). Most South African research on *A. mearnsii* and other IAPs in the Fynbos biome have shown that dense stands of invasive acacias can rapidly reduce the abundance and diversity of native plants at the landscape scale (Richardson et al., 1989). Such dense stands of IAPs also lead to a decline in seed banks in the soil of Fynbos native plants, leading to increased probability of extinctions of native species (Vosse et al., 2008). In addition, IAPs greatly increase biomass (Milton, 1981), affect fire regimes (Van Wilgen et al., 2008) change nutrient cycles (Witkowski, 1991) and reduce arthropod richness (reviewed by Litt et al., 2014).

Clearing of IAPs can lead to recovery of vegetation communities under certain conditions (Blanchard and Holmes, 2008) and it is expected that removal of *A. mearnsii* from riparian systems would also help maintain the high arthropod species diversity that characterizes CFR riparian communities (Samways et al., 2011). Williams (1993), Longcore (2003) and McCall and Pennings, (2012) have shown that arthropod richness (alpha-diversity) and abundance can recover after restoration efforts on disturbed natural riparian ecosystems in various countries. Gratton and Denno, (2005) observed that removal of invasive *Phragmites* resulted in the return of dominant native vegetation and the re-establishment of arthropod species assemblages. However, the effect of clearing of IAPs from CFR riparian ecosystems on arthropod diversity has not yet been assessed.

Although the advantages of the removal of IAPs are apparent, the process itself represents yet another disturbance to river ecosystems. Recent research has shown that it can result in unexpected changes to ecosystem processes that may affect arthropod survival. For example, removal of IAPs alters canopy characteristics, which directly affects the interior environments of ecosystems (i.e., temperature, humidity, and radiation) (Walker and Smith, 1997; Ziesche and Roth, 2008). This, in turn, leads to changes in arthropod richness and abundance (Ziesche and Roth, 2008). Apart from microclimate, altered architectural habitat complexity (Schowalter and Crossley, 1988) and changes in plant nutritional quality (Maridet et al., 1998; Fischer et al., 2010), IAP removal may also influence arthropod communities by limiting their dispersal ability by creating isolated patches (Schowalter and Crossley, 1988).

The measurement of arthropod species richness (alpha-diversity) and species turnover (beta-diversity) under different management scenarios aids our understanding of the effect of specific management actions for biodiversity conservation (Kessler et al. 2009). This study tests the effect of an invasive alien tree on arthropod alpha-and beta-diversity in riparian systems of the CFR and whether measures of arthropod alpha- and beta-diversity can indicate a post-IAP removal recovery trajectory. The expectation was to see differences in arthropod alpha- and beta-diversity among areas with the three different invasion treatments (near pristine, heavily invaded by *A. mearnsii* and *ca*. 7 years after removal of *A. mearnsii*), with major differences between the near pristine habitats and those that have been restored reflecting the time it takes for arthropod assemblages to fully recover after invasion by IAPs.

2.2. METHODS

2.2.1. Study area and species

This study was conducted in the mountain stream and foothill sections of several riparian systems within the Western Cape, South Africa (Fig. **1.2** in Chapter 1). The selected river sections are on quartzitic sandstone that is characteristically acidic and low in nutrients and dissolved solids (Day and King, 1995). Vegetation is largely shrubby Fynbos and includes a variety of tree taxa that form forest pockets (Goldblatt and Manning, 2000). Three different river systems (Molenaars, Dwars and Wit) were selected in order to represent the CFR as a whole.

Nine study sites in the three different rivers listed above were identified: three near pristine sites (NP) (reference sites), three heavily invaded (HI) sites (predominantly by *A. mearnsii*); and three restored sites (R) (formerly invaded sites that had been cleared of IAPs more than 7 years prior to this study). In restored sites, IAPs were felled as close to the base as possible and herbicide was applied to stumps. After felling, slash was left on site and burned *ca*. six months to a year later as this kills non-re-sprouting aliens, reduces alien soil-stored seed, removes excessive above-ground biomass and initiates indigenous Fynbos vegetation recovery (Blanchard and Holmes, 2008). Potential sites were identified using information obtained from previous studies (Reinecke et al., 2007; Blanchard and Holmes, 2008) and confirmed by discussions with CapeNature Conservation managers, members of WfW and private landowners.

For the purposes of this study, two tree species endemic to Fynbos riparian zones, *Brabejum stellatifolium* (L.) (Proteaceae) and *Metrosideros angustifolia* (L.) (Myrtaceae), were selected. These trees are naturally confined to the Fynbos (Richardson et al., 1992; Thuiller et al., 2006) where they prefer moist areas and therefore commonly occur along streams (Mucina and Rutherford, 2006). These tree species are abundant and important components of riparian habitats and considered key species in south-western Cape Mediterranean-type riparian systems (Galatowitsch and Richardson, 2005). In addition to these two native species, the invasive woody species *Acacia mearnsii* DeWild (L.) (Fabaceae) that commonly invades habitats dominated by *B. stellatifolium* and *M. angustifolia* was selected.

2.2.2. Arthropod collection

As CFR arthropods show substantial seasonal variation (Pryke and Samways, 2010, 2011; Roets and Pryke, 2013), sampling was conducted once during summer (2011), autumn (2011), winter (2012), and spring (2012) and the data from all four seasons were combined for analyses. Arthropods associated with the foliage of the three tree species were sampled using a modified petrol-driven Blow and Vac (Stihl, Germany) suction apparatus (Stewart and Wright, 1995). Five individuals of each of the three tree taxa of similar height and stem diameter were selected at random at each site and arthropods collected from their crowns by inserting tips of branches into the nozzle for 30s. This process was repeated 70 times on different branches for each individual tree. Catches per individual tree were kept separate. Collected arthropods were transferred to re-sealable plastic bags and stored at -20°C. All arthropods collected were assigned to morphospecies and taxonomic order (Oliver and Beattie, 1996b). Reference material was stored in 70% ethanol and is held at the University Stellenbosch Insect Collection (USEC), Stellenbosch, South Africa.

2.2.3. Statistical analyses

To establish sampling-representativity, a non-parametric richness estimator was selected, because most arthropod assemblages normally have large number of rare species (Novotny and Basset, 2000; Hortal et al., 2006). The Chao2 estimator was used as it is considered to be the least biased and most precise estimator when working with small sample sizes (Colwell and Coddington, 1994; Walther and Morand, 1998). Values were calculated using EstimateS (Colwell, 2009).

Arthropod alpha-diversity (α) (or species richness) for heavily invaded, restored and near pristine riparian invasion treatments was compared using Generalised Linear Models (GLMs). These variables were fitted to a Poisson distribution model with a log-link function using generalised estimating equations (Allison, 1999; O'Hara, 2009; Zuur et al., 2010) in Proc Genmod of SAS 9.1 (SAS Institute Inc., Cary, USA). The Poisson distribution type was selected to minimize the deviance statistic (Johnson et al., 2006). Test statistics were calculated using the penalised quasi-likelihood technique, as variances showed no over-dispersion (Bolker et al. 2008). Separate analyses were run for the three host tree species, sites within each invasion treatment type, as well as for the eight most species rich arthropod taxonomic groups (Araneae, Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera, Formicidae, and Orthoptera). Significant differences under this model are reported where $P \leq 0.05$.

Two measures of beta-diversity were assessed in this study: (i) β1[2]= species turnover among sites of the same invasion treatment (Anderson, 2006) and (ii) β2[3] = assemblage compositional changes between sites with different management histories (Anderson, 2006; Pryke et al., 2013). Species turnover among sites of the same invasion treatment (β1) was calculated using a resemblance matrix based on the Jaccard measure. The Jaccard dissimilarity measure uses only compositional (presence/absence) information and is directly interpretable as the percentage of unshared species among samples (Terlizzi et al., 2009). To determine the variability in species composition within the study sites, PERMDISP routine in the PERMANOVA+ extension in PRIMER 6 was conducted. PERMDISP (β1-diversity) determines the mean distance of samples to the geometric centre (centroid) of each predefined group (e.g. arthropods associated with *A. mearnsii* from near pristine sites) in three dimensional space (Anderson, 2006). This allows for comparisons between the mean distances to various centroids (e.g. arthropods associated with *A. mearnsii* from near pristine, restored and heavily invaded sites respectively) using ANOVA to determine F- and p-values (Anderson, 2006) and allows for pair-wise testing. These analyses were performed in PRIMER 6 (PRIMER-E 2008) with 9,999 permutations (Anderson, 2006).

Assemblage compositional differences among different invasion treatments (near pristine, heavily invaded and restored) and sites within each invasion treatment (β2, Anderson, 2006) were compared using Permutational Multivariate Analysis of Variance (PERMANOVA+) in PRIMER 6. The F and p- values for main test (as well as t values for pair-wise differences) for similarity of the eight taxonomic groups listed above between each management type and the three host trees were calculated using 9,999 permutations. Hierarchical agglomerative clustering analyses were performed using Bray-Curtis similarity (Bray and Curtis, 1957) after fourth-root transformation of data to reduce the influence of common species (Anderson, 2001). Results were visually represented using non-metric Multidimensional Scaling (nMDS) (Clarke, 1993) in PRIMER 6. All diversity indices were compared for all tree taxa combined, for each individual tree species and for the eight most species rich arthropod taxonomic groups.

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[2] **β1 = species turnover:** the variation in species richness between arthropods communities as influenced by invasion treatment of riparian sites sampled.

[3] **β2 = assemblage compositional:** variability in species composition of arthropods assemblages among invasion treatments (e.g. among near pristine and heavily invaded).

2.3. RESULTS

2.3.1. Arthropod alpha-diversity

A total of 32,341 arthropod individuals representing 967 morphospecies from 15 orders were collected. The most abundant orders were the Coleoptera (14,253), Hemiptera (5197), Diptera (3359), Araneae (1734), Hymenoptera (excluding the Formicidae) (1710), Formicidae (470), Lepidoptera (237), and Orthoptera (388). The near pristine sites had the highest number of observed and estimated species, while the heavily invaded sites had the lowest number of observed and estimated species (Table 2.1). The restored sites had intermediate numbers for observed and estimated number of species (Table 2.1). For *M. angustifolia* the estimated numbers of species at heavily invaded sites were similar to those at restored sites (Table 2.1). For *B. stellatifolium* and *A. mearnsii* the estimated number of species varied across all the riparian sites (Table 2.1).

Table **2.1**: Number of collected arthropod species (S_{obs}) and individuals as well as the estimated number of species (Chao2 = second order Chao estimator) from three tree taxa at sites that differ in invasive trees treatment (near pristine, heavily invaded and restored).

Site	S_{obs}	Individuals	Chao2
Overall	967	32341	1215 (SD±34.08)
Near pristine	868	9798	995.3 (SD±44.6)
B. stellatifolium	479	5234	679.5 (SD±32.3)
M. angustifolia	340	2373	609.3 (SD±59.3)
A. mearnsii	346	2191	488.9 (SD±31.8)
Heavily invaded	550	7667	666.9 (SD±30.8)
B. stellatifolium	250	4306	453.9 (SD±51.1)
M. angustifolia	295	2012	416.5 (SD±30.28)
A. mearnsii	257	1349	510.8 (SD±65.1)
Restored	615	12346	857.9 (SD±45.8)
B. stellatifolium	280	7976	610.5 (SD±101.7)
M. angustifolia	297	2020	435.3 (SD±33.0)
A. mearnsii	338	2350	486.9 (SD±32.4)

Generalised linear models indicated that for all arthropods from all three tree taxa combined, near pristine sites had significantly higher alpha-diversity than heavily invaded sites and intermediate alpha-diversity at restored sites. This was true for all host trees separately (Table 2.2). Species richness (for all tree species combined) was highest at near pristine sites for most arthropod orders (excluding the Formicidae and Lepidoptera), followed by restored sites, with heavily invaded sites usually containing the least number of species (Table 2.2). However, for most orders the differences in alpha-diversity among invasion treatments for individual tree species were not significant. Araneae alpha-diversity was significantly lower at heavily invaded sites for all tree species combined and for those associated with *B. stellatifolium*. The Formicidae were more species rich at restored sites, but only significantly so for all tree taxa combined and for *B. stellatifolium* (Table 2.2). Trees at different sites (individual species and data for tree taxa combined) often differed in their arthropod alpha-diversity (Table A2.1).

Table **2.2**: Summary results for Generalised linear models (Poisson distribution and log-link function) on species richness data for the overall, and eight most species-rich and abundant taxonomic groups.

Dependent variable	Overall	Tree species		
		B. stellatifolium	*M. angustifolia*	*A. mearnsii*
Species Richness				
Overall	NP = R ≥ HI	NP > R = HI	NP = R ≥ HI	NP = R ≥ HI
Araneae	NP = R > HI	NP > R > HI	NP = R = HI	NP = R = HI
Coleoptera	NP > R = HI	NP > R = HI	NP = R = HI	NP = R = HI
Diptera	NP = HI = R	NP = HI ≥ R	NP = HI = R	NP = HI = R
Hemiptera	NP > R = HI	NP > HI = R	NP = R = HI	NP = R = HI
Hymenoptera[a]	NP > R = HI	NP > R = HI	NP = R = HI	R = NP = HI
Lepidoptera	R = HI = NP	NP = R = HI	HI = R = NP	R = NP = HI
Formicidae	R > NP = HI	R > NP = HI	R = NP = HI	R = NP = HI
Orthoptera	NP = R = HI	NP = R = HI	NP = R = HI	NP = R = HI

Sites are ordered with those with the highest means on the left and the lowest on the right.

[a] All members of Hymenoptera except the Formicidae.

NP = Near Pristine, HI = Heavily Invaded, R = Restored riparian habitat types,

= signifies no significant differences, > signifies that habitats to the left are significantly more species-rich; ≥ signifies that the first habitat is significantly more species-rich than the last habitat.

2.3.2. Species turnover among sites (β1)

When combining all arthropods collected, near pristine sites had significantly higher β1-diversity (species turnover among sites) than heavily invaded and restored sites, which were statistically similar (Table 2.3). However, the influence of invasion treatment of riparian zones on β1-diversity was non-significant for most arthropod taxa separately (except Orthoptera). Orthopteran β1-diversity was significantly lower at heavily invaded sites than at near pristine and restored sites. β1-diversity for the different orders associated with specific tree species varied little among sites with differing invasion treatment (Table 2.3).

When β1-diversity of specific sites within each invasion treatment was compared, little variation was evident. The most pronounced variations were found for arthropods associated with *B. stellatifolium*. For this species, β1-diversity varied for most taxonomic orders between near pristine sites. This variability was absent at heavily invaded sites but detected again at restored sites (Table A2.2).

Table **2.3**: Results of tests for β1-diversity for host trees using the Jaccard resemblance measure.

		Tree species		
	Invasion treatment	*B. stellatifolium*	*M. angustifolia*	*A. mearnsii*
All	NP > R = HI	R = NP = HI	NP = R = HI	NP = R = HI
Araneae	NP = R = HI	HI = R = NP	R = HI = NP	R = NP = HI
Coleoptera	R = HI = NP	HI = NP = R	NP = HI = R	NP = R = HI
Diptera	NP = R = HI	HI = R = NP	HI = R = NP	HI = NP = R
Hemiptera	NP = HI = R	HI = R = NP	R = NP = HI	HI = R = NP
Hymenoptera[a]	HI = R = NP	HI = R = NP	R = NP = HI	NP = R = HI
Lepidoptera	R = NP = HI	HI = NP = R	NP = R = HI	HI = R = NP
Formicidae	NP = R = HI	R = NP = HI	R = NP = HI	HI = NP = R
Orthoptera	NP = R ≥ HI	NP = R = HI	NP = HI = R	R = HI = NP

Sites are ordered with those with the highest means on the left and the lowest on the right. [a]All members of Hymenoptera except the Formicidae. NP = Near Pristine, HI = Heavily Invaded, R = Restored riparian habitat types, = signifies no significant differences, > signifies that habitats to the left are significantly more species-rich/abundant; ≥ signifies that the first habitat is significantly more species-rich/ abundant than the last habitat.

2.3.3. Arthropod assemblage composition among sites that differ in invasion treatment (β2)

PERMANOVA analyses revealed that invasion treatment of riparian habitats significantly influenced arthropod assemblage composition when data from all trees were combined, with the exception of the Lepidoptera and Diptera (Table **2.4**, Fig **2.1a**). However, nearly all pair-wise comparisons among sites for each arthropod taxon separately (combined tree species data) did not differ significantly (Table **2.4**), except for the Coleoptera and Hemiptera.

Invasion treatment of the riparian habitat significantly influenced overall assemblage composition for *B. stellatifolium* and *A. mearnsii* (Table **2.4**). For *B. stellatifolium*, pair-wise comparisons indicated that the significant divergence between communities at near pristine- and restored sites drove its overall pattern (Table **2.4**). For *M. angustifolia*, no significant differences were detected in overall arthropod community assemblages but for a few taxa differences were observed. For *A. mearnsii* no differences were found for pair-wise comparisons among the different management types, but overall management type had a significant influence on arthropod assemblages (Table **2.4**). Pair-wise comparisons between arthropods from restored and heavily invaded habitats never differed significantly, but comparisons between near pristine and restored, and near pristine and heavily invaded habitats sometimes did (Table **2.4**). Comparisons of sites within each invasion treatment for individual host tree species also indicated that invasion treatment of riparian habitats significantly influenced arthropod assemblage composition, except for a few taxonomic groups (Table **2.5**, Table **A2.3**).

From the nMDS plot it was evident that when considering all arthropods from all three host tree species combined, sites grouped strongly according to invasion treatment (Fig **2.1a**). This was also evident when considering arthropods collected from the two native tree taxa respectively, but less so when considering the arthropod communities associated with *A. mearnsii* (Fig **2.1d**). Near pristine sampling units for *B. stellatifolium* were more closely grouped than heavily invaded and restored sampling units that were more intermixed (Fig **2.1b**). For *M. angustifolia*, heavily invaded units separated out with near pristine and restored sites intermixed (Fig **2.1c**). When considering collection of sites, samples from specific sites tended to group together for all three host trees (Fig **2.1a**).

Table **2.4**: Arthropod assemblage beta- diversity (β2) from PERMANOVA to determine similarity in the composition of arthropod assemblages among riparian habitats that differ in invasion treatment for three tree species and for the eight most species-rich and abundant taxonomic groups.

	Invasion treatment	NP versus HI	NP versus R	HI versus R
All	1.98**	1.46	1.36	1.39
Araneae	1.67**	1.34	1.29	1.26
Coleoptera	2.26**	1.59	1.68*	1.21
Diptera	0.99	0.99	1.11	0.88
Hemiptera	1.63**	1.21*	1.28*	1.37
Hymenoptera[a]	1.87**	1.45	1.45	1.21
Lepidoptera	0.78	0.92	0.97	0.73
Formicidae	1.96*	1.72	1.33	1.05
Orthoptera	2.77**	1.91	1.78	1.28
B. stellatifolium				
Overall	1.69**	1.29	1.4*	1.17
Araneae	1.18	1.15	1.09	1.01
Coleoptera	2.44**	1.53	1.79*	1.33
Diptera	0.93	1.06	1.01	0.81
Hemiptera	1.76**	1.37	1.29	1.31
Hymenoptera [a]	1.80**	1.36	1.36	1.31
Lepidoptera	0.94	1.04	0.89	0.95
Formicidae	1.27	1.65	0.63	1.08
Orthoptera	1.74	1.64	1.16	1.12
M. angustifolia				
Overall	1.41	1.17	0.99	1.39
Araneae	1.42*	1.27	1.21	1.09
Coleoptera	1.77**	1.44*	1.35*	1.21
Diptera	1.33*	1.27	1.31	0.85
Hemiptera	1.72**	1.24	1.43*	1.26
Hymenoptera[a]	1.65	1.44	1.22	1.18
Lepidoptera	1.16	1.02	1.05	1.16
Formicidae	1.91	1.69	1.35	1.08
Orthoptera	2.20*	1.52	1.92	0.82
A. mearnsii				
Overall	1.57*	1.37	1.25	1.13
Araneae	1.18	1.05	1.10	1.09
Coleoptera	1.44*	1.23	1.24	1.12
Diptera	1.36	1.14	1.34	1.01
Hemiptera	1.36*	1.23	1.11	1.15
Hymenoptera[a]	1.58*	1.35	1.27	1.14
Lepidoptera	1.00	0.96	1.28	0.75
Formicidae	1.40	1.08	1.35	1.13
Orthoptera	1.87*	1.62	1.54	0.93

Figures represent F- and t- values, number of permutations for each analysis = 9,999.

[a] All members of Hymenoptera with the exception of Formicidae. NP = Near Pristine, HI = Heavily Invaded, R = Restored riparian habitats, * $P < 0.05$, ** $P < 0.01$

Table **2.5**: Main test of arthropod assemblage beta- diversity (β2) from PERMANOVA to determine similarity in the composition of arthropod assemblages among riparian sites that are similar in invasion treatment for three tree species and for the eight most species-rich and abundant taxonomic groups.

	Near pristine	Heavily invaded	Restored
B. stellatifolium			
Overall	3.64***	3.39***	3.22***
Araneae	2.87***	1.58*	2.83***
Coleoptera	4.22***	3.19***	4.16***
Diptera	3.83***	2.24***	2.89***
Hemiptera	2.61***	2.43***	1.51*
Hymenoptera [a]	3.45***	3.40***	3.22***
Lepidoptera	1.74*	1.35	1.66*
Formicidae	1.98*	1.81*	1.81*
Orthoptera	1.75*	1.49	0.87
M. angustifolia			
Overall	3.24***	2.91***	4.07***
Araneae	2.12**	1.98***	3.59***
Coleoptera	2.97***	3.48***	3.03***
Diptera	2.72***	2.28***	3.42***
Hemiptera	3.68***	2.17***	4.02***
Hymenoptera[a]	2.45**	4.21***	3.13***
Lepidoptera	1.74*	1.57	2.21**
Formicidae	1.21	1.07	5.74***
Orthoptera	1.03	1.26	1.72
A. mearnsii			
Overall	3.63 ***	3.89***	3.39***
Araneae	1.60**	4.85***	1.68***
Coleoptera	6.45***	3.00***	3.44***
Diptera	4.73***	4.02***	4.46***
Hemiptera	2.48***	3.14***	3.31***
Hymenoptera[a]	4.52***	3.02***	2.81***
Lepidoptera	1.83*	1.88**	1.39
Formicidae	0.95	2.15**	1.38
Orthoptera	1.10	1.61	1.07

Figures represent F- values, number of permutations for each analysis = 9,999.
[a] All members of Hymenoptera with the exception of Formicidae.
* $P < 0.05$, ** $P < 0.01$, *** $P \leq 0.001$

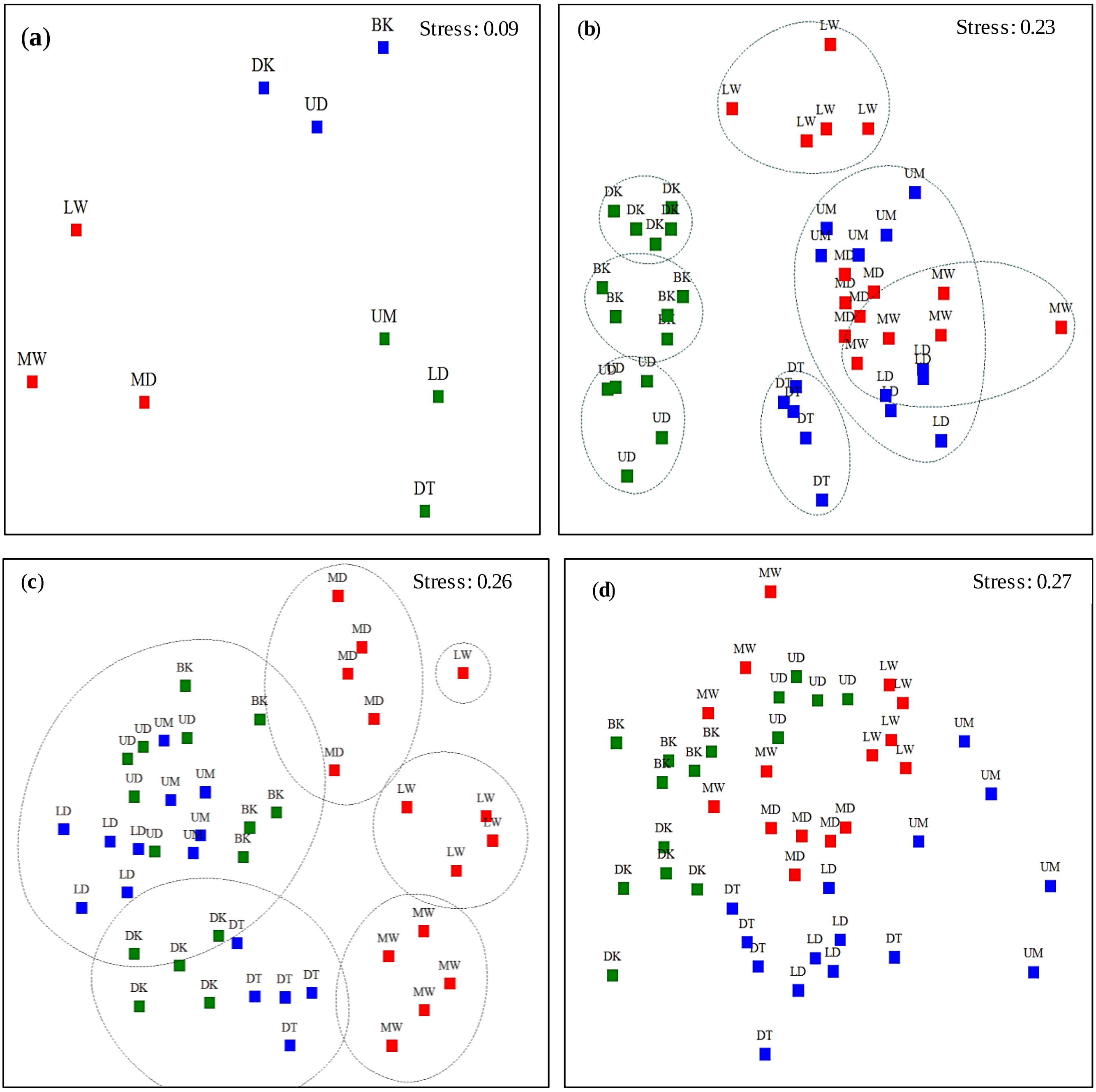

Figure **2.1**: non-metric Multidimensional Scaling (nMDS) plots of arthropod assemblages from near pristine (green), heavily invaded (red), and restored (blue) riparian habitats for (**a**) all arthropods from all host trees combined, (**b**) arthropods collected from, *B. stellatifolium* (**c**) arthropods collected from *M. angustifolia* and (**d**) arthropods collected from *A. mearnsii*. The ellipses represent sampling units which were 25% similar. UD = Upper Dwars, DK = Du toits Kloof, BK = Bains Kloof, MD = Mid Dwars, LW = Lower Wit, MW = Mid Wit, LD = Lower Dwars, DT = Du Toit, and UM = Upper Molenaars collection sites.

Considering all arthropods collected, near pristine sites had proportionately higher numbers of unique species 317 (29.8%), higher than either the restored 282 (29.2%) or heavily invaded 217 (22.4%) riparian habitats (Table **2.6**). This was true for all separate tree species. Araneae had proportionately higher numbers of unique species 56 (5.8%) in restored sites, higher than either the near pristine 46 (4.8%) or heavily invaded 35 (3.6 %) riparian habitats (Table **2.6**) for all separate tree species.

Table **2.6**: Number of common arthropod species (considering only those with more than four individuals collected throughout the study period) that were unique to a specific habitat or tree species, for various assemblages collected from CFR riparian habitats. (Percentage of total in parenthesis).

	Near Pristine	Heavily Invaded	Restored
All	317 (29.8)	217 (22.4)	282 (29.2)
Araneae	46 (4.8)	35 (3.6)	56 (5.8)
Coleoptera	63 (6.5)	49 (5.1)	52 (5.4)
Diptera	48 (4.9)	23 (2.4)	35 (3.6)
Hemiptera	74 (7.7)	43 (4.4)	41 (4.2)
Hymenoptera[a]	35 (3.6)	28 (2.9)	30 (3.1)
Lepidoptera	17 (1.8)	17 (1.8)	28 (2.9)
Formicidae	7 (0.7)	4 (0.4)	7 (0.7)
Orthoptera	10 (1.0)	6 (0.6)	10 1.0)
B. stellatifolium			
Overall	270 (39.4)	188 (27.4)	219 (31.9)
Araneae	60 (8.8)	27 (3.9)	46 (6.7)
Coleoptera	58 (8.5)	50 (7.3)	45 (6.6)
Diptera	42 (6.1)	31 (4.5)	29 (4.2)
Hemiptera	57 (8.3)	32 (4.6)	40 (5.8)
Hymenoptera[a]	22 (3.2)	16 (2.3)	20 (2.9)
Lepidoptera	7 (1.0)	8 (1.2)	10 (1.5)
Formicidae	4 (0.6)	3 (0.4)	7 (1.0)
Orthoptera	3 (0.4)	2 (0.3)	1 (0.1)
M. angustifolia			
Overall	241 (38.9)	199 (32.1)	220 (35.5)
Araneae	49 (7.9)	32 (5.2)	54 (8.7)
Coleoptera	47 (7.6)	49 (7.9)	52 (8.4)
Diptera	34 (5.5)	39 (6.3)	29 (4.7)
Hemiptera	56 (9.0)	36 (5.8)	35 (5.7)
Hymenoptera[a]	32 (5.2)	22 (3.6)	23 (3.7)
Lepidoptera	4 (0.6)	6 (1.0)	3 (0.5)
Formicidae	3 (0.5)	1 (0.2)	5 (0.8)
Orthoptera	0 (0.0)	2 (0.3)	4 (0.6)
A. mearnsii			
Overall	256 (38.7)	187 (28.3)	262 (39.6)
Araneae	49 (7.4)	31 (4.7)	54 (8.2)
Coleoptera	53 (8.0)	35 (5.3)	59 (8.9)
Diptera	35 (5.3)	31 (4.7)	28 (4.2)

Hemiptera	49 (7.4)	30 (4.5)	40 (6.1)
Hymenoptera[a]	29 (4.4)	25 (3.8)	29 (4.4)
Lepidoptera	5 (0.8)	11 (1.7)	13 (1.9)
Formicidae	7 (1.1)	6 (0.9)	3 (0.5)
Orthoptera	2 (0.3)	4 (0.6)	5 (0.8)

[a] All members of Hymenoptera with the exception of Formicidae.

2.4. DISCUSSION

Many studies have investigated the effects of Invasive Alien Plants (IAPs) on species richness of arthropods. Although some reported no effect on certain taxa (e.g. Robertson et al., 2011), the vast majority indicated that IAPs have a negative effect on certain arthropod taxa (e.g. Samways and Moore, 1991). Three main invasion treatments of riparian habitats compared in this study were found to differ in alpha diversity of arthropods. Near pristine sites had higher species richness than restored sites, with heavily invaded sites housing fewest species. Therefore, after removing IAPs the habitats can be recolonised by arthropods, with alpha diversity returning to near pristine riparian habitat levels. Low arthropod species richness in heavily invaded sites was expected given similar results from other studies that have investigated the impacts of invasive alien plants on arthropod populations and communities across a wide variety of habitats; both within South Africa (Samways and Moore, 1991; Samways et al., 1996; Steenkamp and Chown, 1996; Ratsirarson et al., 2002; Samways and Taylor, 2004; Coetzee et al., 2007; Samways et al., 2011; Roets and Pryke, 2013) and elsewhere (Toft et al., 2001; Greenwood et al., 2004; Ernst and Cappuccino, 2005; Bultman and Dewitt, 2008). However, unlike these studies the focus of this study was on arthropods associated with particular trees rather than arthropods associated with the entire ecosystems. Loss in some arthropod species in invaded sites may therefore be independent of changes in plant diversity, vegetation structure and microclimatic conditions (see Litt et al., 2014). These changes would be worth exploring in future studies.

When analysing alpha diversity of the various arthropod taxa, Coleoptera, Hemiptera and Hymenoptera showed significantly higher species richness in near pristine sites than in heavily invaded and restored sites. Low species richness of Hymenoptera in restored sites was unexpected, as one would predict that these highly mobile insects would rapidly immigrate to restored sites and increase their numbers through time (for example see Williams, 1993). Heavily invaded sites may lack the abundance of native flowering plants usually pollinated by hymenoptera species, resulting in a migration of these species to other areas where native plants are more abundant (Lopezaraiza-Mikel et al., 2007). No significant change in species richness of Formicidae was detected between near pristine and heavily invaded sites. Similarly, French and Major, (2001) found no significant differences in the species richness of Formicidae (ants) between areas of South African Fynbos invaded by *Acacia saligna* and native sites. Contrary to invaded sites, restored sites supported significantly higher species richness of Formicidae. This suggests that restored sites appear to be benefiting ants, although the mechanisms behind this pattern are unclear.

For Araneae, near pristine and restored sites were significantly more species rich than heavily invaded sites were. Mgobozi et al., (2008) also found significant negative impacts of an invasive shrub (*Chromolaena odorata*) on Araneae assemblage richness in South African savannah. In contrast, Robertson et al., (2011) showed that species richness of Araneae did not significantly differ between invaded and uninvaded fragments in Kruger National Park of South Africa. The reduced richness of Araneae in heavily invaded sites could imply reduced predation pressure of folivorous insects (members of Hemiptera and Coleoptera) (Simao et al., 2010), eventually exacerbating folivore damage to native plant species (Halaj and Wise, 2001). This decline in Araneae richness in heavily invaded sites suggests that it would be beneficial to quantify damage levels to plants across all invasion treatments to explore the possible consequences of altered Araneae richness.

Contrary to Araneae species richness, no differences in richness were detected for Diptera, Lepidoptera and Orthoptera, across all invasion treatments. These results suggest that current management practices in riparian zones in South Africa are not having a major impact on their species richness and that these orders may be less important as indicator groups when assessing IAP management. These findings are similar to Harris et al.'s (2004) argument that invasive plants do not necessarily have to impact biodiversity negatively. In their study, *Ulex europaeus* (an exotic invasive shrub in New Zealand) supported more species of some taxonomic groups than did native Kanuka trees (*Kunzea ericoides*).

Considering all arthropods, restored and heavily invaded sites had much more homogenous arthropod communities as compared to near pristine sites. This pattern was strongly evident for arthropods associated with *B. stellatifolium*. For this species, different near pristine sites varied considerably with regards to β1-diversity. This variability was lost somewhat in invaded sites, but again returned for some taxonomic groups after restoration. However, although arthropod alpha-diversity at restored sites is similar overall to near pristine sites, much natural variability that is expected over the landscape is lost despite these restoration efforts. This suggests that after restoration of a riparian ecosystem, a site is usually recolonised by a community consisting of similar, abundant arthropod taxa while the rarer taxa fail to recolonize. It is possible that, given enough time, rarer arthropod taxa would also recolonise the restored habitats and ultimately increase variability between these areas. Possible reasons for significantly higher β1-diversity for arthropods in near pristine sites are numerous: (i) higher heterogeneity in both plant species composition and structure (Walz, 2011). There is current evidence that diverse habitats support higher biological diversity than monotypic ones, thus allowing more species to coexist (Mlambo et al., 2011), (ii) spatial autocorrelation i.e. sites that are further apart have a tendency to differ drastically in arthropod species composition (Horak, 2013).

Interestingly, not all native host taxa reacted similarly in terms of the response of their arthropod communities to invasion and restoration. For example, β2-diversity of arthropod communities on *B. stellatifolium* were fairly similar between restored and invaded sites, while on *M. angustifolia* the arthropod communities from restored sites were more similar to near pristine sites. Restoration success therefore seems to vary considerably between different plant taxa and their respective arthropod communities and they need different lengths of time to regenerate. The reason for this is unclear, but may be due to changes in plant characteristics (e.g. physical structure, host abundance) associated with plant invasion (for example see Lathrop et al., 2003). It is possible that plant characteristics that may alter quality of habitats for arthropods for *M. angustifolia* did not change in the presence of IAPs, hence arthropods were quick to recolonise *M. angustifolia* individuals after removal of IAPs. Conversely, IAPs appear to have heavily influenced the characteristics of *B. stellatifolium* thereby delaying the return of arthropod communities to their original state.

When considering arthropod communities from *A. mearnsii*, β2-diversity varied substantially among different collection sites and among invasion treatments. This was also evident for numerous taxa. However, invasion treatment had a lesser effect on grouping of communities than it did on collection sites. This can be explained if one considers that most arthropods associated with *A. mearnsii* are actually associated with the surrounding vegetation rather than *A. mearnsii* itself (as can be expected from a non-native plant in accordance with the enemy release hypothesis (Wolfe, 2002; Siemann and Rogers, 2003)). Although limited information exists on the arthropod communities of *A. mearnsii* in its invaded range, it is colonised almost exclusively by native arthropods within forestry plantations (Govender, 2007, DEA, 2009). The arthropod communities associated with *A. mearnsii* are therefore expected to reflect the general communities associated with the specific sites where it is found.

The results of this study are largely in accordance with the findings of other studies (e.g. Wishart et al., (2002), King and Schale, (2001), and Samways et al., (2011)) that found that individual rivers of Fynbos bioregions of the Western Cape have specific arthropod communities. This is not surprising, given the high spatial variability in Mediterranean-type ecosystems (Caterino, 2007). Interestingly, the three Fynbos studies mentioned were limited to aquatic invertebrates while this study focused on terrestrial invertebrates. Thus, the phenomenon of specific river catchment arthropod communities may prevail even when the organisms in these systems are not directly dependant on the water itself.

The results further highlighted the importance of conserving and maintaining near pristine sites for sustaining overall diversity in riparian habitats as these contain numerous unique species (particularly Hemiptera and Coleoptera). Unique species are perceived as important in ecological systems and their preservation is often the ultimate aim of biological monitoring (Lenat and Resh, 2001). The recolonisation of restored habitats by particularly rare arthropods will also depend greatly on the availability of nearby suitable habitat. For example, it has also previously been demonstrated that irrespective of fragment size, all fragments of natural habitat in CFR are important for the conservation of many endemic species (Kemper et al., 1999).

To conclude, the above results clearly underscore that alpha and beta-diversity of arthropods are greatly impacted by different management practices of riparian habitats. Removal of IAPs appears to benefit species richness of the majority of taxonomic groups and complete removal of IAPs patches in riparian habitats is a difficult but crucial task (van Wilgen et al., 2012). Arthropod beta diversity demonstrated that a change in species composition may be a better measure than alpha diversity to detect shifts in arthropod communities induced by different management practices of riparian habitats than species richness alone (e.g. Pryke et al., 2013). These changes in community composition may have profound influences on the normal functioning of riparian ecosystems. Restoration success should also be evaluated on a per species basis when considering arthropods associated with foliage as different hosts vary with regards to restoration success of their associated organisms.

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APPENDIX **2**

Table **A2.1**: Summary results for Generalised linear models (Poisson distribution and log-link function) (for each host tree) on comparison of species richness data for the overall, and eight most species-rich and abundant taxonomic groups among sites within each invasion treatment.

		Tree species		
Invasion treatment	Dependent variable	*B. stellatifolium*	*M. angustifolia*	*A. mearnsii*
Near pristine	Overall	DK = UD > BK	UD >BK = DK	BK = DK > UD
	Araneae	DK ≥ UD = BK	UD = BK = DK	BK = DK = UD
	Coleoptera	DK > UD = BK	DK = BK = UD	DK = BK > UD
	Diptera	UD > DK = BK	UD ≥ DK = BK	DK = BK = UD
	Hemiptera	DK = UD = BK	UD = BK = DK	BK = DK = UD
	Hymenoptera[a]	DK = UD = BK	DK = UD = BK	BK = UD = DK
	Lepidoptera	UD = DK = BK	UD = BK = DK	BK = UD = DK
	Formicidae	UD = DK = BK	DK = UD = BK	DK = UD = BK
	Orthoptera	DK = UD = BK	BK = DK = UD	DK = UD = BK
Heavily invaded	Overall	LW = MD = MW	MD > MW = LW	MD > LW > MW
	Araneae	MD = LW = MW	MD = MW > LW	MD > MW = LW
	Coleoptera	MD = LW = MW	MD > LW = MW	MD > LW = MW
	Diptera	LW ≥ MW = MD	MD > LW = MW	MD = LW > MW
	Hemiptera	MW ≥ LW = MD	MW = MD = LW	MD > MW = LW
	Hymenoptera[a]	MW = MD = LW	MD ≥ MW = LW	MD = LW ≥ MW
	Lepidoptera	LW= MW = MD	MD = LW = MW	MD = LW = MW
	Formicidae	MD = MW = LW	MW = MD = LW	LW > MW = MD
	Orthoptera	MW = LW = MD	MD = MW = LW	MD = LW = MW
Restored	Overall	DT ≥ LD = UM	UM = LD = DT	LD = DT > UM
	Araneae	DT ≥ LD = UM	LD = UM = DT	LD = DT > UM
	Coleoptera	UM = DT = LD	UM = DT = LD	LD > DT > UM
	Diptera	DT > LD = UM	DT = UM = LD	DT = LD = UM
	Hemiptera	LD = DT = UM	UM = DT = LD	LD = DT > UM
	Hymenoptera[a]	DT > LD = UM	LD = DT = UM	DT = LD ≥ UM
	Lepidoptera	DT = LD = UM	DT = UM = LD	DT = LD = UM
	Formicidae	DT = LD = UM	UM = LD = DT	DT = LD = UM
	Orthoptera	LD = UM = DT	UM = LD = DT	LD = UM = DT

Sites are ordered with those with the highest means on the left and the lowest on the right. [a] All members of Hymenoptera except the Formicidae. UD = Upper Dwars, DK = Du toits Kloof, BK = Bains Kloof, MD = Mid Dwars, LW = Lower Wit, MW = Mid Wit, LD = Lower Dwars, DT = Du Toit, and UM = Upper Molenaars rivers; = signifies no significant differences, > signifies that habitats to the left are significantly more species-rich/abundant; ≥ signifies that the first habitat is significantly more species-rich/ abundant than the last habitat.

Table **A2.2**: Comparisons of β1-diversity of sites within each invasion treatment for each host tree using the Jaccard resemblance measure.

		Tree species		
Invasion treatment	Dependent variable	*B. stellatifolium*	*M. angustifolia*	*A. mearnsii*
Near pristine	Overall	BK ≥ UD = DK	DK = BK = UD	BK = DK = UD
	Araneae	BK ≥ UD = DK	UD = DK = BK	DK = BK = UD
	Coleoptera	UD ≥ BK = DK	DK = BK = UD	BK = DK = UD
	Diptera	DK = BK ≥ UD	BK = DK = UD	UD = BK = DK
	Hemiptera	BK ≥ UD = DK	DK = BK = UD	DK = UD = BK
	Hymenoptera[a]	DK ≥ BK = UD	BK = UD = DK	BK = DK = UD
	Lepidoptera	BK = DK = UD	DK = UD = BK	UD = DK = BK
	Formicidae	BK = DK = UD	BK = DK = UD	BK = DK = UD
	Orthoptera	BK = DK = UD	DK = UD = BK	BK = UD =DK
Heavily invaded	Overall	MW = LW = MD	LW = MD = MW	MW = LW > MD
	Araneae	MW = LW = MD	LW = MW = MD	MD = MW = LW
	Coleoptera	MW = MD = LW	LW = MD = MW	LW = MW = MD
	Diptera	MD = MW = LW	MW = LW = MD	MW = LW = MD
	Hemiptera	LW = MD = MW	MD = LW = MW	LW = MW = MD
	Hymenoptera[a]	MW = LW = MD	MW = LW = MD	MD = MW = LW
	Lepidoptera	MD = LW = MW	MD = LW = MW	LW = MD = MW
	Formicidae	LW = MW = MD	MD = MW = LW	MD = MW ≥ LW
	Orthoptera	LW = MD = MW	LW = MD = MW	MW = LW = MD
Restored	Overall	DT = LD = UM	DT = LD = UM	UM = DT = LD
	Araneae	LD = DT = UM	DT = LD = UM	UM ≥ DT = LD
	Coleoptera	DT ≥ LD= UM	UM = DT = LD	UM = LD = DT
	Diptera	UM = LD = DT	LD = DT = UM	UM = LD = DT
	Hemiptera	DT = LD = UM	LD = DT = UM	DT = UM = LD
	Hymenoptera[a]	UM ≥ LD = DT	UM = DT = LD	DT = UM = LD
	Lepidoptera	UM = LD = DT	LD = DT = UM	UM = DT = LD
	Formicidae	DT = LD = LD	LD = UM = DT	UM = LD = DT
	Orthoptera	DT > LD > DT	DT = UM = LD	LD = DT = UM

Sites are ordered with those with the highest means on the left and the lowest on the right. [a] All members of Hymenoptera except the Formicidae. UD = Upper Dwars, DK = DutoitKloof, BK = BainsKloof, MD = Mid Dwars, LW = Lower Wit, MW = Mid Wit, LD = Lower Dwars, DT = DuToit, and UM = Upper Molenaars rivers; = signifies no significant differences, > signifies that habitats to the left are significantly more species-rich/abundant; ≥ signifies that the first habitat is significantly more species-rich/ abundant than the last habitat.

Table **A2.3**: Pairwise test of arthropod assemblage beta- diversity (β2) from a Permutation multivariate analysis (PERMANOVA) to determine similarity in the composition of arthropod assemblages among riparian sites that are similar in invasion treatment for three tree species and for the eight most species-rich and abundant taxonomic groups.

	Near pristine			Heavily invaded			Restored		
Dependent variable	UD vs DK	UD vs BK	DK vs BK	MD vs LW	MD vs MW	LW vs MW	DT vs UM	DT vs LD	UM vs LD
B. stellatifolium									
Overall	2.09**	1.82**	1.81**	1.95**	1.78**	1.80**	1.85**	1.67**	1.87**
Araneae	1.91**	1.46 **	1.71**	1.38*	1.17	1.22	1.94**	1.46**	1.65**
Coleoptera	2.19**	1.92**	2.05**	2.24**	1.54**	1.60**	2.11**	1.50*	2.51**
Diptera	1.77**	2.31**	1.81**	1.44**	1.27*	1.81**	1.74**	1.63**	1.72**
Hemiptera	1.96**	1.48**	1.42**	1.53**	1.58*	1.57*	1.09	1.27*	1.31*
Hymenoptera[a]	1.96**	1.83**	1.79**	1.75**	1.98*	1.80*	1.71**	2.03**	1.68*
Lepidoptera	1.22	1.41*	1.32	1.12	1.12	1.25	1.29	1.50*	1.05
Formicidae	1.78*	1.32	1.15	1.65*	1.49	0.92	1.05	1.84*	1.13
Orthoptera	1.49	1.36	1.12	1.05	1.36	1.27	1	0.9	0.89
M. angustifolia									
Overall	1.89**	1.83**	1.68**	1.63**	1.85**	1.64**	2.13**	1.91**	2.02**
Araneae	1.18	1.45*	1.72**	1.46**	1.45*	1.31*	1.83*	1.79**	2.09**
Coleoptera	1.76**	1.97**	1.44*	1.84**	1.99**	1.79**	1.69*	1.91**	1.64**
Diptera	2.01**	1.72**	1.25*	1.67**	1.56**	1.31*	2.15**	1.65**	1.85**
Hemiptera	1.92**	2.08**	1.77**	1.25*	1.72**	1.48**	2.37**	1.81**	1.88**
Hymenoptera[a]	2.07*	1.31	1.41*	1.91**	2.18**	2.05**	1.77**	2.07**	1.48*
Lepidoptera	1.31	1.10	1.48*	0.86	1.41	1.42	1.66*	1.08	1.77*
Formicidae	1.23	0.95	1.11	1.13	1.11	0.83	3.69**	2.83**	0.87
Orthoptera	1	0.9	1.12	1.12	0.85	1.33	1.25	1.07	1.63
A. mearnsii									
Overall	2.09**	1.89**	1.73**	2.11**	1.96**	1.86**	1.83**	1.89**	1.82**
Araneae	1.19	1.39**	1.21	2.57**	1.87**	2.18**	1.24**	1.36*	1.31**

Coleoptera	3.05**	2.94**	1.65**	1.69**	1.87**	1.66**	1.75**	2.00 **	1.83**
Diptera	2.09**	1.99**	2.48**	2.33**	1.94**	1.81**	2.39**	2.26**	1.74**
Hemiptera	1.73**	1.43*	1.51**	1.91**	1.69**	1.72**	1.83**	1.54**	2.11**
Hymenoptera[a]	2.83**	1.69**	1.92**	1.94**	1.55**	1.74**	1.63**	1.46*	1.92**
Lepidoptera	0.94	1.44*	1.67*	1.20	1.62*	1.29	0.84	1.36	1.31
Formicidae	0.92	1	1	1.66*	1.33	1.43	1.28	1.32	0.92
Orthoptera	1.03	0.89	1.21	1.68*	1.02	1.01	1	1.09	1.00

Figures represent t- values, number of permutations for each analysis = 9,999.

[a] All members of Hymenoptera with the exception of Formicidae.

vs = versus

UD = Upper Dwars, DK = Du toits Kloof, BK = Bains Kloof, MD = Mid Dwars, LW = Lower Wit, MW = Mid Wit, LD = Lower Dwars, DT = Du Toit, and UM = Upper Molenaars rivers

$P < 0.05$, ** $P < 0.01$, *** $P \leq 0.001$

Table **A2.4**: Numbers of arthropods for the eight most species-rich and abundant taxonomic groups caught in each site.

	Near pristine			Heavily invaded			Restored		
	UD	DK	BK	MD	LW	MW	DT	UM	LD
Abundance									
Araneae	265	205	185	137	81	99	167	399	196
Coleoptera	1169	1184	1514	1847	698	676	4094	1124	1947
Diptera	436	364	356	406	390	193	311	519	384
Hemiptera	880	461	1007	399	312	289	867	415	567
Hymenoptera[a]	244	319	304	101	94	243	108	99	198
Lepidoptera	38	11	23	21	31	26	25	33	29
Formicidae	30	13	4	14	72	114	120	27	76
Orthoptera	67	40	55	42	15	43	53	14	59
Species richness									
All insects	329	342	313	230	248	200	218	244	298
Araneae	70	66	55	36	34	29	36	59	62
Coleoptera	67	86	73	66	66	46	61	48	73
Diptera	43	52	47	38	44	31	32	31	42
Hemiptera	83	76	81	50	52	55	46	52	61
Hymenoptera	37	34	34	22	26	21	19	29	30
Lepidoptera	10	6	8	9	10	8	7	13	9
Formicidae	6	8	3	4	4	7	5	8	6
Orthoptera	12	12	11	9	7	7	9	6	13

[a] All members of Hymenoptera with the exception of Formicidae.

UD = Upper Dwars, DK = Du toits Kloof, BK = Bains Kloof, MD = Mid Dwars, LW = Lower Wit, MW = Mid Wit, LD = Lower Dwars, DT = Du Toit, and UM = Upper Molenaars rivers

CHAPTER 3

Invasion, alien control and restoration: legacy effects linked to folivorous insects and fungal pathogens.

ABSTRACT

Invasive alien trees compete with native trees for resources and therefore increase native plant stress. Native trees in invaded habitats may therefore become more vulnerable to attack by folivorous insects and fungal pathogens. Restoration of invaded habitats (i.e. alien tree removal) is expected to ameliorate these negative impacts and the damage caused by these organisms should return to pre-invasion levels unless there are legacy effects. This was tested at sites subject to different invasion treatments (near pristine, heavily invaded by *A. mearnsii* and restored (>7 years after *A. mearnsii* removal)). At each site, the levels of damage caused by leaf fungal pathogens and folivorous insects were determined for two native trees (*Brabejum stellatifolium* and *Metrosideros angustifolia*) and one invasive species, *Acacia mearnsii*. The observed differences in levels of leaf damage were then correlated to changes in native plant diversity and/or host abundance across sites. For *B. stellatifolium*, folivore and pathogen damage was significantly higher at invaded sites than at near pristine sites. At restored sites, pathogen and folivore damage was still significantly higher than at near pristine sites, with folivore damage levels even higher than at heavily invaded sites. For *M. angustifolia*, damage caused by leaf fungal pathogens and folivorous insects was also higher at restored sites than at heavily invaded and near pristine sites. For *A. mearnsii*, damage caused by both folivorous insects and leaf fungal pathogens increased at heavily invaded sites, but at restored sites it returned to levels similar to near pristine sites. Differences in native plant diversity did not explain these patterns, as restored sites had similar diversity levels to the near pristine sites. Contrary to expectations, as native host abundance increased, damage caused by leaf fungal pathogens and folivorous insects decreased. For *A. mearnsii*, increased host abundance was positively correlated to increased folivore and pathogen damage. Overall, the results showed that, although plant species richness often recover after invasive alien removal, native trees continue to be damaged by folivorous insects and fungal pathogens resulting in damage levels in excess of that seen at invaded sites. The impact of invasive plants and their control may therefore have many unexpected ecological consequences that are still poorly understood.

Keywords: Folivorous insect, Leaf pathogen, Host plant abundance, Riparian vegetation, Invasion treatment.

3.1. INTRODUCTION

In terrestrial ecosystems, folivorous insects and fungal pathogens have strong impacts on their host plants, altering reproduction, growth and survival and eventually distribution and community composition of plant species (Dobson and Crawley, 1994; Weste et al., 2002). These impacts translate into economic costs, for example forest pests in the USA, regularly damage up to 20 million ha of forest with economic costs that exceed $1 billion/year (Ayres and Lombardero, 2000). Pest and pathogen loads are highly variable, both in space and in time. Variability exists due to host plant stress levels (Gilbert and Hubbell, 1996), plant species richness (Scherber et al., 2010b) host abundance (Knops et al., 1999; Mitchell et al., 2002; Bell et al. 2006; Emiko and Meentemeyer, 2007) and plant nutritional status (Forkner and Hunter, 2000; Moran and Scheidler, 2002; Gnuner et al, 2005).

Native plant stress induced by presence of Invasive Alien Plants (IAPs) has been hypothesised to negatively impact on the susceptibility of host plants to attack by natural enemies (folivorous insects and fungal pathogens) since IAPs alter factors such as water, sunlight and nutrients (Mack et al., 2000; Daehler, 2003; Brooks et al., 2004). These factors have been shown to affect many aspects of host-plant/pest (and pathogen) dynamics (Weisser and Siemann, 2004; White et al., 2006). Habitat fragmentation is also considered a driver of host-plant/pest dynamics as shown in the tree *Micropholis venulosa* in tropical rainforest fragments which suffered increased fungal and folivore damage due to stress associated with forest fragmentation (Benítez-Malvido et al., 1999).

In South Africa, woody invasive alien trees have been targeted for removal in riparian areas, in a 15 year old landscape level restoration initiative led by the extended public works programme, Working for Water (WfW). A body of evidence relating to the lack of effectiveness of the WfW initiative is emerging (Beater et al., 2008; De Lange and van Wilgen, 2010; van Wilgen et al., 2012). Clearing of IAPs, through manual control measures, can affect the interactions between plants and their associated organisms through several direct and indirect pathways. Clearing results in gap formation, alters the ecosystem microenvironment (including light, moisture, and temperature regimes) and increases patchiness and habitat heterogeneity (Torchin and Mitchell, 2004). As folivore populations may directly depend on these factors, their impact on host plants is also likely to change in response to the altered conditions (Folgarait et al., 1995; Facelli et al., 1999).

The influence of plant species diversity on performance of natural enemies has been a focus of several experimental studies (Pfisterer et al., 2003; Agrawal et al., 2006; Cornelissen et al., 2008; van Hezewijk et al., 2008; Scherber et al., 2010b). Specifically, many studies have found that native plant diversity can increase or decrease stability, diversity and performance of natural enemies (Mitchell et al., 2002; LoGiudice et al., 2003; Keesing et al., 2006, Jactel and Brockerhoff, 2007; Johnson et al., 2008). Two hypotheses have been proposed to explain such differences (Root, 1973). The 'resource concentration' hypothesis predicts that folivores especially specialist folivores increase in abundance with increasing concentration of preferred resources in homogenous stands of host plants as compared heterogeneous stands (Grez and González, 1995). Higher abundance of folivores in plant monocultures may result in higher levels of folivory on host plants. The 'enemies' hypothesis states that the lower folivory in heterogeneous communities is a result of effectiveness of predators and parasitoids in controlling insect pests densities (Bach, 1980a; Riihimäki et al., 2005).

Apart from plant species diversity, host abundance can also influence the prevalence of folivorous arthropods and fungal pathogens (as reviewed by Agrawal et al., 2006). Numerous hypotheses relating to host abundance and folivore attack rates generally predict increased attack rates as host abundance increases (reviewed by Cook and Holt, 2006; Agrawal et al., 2006). Furthermore, some studies have found evidence that natural enemy attack is reduced on plants in dense stands compared to plants in sparse stands (Bach, 1980a; Schowalter, 1996; Garrett et al., 2000). Changes in host abundance might influence: (i) the quality and quantity of resources (host plant individuals) available for fungal pathogens or folivores to infest (Bach, 1980a; Cook and Holt, 2006), and (ii) the spatial arrangement of host plants (Alexander 1992; Folgarait et al., 1995). There is evidence that some folivore species find host plants more efficiently, and stay for a longer period of time, in dense stands than they do in sparse stands (Kelty, 2000) and (iii) the microclimatic conditions under which the folivore/pathogen and host interact (Folgarait et al., 1995; Facelli et al., 1999).

How host plant abundance and native tree species richness affects plant exposure to fungal pathogen and folivorous insect attack in a restoration scenario has rarely been tested (Covington et al., 1997; Werner et al., 2005). The aim of this study was to investigate whether differences exist in levels of leaf damage by folivores and fungal pathogens on an invasive alien plant (*A. mearnsii* DeWild (L.)) and two native plants (*B. stellatifolium* (L.) and *M. angustifolia* (L.)) in riparian sites with different invasion treatments: a) near pristine (few *A. mearnsii* individuals), b) heavily invaded (*A. mearnsii* dominated communities); and c) restored sites that were cleared of invasive *A. mearnsii* more than seven years ago. The roles of differences in host abundance and native tree species richness in explaining observed differences in folivore and pathogen damage levels were evaluated. These data may provide tools for evaluating restoration protocols in the future.

3.2. METHODS

3.2.1. Focal species and study sites

The study was conducted in mountain stream and foothill sections of three riparian systems within the south-western Cape, South Africa (Fig. **3.1**). The area is characterised by a Mediterranean–type climate with cool rainy winters (June-August) and hot dry summers (December–February) (Deacon et al., 1992). The riparian ecosystems contain some Fynbos vegetation (dominated by Ericaceae, Proteaceae and Restionaceae) (Mucina and Rutherford, 2006) and include a variety of tree taxa that may form forest pockets (Goldblatt and Manning, 2000). Nine study sites in three different catchments were selected based on invasion treatment: three near pristine sites (N) (reference sites), three heavily invaded (H) sites (dominated by *A. mearnsii*), and three restored sites (R) (formerly invaded sites that had been cleared of invasive plants more than 7 years prior to this study) (Fig. **3.1**). Reference sites are frequently used in restoration to provide direction for restoration goals (Blanchard and Holmes, 2008). Potential sites were located using information from previous studies (Reinecke et al., 2007; Blanchard and Holmes, 2008) and through discussions with Western CapeNature Conservation managers, members of WfW and private landowners.

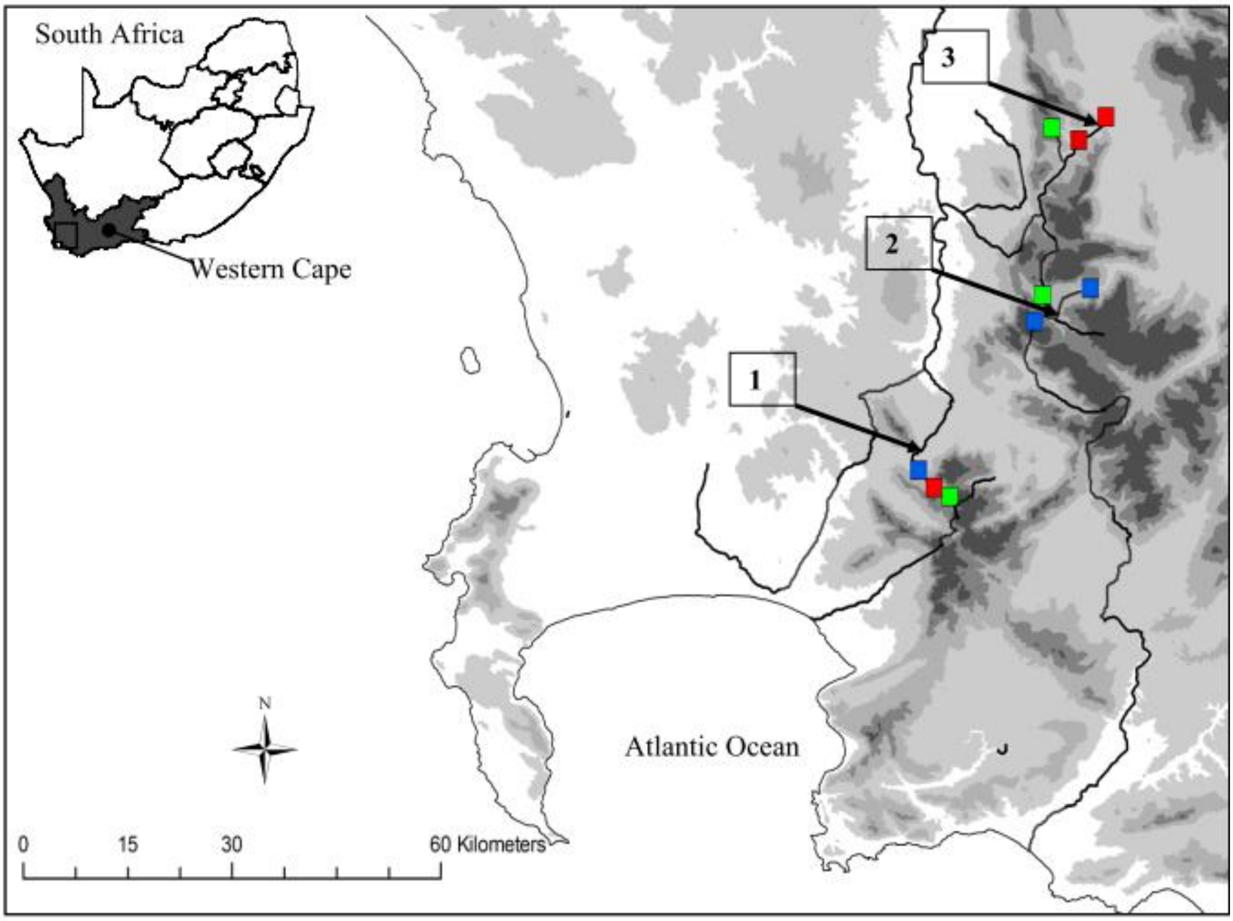

Figure **3.1**: Location of the three Western Cape rivers: 1= Dwars, 2 = Molenaars, and 3 = Wit and the nine sites (green: near pristine, red: heavily invaded and blue: restored) used in this study.

Two species native to Fynbos riparian zones, *Brabejum stellatifolium* (Proteaceae) and *Metrosideros angustifolia* (Myrtaceae) (Reinecke and King, 2007) (Fig. **3.2 D and E**), were selected as they are common components of these riparian habitats and considered key species in south-western Cape Mediterranean-type riparian systems (Galatowitsch and Richardson, 2005). Within the riparian ecosystems studied, invasive taxa include numerous Australian acacias such *A. cyclops* (A. Cunn. ex G. Don), *A. longifolia* (Andrew) Wild), *A. saligna* ((Labill.) H.L.Wendl.) and *A. melanoxylon* (R.Br). However, *A. mearnsii* (Fabaceae) (Fig. **3.2 F**) is by far the most abundant and was subsequently selected for comparisons with the selected native species. It is also considered the most problematic species in these riparian systems (Dye and Jarmain, 2004).

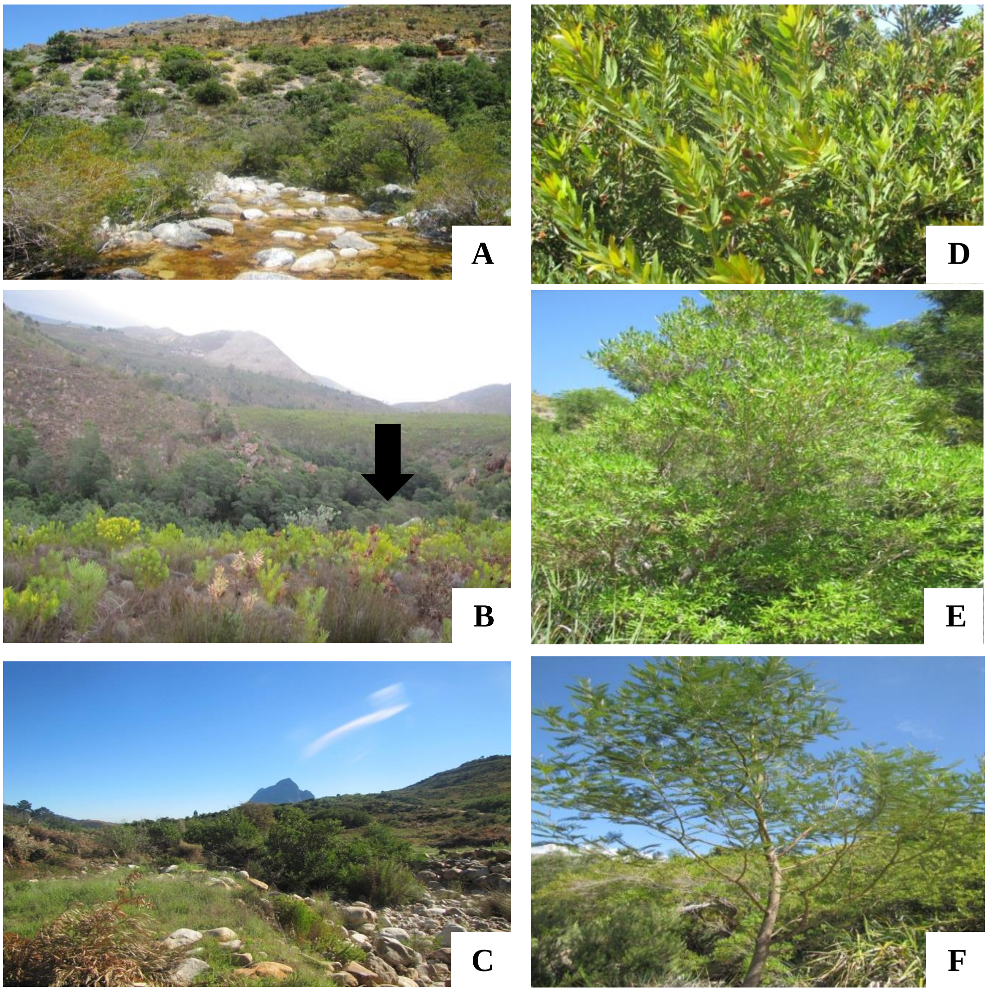

Figure **3.2**: Images of representative sites that differ in invasion treatment (**A**) near pristine, (**B**) heavily invaded (black arrow signifies heavy infestation of *A. mearnsii*) and (**C**) restored and the three focal tree taxa used in this study (**D**) *Brabejum stellatifolium*, (**E**) *Metrosideros angustifolia*, and (**F**) *Acacia mearnsii*.

3.2.2. Extent of folivorous insect and pathogen damage

At each of the nine sites, five individuals of each of the three focal tree taxa were randomly selected for comparisons of extent of damage caused by folivorous insects and fungal pathogens to leaves. Selected individuals had similar heights and stem diameters for each species respectively across all sites (to minimize difference in susceptibility due to plant age) and all were within 5 meters of the water's edge (to minimize differences in susceptibility due to water stress) (Crous et al., 2012).

During August and October 2011, five small branches (*ca*. 5 cm diameter and 30 cm long) were randomly collected from each individual tree at canopy level. All leaves were detached from branches and mature leaves were combined into a single bulk sample per individual tree in a brown paper bag. From these bulk samples, 100 leaves for each individual were randomly chosen (blindly drawing from brown paper bag) in the laboratory. Symptom assessments were conducted for each leaf of each tree in the laboratory using a dissecting microscope, allowing careful confirmation of damage type.

Leaves were assigned to one of the following damage types: (i) Fungal damage alone (F), (ii) folivore damage alone (H) and (iii) intact leaves (I) (García-Guzmán and Dirzo, 2001). Wounds created by folivorous insects are often entry points for fungal pathogens (Agrios, 2005). Therefore, leaves with both fungal and insect damage were not included in analyses as this would bias fungal pathogen damage. Insect damage was categorised into two types – that caused by insects with chewing mouthparts and those with piercing and sucking mouthparts. Damage caused by leaf mining insects was negligible and not included in the analyses. Leaves colonised by fungal pathogens were separated according to four predominant symptoms (blight, chlorotic areas, necrotic spots and black mould). Identifications of the fungal pathogens that caused these main symptoms were based on a combination of characters including: the symptoms themselves, fungal micro-morphology (studied with the aid of a light- and dissecting microscope), cultural characteristics (as grown for two weeks in the dark at 25°C on Malt Extract agar, Biolab, South Africa) and published literature (Doidge and Bottomley, 1931; Morris et al., 1988; Arzanlou et al., 2007). These preliminary identifications were confirmed by comparisons of ITS DNA sequence data (obtained using protocols of Gardes and Bruns, (1993) and White et al., (1990) to those of taxa published on GenBank (http://www.ncbi.nlm.nih.gov/genbank/) using standard BLAST searches.

3.2.3. Severity of pest and pathogen damage

Damage severity was defined as the percentage of leaf area visibly infected by a pathogen or area damaged by feeding activities of arthropods. The percentage of area lost to fungal disease or folivory was determined using a practiced estimate of lamina damage (1 = intact; 2 = 1–11%; 3 = 12–49% and 4 = 50–100% of leaf area damaged (LAD)) based on previous studies (Benítez-Malvido, 1999; García-Guzmán and Dirzo, 2001; García-Guzmán and Dirzo, 2004; Eichhorn et al., 2010). Leaf damage for all tree individuals was scored by the same person. The estimates of leaf area lost were based on comparisons with a standard leaf of the same size as that being scored.

To test the effects of invasion treatment on numbers of leaves damaged (extent of damage) and categories of damage severity, one-way analysis of variance (ANOVA) was used since data were normally distributed. Shapiro-Wilk's W test was used to test for normality because of its rigour as compared to a wide range of alternative tests (Shapiro et al., 1968). The data were analysed using the STATISTICA version 11 software package (Statsoft Corporation Tulsa, U.S.A). Sample means were separated using a Tukey's HSD test (Guzmán and Dirzo, 2001).

3.2.4. Effect of native tree species richness and host abundance on host damage

At each of the nine sites, two transects were established measuring 50 m in length (parallel to the river) and 5 m in width (perpendicular to the river crossing both wet and dry bank zones). In these, native tree species diversity (number of tree taxa present) and host abundance were determined. Data from the two transects were combined per site, and compared among sites with different invasion treatments using ANOVA in STATISTICA version 11 software package (Statsoft Corporation Tulsa, U.S.A). Plant diversity using the Shannon-Wiener index was also calculated (Hutcheson, 1970). This index was chosen as it gives an equivalent number of species, *i.e.* the number of species that yield the index if all species contain the same number of individuals. In order to investigate the interactions between host species abundance and native plant species richness on extent of folivorous insect and pathogen damage, regression analyses were performed with percentage of leaves damaged by the respective organism groups as the response variables and native tree species richness and host abundance as predictors. Analyses were performed using the STATISTICA version 11 software package. Unadjusted $P < 0.05$ values are reported.

3.3. RESULTS

3.3.1. Differences in folivorous insect and fungal damage levels

All three tree species exhibited significant differences in levels of folivory in relation to invasion treatment of the site. Levels of folivory on *B. stellatifolium* were significantly higher at restored sites ($F_{(2, 42)} = 23.9$, $P < 0.000001$) compared to heavily invaded and near pristine sites (Fig. **3.3a**). *Metrosideros angustifolia* had significantly more leaves damaged by folivorous insects at restored and heavily invaded sites ($F_{(2, 42)} = 23.9$, $P < 0.000001$) compared to near pristine sites (Fig. **3.3a**). *Acacia mearnsii* showed a different pattern, with significantly more leaves damaged by folivorous insects at heavily invaded sites compared to near pristine sites and restored sites ($F_{(2, 42)} = 7.37$, $P = 0.002$), which were statistically similar (Fig. **3.3a**).

In addition to damage caused by folivorous insects, the only other organisms causing significant leaf damage to the study plants were fungi. Each host species had at least one species of fungal pathogen affecting leaves. Different plant species differed in terms of the fungal taxa that infected them: *B. stellatifolium* was infected by *Periconiella velutina* (G.Winter) that causes black mould and *Phyllosticta owaniana* (G.Winter) that causes leaf spot. *Metrosideros angustifolia* was infected by an *Alternaria* sp. that caused necrotic spots. *Acacia mearnsii* was infected by *Uromycladium alpinum* (McAlpine) that caused chlorotic spots.

For *B. stellatifolium* the extent of fungal disease was significantly higher at restored sites and heavily invaded sites ($F_{(2,\ 42)} = 3.97$, $P = 0.03$) compared to near pristine sites (Fig. **3.3b**). For *M. angustifolia*, extent of fungal disease was higher in restored riparian vegetation ($F_{(2,\ 42)} = 10.87$, $P = 0.00015$) than in near pristine and heavily invaded sites which did not differ significantly (Fig. **3.3b**). For *A. mearnsii*, fungal disease was higher in heavily invaded sites ($F_{(2,\ 42)} = 6.02$, $P = 0.005$) than in restored and near pristine sites, which were statistically similar (Fig. **3.3b**).

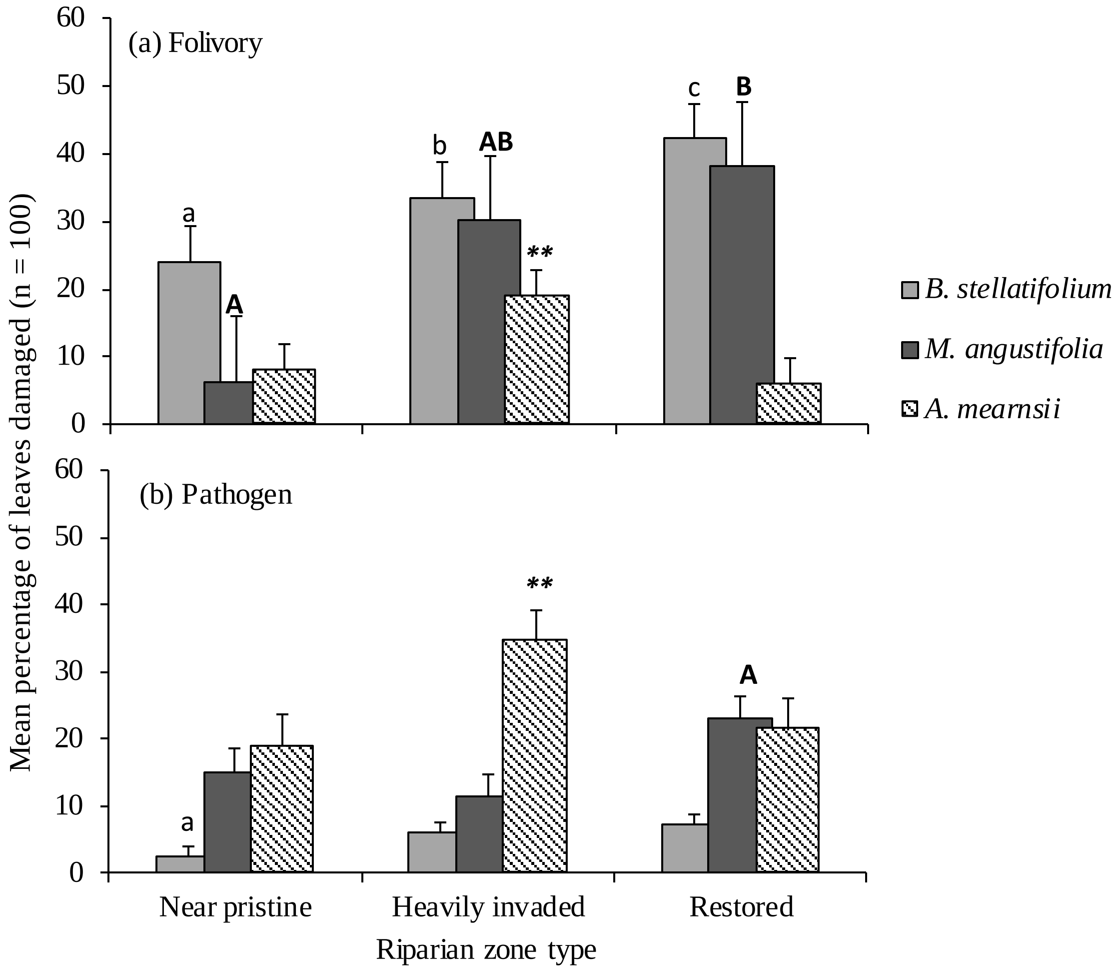

Figure **3.3**: Mean percentage (+SE) of leaves with damage caused by either folivorous insects (**a**) or pathogens (**b**) for three test tree taxa (*B. stellatifolium*, *M. angustifolia*, *A. mearnsii*) from sites with different invasion treatments: near pristine, heavily invaded and restored. Significant differences in damage caused are indicated above error bars by different lower case letters for *B. stellatifolium*, different uppercase letters for *M. angustifolia* and differences in the number of asterisks for *A. mearnsii*. (n = 15 for each tree species in each invasion treatment).

Numerous significant differences were detected in the levels of folivore damage and leaf pathogen damage when comparing individuals from sites of the same invasion treatment (Table 3.1). However, damage levels by leaf fungal pathogens between restored sites did not differ for any host. Similarly, folivore levels did not significantly differ between restored sites for all tree taxa excluding *A. mearnsii* (Table 3.1).

Table **3.1**: A summary of results for one-way ANOVA's comparing levels of folivore damage and leaf fungal diseases on *B. stellatifolium*, *M. angustifolia* and *A. mearnsii* at sites with similar invasion treatment (n = 15).

Species name	Invasion treatment	Folivory	Fungal diseases
Brabejum stellatifolium	Near pristine	0.61 *NS*	7.03**
	Heavily Invaded	4.22*	8.80**
	Restored	2.97 *NS*	1.34 *NS*
Metrosideros angustifolia	Near pristine	7.41**	8.36**
	Heavily Invaded	15.65***	4.8*
	Restored	1.68 *NS*	2.59 *NS*
Acacia mearnsii	Near pristine	4.97*	9.64**
	Heavily Invaded	51.37***	38.43***
	Restored	21.03***	2.41 *NS*

Figures represent F- values
Significance: * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; *NS* $P > 0.05$.

Brabejum stellatifolium mostly incurred damage caused by folivorous insects with chewing mouthparts (Fig. **3.4a**), while *M. angustifolia* and *A. mearnsii* were almost equally damaged by insects with chewing- and those with piercing-sucking mouthparts (Fig. **3.4b** and **c**). Damage levels caused by both chewing and sucking insects followed a similar pattern to those observed for the combined data presented in figure **3.3a**. Similarly, for *B. stellatifolium*, both fungal taxa showed the same pattern (Fig. **3.4d**) as was found for their combined effect shown in figure **3.3b**.

Leaf chewing damage was significantly different among *B. stellatifolium* and *A. mearnsii* individuals in near pristine and restored sites (Table **A3.1**). This was true for *M. angustifolia* individuals in both near pristine and heavily invaded sites (Table **A3.1**). Sap-sucking damage was significantly different among *A. mearnsii* individuals in both restored and heavily invaded sites (Table **A3.1**). Considering different types of leaf fungal diseases, leaf spot varied significantly on *B. stellatifolium* in both heavily invaded and restored sites. This was true for *M. angustifolia* individuals in all invasion treatments (Table **A3.2**). No significant difference was detected in leaf disease on *A. mearnsii* and black mould on *B. stellatifolium* leaves in near pristine and heavily invaded sites (Table **A3.2**).

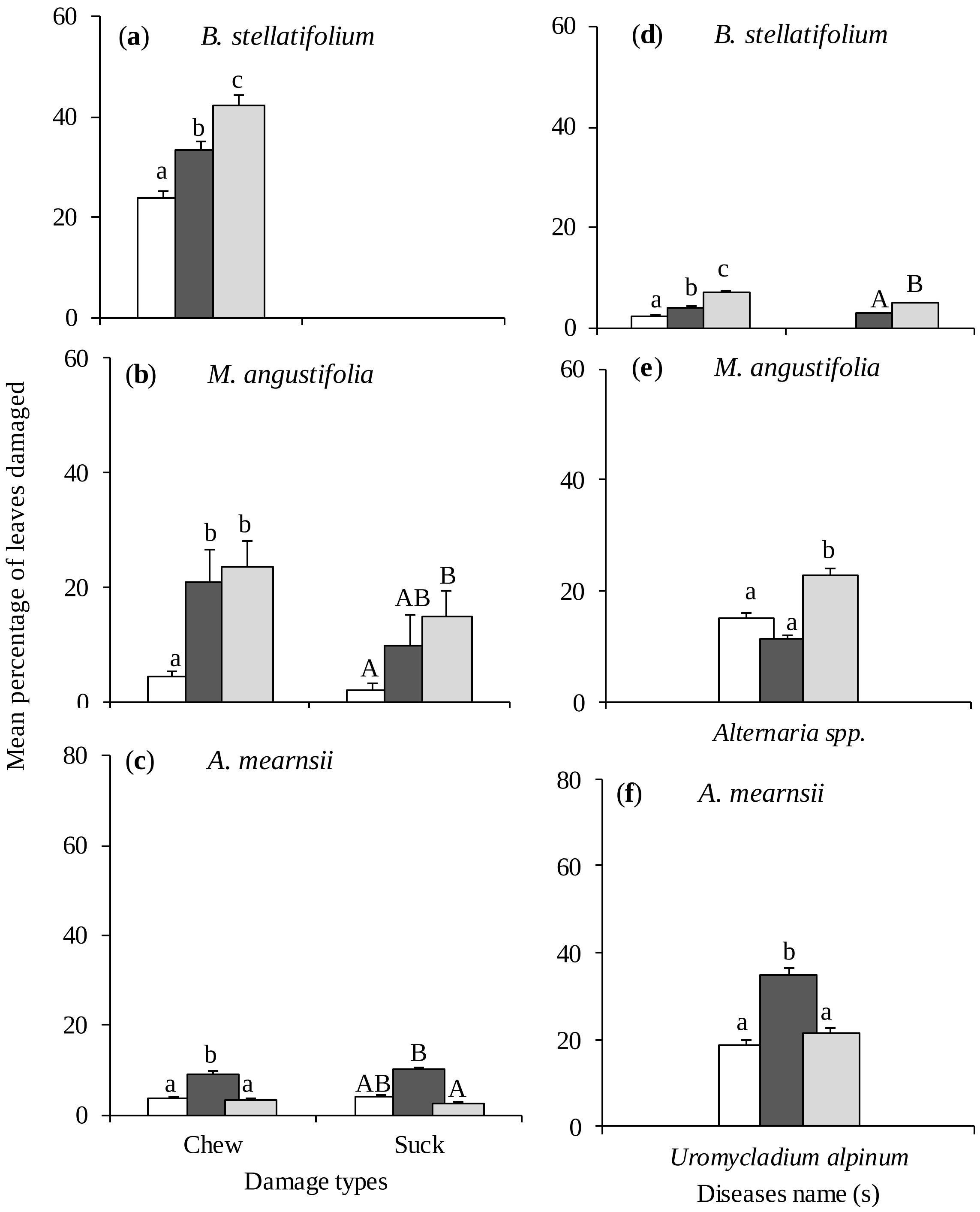

Figure **3.4**: Frequency of leaves damaged by folivorous insects with chewing and sucking mouthparts (**a, b and c**) and diseases caused by four fungal pathogens (**d, e and f**) for three host tree species (*B. stellatifolium*, *M. angustifolia* and *A. mearnsii*) at sites that differ in their invasion treatment: Near pristine (*clear bars*); Heavily invaded (*dark grey bars*); and Restored riparian zones (*light-grey bars*). Different letters indicate a significant difference between groups within each damage type.

3.3.2. Severity of pest and pathogen damage

Frequencies of leaf damage severity are presented in figure 3.5. For *B. stellatifolium* and *M. angustifolia*, damage severity caused by both folivorous insects and fungal pathogens was higher at heavily invaded and restored sites than at near pristine sites, but for both taxa, damage severity tended to be highest at the restored sites (Figs. 3.5a,b,d, and e). *Acacia mearnsii* tended to be more severely damaged by both folivorous insects and fungal pathogens at heavily invaded sites (Figs. 3.5c and f).

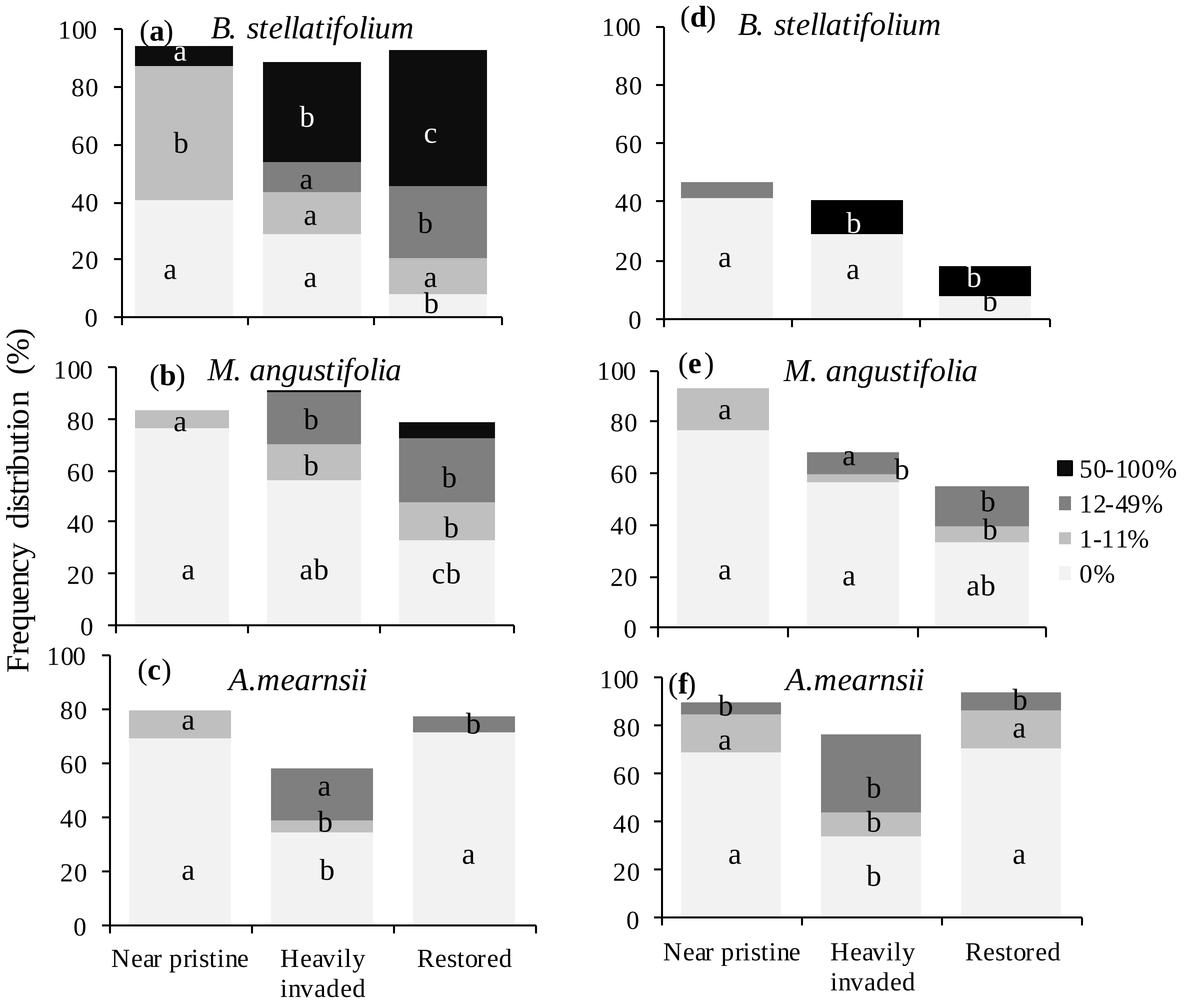

Figure 3.5: Frequency distributions of percentage of leaf area damaged by folivorous insects (**a, b** and **c**) and fungi (**d, e** and **F**). The darker the bars, the more severe the damage caused by the various organisms were. Categories of leaf area damaged were as follows: 0%; 1-11% (light grey); 12-49% and 50-100% (dark grey). Significant differences across sites for each category are denoted by different letters.

Comparisons between sites within each invasion treatment revealed significant differences in numbers of undamaged leaves for both *B. stellatifolium* and *M. angustifolia* individuals growing in near pristine heavily invaded and restored sites (Table **A3.3**). Numbers of undamaged leaves for *A. mearnsii*, only differed significantly among individuals growing in near pristine sites and in restored sites (Table **A3.3**). *Brabejum stellatifolium* leaves with 1–11% of their leaf-tissue removed/consumed by folivores or diseased, significantly differed across heavily invaded sites. This was also true for *M. angustifolia* in both near pristine, heavily invaded and restored sites, and for *A. mearnsii* in both heavily invaded and restored sites (Table **A3.3**). *Brabejum stellatifolium* leaves with 12–49% of their leaf-tissue diseased also differed significantly across heavily invaded sites; while in the same 12–49% leaf-tissue diseased category *M. angustifolia* differed significantly across restored sites (Table **A3.3**). Again, across restored sites *M. angustifolia* had significantly different numbers of leaves having 50-100% of their leaf-tissue consumed by folivores and diseased as did *A. mearnsii* in heavily invaded sites (Table **A3.3**).

3.3.3. Effect of native tree species richness and host abundance on host damage

Mean native tree species richness was significantly lower at heavily invaded sites than at near pristine sites ($F_{(2,\ 6)} = 6.86$, $P = 0.03$), but returned to pre-invasion levels after alien removal. This was also true for species diversity as measured using the Shannon-Wiener index (Table **3.2**). The abundance of *A. mearnsii* was the highest at the heavily invaded sites ($F_{(2,\ 6)} = 6.09$; $P = 0.05$) but abundance was similar between the near pristine and restored sites (Table **3.2**). Abundance of *B. stellatifolium* was highest at the near pristine sites ($F_{(2,\ 6)} = 16.89$; $P = 0.003$) with no difference detected between the restored and invaded sites (Table **3.2**). Abundance of *M. angustifolia* was highest at the near pristine sites and restored sites while at heavily invaded sites, abundance was significantly lower ($F_{(2,\ 6)} = 6.31$; $P = 0.03$) (Table **3.2**).

Acacia mearnsii was the only host species that showed significant differences in size of individuals at sites of the same invasion treatment (only for individuals from different heavily invaded sites) (Table **A3.4**). This species also showed differences in size of individuals from sites that differ in invasion treatment, with individuals at restored sites significantly smaller than those at near pristine or invaded sites (Table **3.2**). The size of *B. stellatifolium* did not vary across sites of the same, or among sites that differ in, invasion treatment (Tables **A3.4** and **3.2**). *Metrosideros angustifolia* did not vary across sites of the same invasion treatment, but showed differences in size of individuals among sites that differ in invasion treatment, with individuals at near pristine sites significantly bigger than those in heavily invaded sites and restored sites (Tables **A3.4** and **3.2**).

Table **3.2**: Differences in native tree species richness and plant diversity among studied sites that vary in their invasion treatment and size classes (diameter (cm) measured at breast height) and host abundance of the three tree species considered in this study. Significant differences in host abundance and size-classes are indicated by different lower case letters for *B. stellatifolium*, different uppercase letters for *M. angustifolia* and differences in the number of asterisks for *A. mearnsii*.

Invasion treatment	Mean native tree species richness ± standard error	Species diversity (Shannon-Wiener index)	Plant species	Host abundance	Mean stem diameter (cm) ± standard error
Near pristine	20 ± 0.9$^{\mathbf{a}}$	2.521	*B. stellatifolium*	25 ± 1.7^{a}	36 ± 1.13^{a}
			M. angustifolia	26 ± 1.0^{A}	31 ± 2.40^{B}
			A. mearnsii	6 ± 0.33*	30 ± 1.30*
Heavily invaded	7 ± 2.3$^{\mathbf{b}}$	1.01	*B. stellatifolium*	11 ± 2.1^{b}	29 ± 0.95 ^{a}
			M. angustifolia	8 ± 2.0^{B}	19 ± 1.05^{A}
			A. mearnsii	63 ± 30.9**	36 ± 2.42*
Restored	17 ± 0.7$^{\mathbf{a}}$	2.44	*B. stellatifolium*	15 ± 1.3^{b}	32 ± 1.27^{a}
			M. angustifolia	17 ± 3.3^{AC}	22 ± 2.15^{A}
			A. mearnsii	6 ± 0.3*	13 ± 0.86**

The regressions between native plant species richness and percentage of leaves damaged by fungal pathogens and insects were not significant in all cases except for damage caused by fungal pathogens on *A. mearnsii* (Table **3.3**); in this case, damage caused by fungal pathogens was negatively correlated to native plant species richness.

Table **3.3**: Regressions for foliar damage caused by fungal pathogens and folivorous insects as response variables versus native tree species richness as the predictor.

Plants species	# of leaves damaged by fungal pathogens			# of leaves damaged by folivorous insects		
	Intercept	Tree species richness	r^2	Intercept	Tree species richness	r^2
B. stellatifolium	8.559**	-0.209 *NS*	0.210	35.752**	-0.192 *NS*	0.017
M. angustifolia	17.16*	-0.459 *NS*	0.002	35.848*	-0.666 *NS*	0.13
A. mearnsii	52.31***	-13.976**	0.590	52.193*	-7.182 *NS*	0.25

Note: Data presented are for the fitted intercept, the regression coefficient (richness), the coefficient of determination (r^2), and sample size (n) = 9

Significance: * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; *NS* $P > 0.05$

Native host tree densities were always negatively correlated to the number of leaves damaged by fungal pathogens or folivorous insects, although only significantly so in the case of damage by folivorous insects (Fig. **3.6**). The exotic *A. mearnsii* showed the opposite with both damage types significantly and positively correlated to host abundance (Fig. **3.6**).

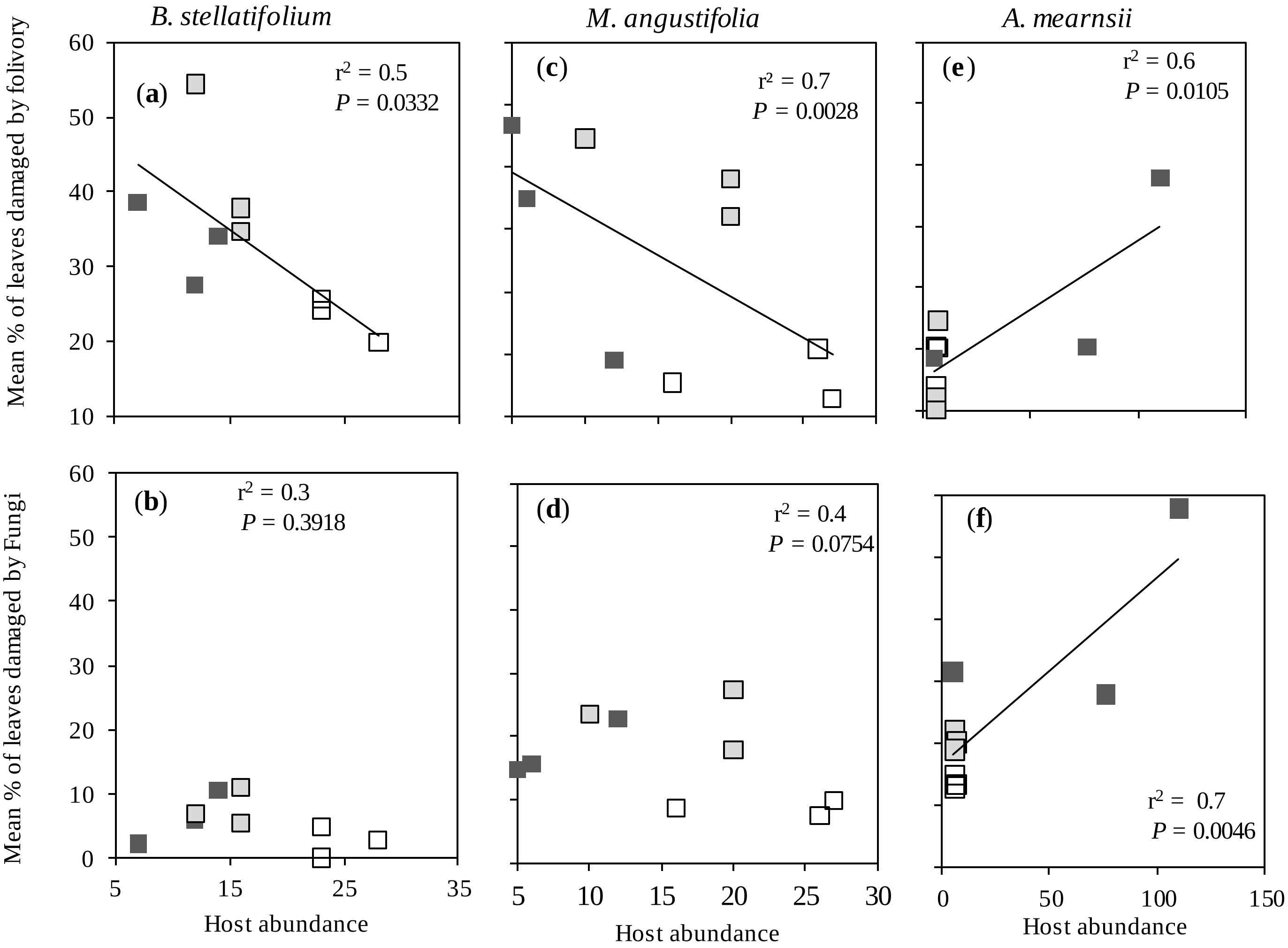

Figure **3.6**: The effects of host abundance on the percentage of leaves damaged by fungal pathogens (**a, c and e**) or folivorous insects (**b, d and f**) for three tree species (*B. stellatifolium*, *M. angustifolia* and *A. mearnsii*) at sites that differ in their invasion treatment: Near pristine (*clear square*); Heavily invaded (*dark grey square*); and Restored (*light-grey square*) riparian zones. Reported P-values are for partial regression coefficients; reported r^2 is for each overall regression. All the trends were linear.

3.4. DISCUSSION

In this study, both native and alien trees in riparian zones varied in their susceptibility towards pathogenic fungal colonisation and folivorous insect (natural enemies) damage across sites, in accordance with differences in invasive alien plant (IAP) invasion treatments. For the native species *B. stellatifolium*, both folivore and pathogen damage was significantly higher at invaded sites than at near pristine sites and these levels did not return to their original levels after restoration. In fact, folivore damage levels were even higher at restored sites than at heavily invaded sites. Similarly, damage caused by leaf fungal pathogens and folivorous insects was also higher for the other native species *M. angustifolia* at restored sites than it was at heavily invaded and near pristine sites. The pattern was different for the invasive *A. mearnsii* where damage caused by both folivorous insects and fungal pathogens increased at heavily invaded sites, but returned to near pristine site levels at restored sites. The high levels of folivory and fungal diseases on *A. mearnsii* could not be credited to biological control agents since the two known biocontrol agents (*Melanterius maculatus* and *Dasineura rubiformis*) do not damage the vegetative parts of *A. mearnsii*, but rather cause reductions in seed availability (Impson et al., 2011). Host plants in riparian sites with similar invasion treatment experienced significantly different levels of damage by fungal pathogen and folivorous insects. Subsequent work is required to find possible causes of site-to site variation of leaf damage by folivory and fungal disease in the invasion treatments concerned. However, these results suggest that despite the sites being managed similarly, they differ in performance of folivores and fungal pathogens. The lower levels of folivory and fungal disease in near pristine sites concurs with the literature which shows that higher heterogeneity in plant species composition and structure of near pristine sites possibly explains observed lower levels of damage by natural enemies (Bach, 1980a; Agrawal et al., 2006). The higher levels of damage by folivorous insects and fungal pathogens on native plants in restored areas is intriguing, particularly because this study was undertaken seven years after restoration efforts.

After removal of IAPs, host abundance of *B. stellatioflium* and *M. angustifolia* (two native trees) remained similar to near pristine sites. However, abundance of these species was low at heavily invaded sites. Host abundances of *A. mearnsii* were clearly greater in heavily invaded sites than it was in restored and near pristine sites. Apart from decreased host abundance of the two native trees in heavily invaded sites, native plant species richness was also lower than that in near pristine sites. Results therefore support previous observations that alien plant species have a negative impact on species richness and host abundance by replacing native plant species in the communities they invade (Seabloom et al., 2003; French et al., 2008;

Hejda et al., 2009; Vilà et al., 2011). Most invasive Australian Acacia spp. are transformers, posing serious threat to structure and functions of pristine Fynbos areas by converting them into species-poor ecosystems (Richardson et al., 2000). Despite these severe impacts, native plant species richness can return to its original level once the IAPs are removed (Holmes and Cowling, 1997b; Blanchard and Holmes, 2008; Marchante et al., 2011; this study). The recovery of native plant species in riparian areas referred to in these studies suggests that, in each case, no "threshold of irreversibility'' (as defined by Aronson et al., 1993) had been reached (see Holmes and Cowling, 1997b) allowing regeneration after clearing. After removal of IAPs, arthropod communities associated with native plants gradually recovered and increasingly began to resemble the situation in equivalent near pristine sites (Chapter 2).

There is a debate over whether or not the levels of leaf damage done by folivorous insects and fungal pathogens depends on native plant species richness, with homogenous tree stands being more damage prone than heterogeneous stands (Balvanera et al., 2006; Mitchell, et al., 2002; Pfisterer et al., 2003; Haas et al., 2011). Consequently, one would expect to see decreasing levels of folivory and fungal disease with decreasing native plant species richness. However, results from this study suggest that activities of folivores and fungal pathogens in diverse riparian habitats (near pristine and restored) and simple habitats (heavily invaded) are independent of plant species richness, a pattern observed in other ecosystems (e.g. Sobek et al., 2009; Scherber et al., 2010b). Bach (1980a) suggested folivore activity is linked to host abundance/density rather than plant species richness. An area which has a high abundance/density of preferred host favours folivore survival more so than does a diverse area where access to the preferred host is more limited; a phenomenon known as 'resource concentration' hypothesis (Grez and González, 1995).

Several studies have tested relationships between levels of leaf folivory and fungal diseases and the abundance of their hosts, with some studies showing positive (Izhaki et al., 1996; Mabry and Wayne, 1997), some negative (Parmesan, 2000; Underwood and Halpern, 2012; Halpern et al., 2014) and some neutral (Rhainds and English-Loeb, 2003) results. In this study, host abundance had a negative effect on native tree folivory and a positive effect on both the levels of folivory and fungal diseases for *A. mearnsii*. The negative relationship between folivory and host abundance for the two native trees could be explained by higher host abundances diluting folivore effects among individual plants (e.g. see Halpern et al., 2014). Alternatively, at low abundance, when host plants are thinly dispersed, folivores may show fidelity to a host, as finding another host both increases its energy use and its exposure to predaceous arthropods (Schowalter, 1996; Schowalter and Lohman, 1999; Schuldt et al., 2010). The positive relationship between host abundance of *A. mearnsii* and levels of folivory and fungal diseases was not expected as it is known that when folivores have not coevolved with a plant, they may not be able to use that plant as a food source (Tallamy, 2004). However, there is some evidence that in their introduced range, alien plants may assemble communities of folivores as abundant and rich as those of native hosts (Frenzel and Brandl, 2003) more especially if IAP is closely related to several native trees (Procheş et al., 2008). The Fabaceae are well represented in South Africa (thirty-six (18%) of the 198 species listed as declared trees in South Africa belong to the Fabaceae (Coates-Palgrave, 2002). Hence, it is possible that a rich regional pool of pre-adapted folivores is available to counteract chemical defenses of IAPs (e.g. see Litt et al., 2014).

Apart from the influences of host abundance and native plant species richness, the observed higher levels of folivory and fungal diseases may be related to changes in leaf chemical components (Chapter 4) due to high rates of nutrient inputs to the ecosystems through the presence of nitrogen-fixing IAPs (e.g. *A. mearnsii* is a putative nitrogen fixer legume (Naudé, (2012)), that are known to elevate soil nutrient levels (van der Waal, 2009). A study by Naudé, (2012), at the same riparian sites used in this study, showed that a legacy remained after clearing IAPs in that soil available nitrogen levels remained high. This implies that host plants in restored sites may be more vigorous than in near pristine sites due to an abundant available resource. This, in turn, may make these plants more attractive to folivore insects and/or fungal pathogens (e.g. see Moran and Scheidler, 2002; Gnuner et al, 2005), thereby increasing the folivore insect population with a correlative increase in damage in riparian systems after alien clearing. These effects should be investigated in future studies in a riparian system context.

In conclusion, the results of this study suggest that, although plant communities often recover after invasive alien removal, native trees continue to be damaged by folivorous insects and fungal pathogens resulting in damage levels in excess of that seen at invaded sites. Restoration efforts of invaded riparian zones therefore alter aspects of interactions among the plants, fungi, and folivorous insects with many unexpected and lasting ecological consequences. Of the factors assessed which explain large differences in levels of leaf folivory and diseases among different invasion treatments of riparian habitats, host abundances (resource concentration) was the most influential. While it is not possible to deduce from these correlative analyses the exact mechanism by which host plant abundance affected insect folivory and fungal disease severity, it is likely that the interplay between insect folivore densities, predaceous arthropod densities and/or plant nutritional status may be important (see Forkner and Hunter, 2000; Moran and Scheidler, 2002; Gnuner et al, 2005).

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APPENDIX 3

Table **A3.1:** Summary of ANOVA statistics comparing leaves damaged by folivorous insects with chewing and sucking mouthparts for three host tree species at sites that are similar in their invasion treatment.

Species name	Invasion treatments	Damage by chewing insects	Damage by insects with sucking mouthparts
Brabejum stellatifolium	NP	3.98*	N/A
	HI	0.25 *NS*	N/A
	R	8.56**	N/A
Metrosideros angustifolia	NP	7.94**	0.47 *NS*
	HI	9.52**	1.68 *NS*
	R	0.05 *NS*	1.22 *NS*
Acacia mearnsii	NP	3.85*	2.89 *NS*
	HI	1.39 *NS*	30.06***
	R	10.73**	11.96**

Figures represent F- values
Significance: * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; *NS* $P > 0.05$, N/A Not Applicable
NP = Near Pristine, HI = Heavily Invaded, R = Restored riparian habitats

Table **A3.2**: Summary of ANOVA statistics comparing leaves diseased by fungal pathogens that cause leaf spot, black mould and leaf rust on leaves three host tree species at sites that are similar in their invasion treatment (n =15).

		Disease type		
Species name	Invasion treatments	Leaf spot	Black	Leaf rust
Brabejum stellatifolium	NP	N/A	1.64 *NS*	N/A
	HI	6.0*	2.09 *NS*	N/A
	R	3.92*	5.03*	N/A
Metrosideros angustifolia	NP	8.73**	N/A	N/A
	HI	5.06*	N/A	N/A
	R	44.91***	N/A	N/A
Acacia mearnsii	NP	N/A	N/A	0.66 *NS*
	HI	N/A	N/A	3.62 *NS*
	R	N/A	N/A	15.19***

Figures represent F- values
Significance: * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; *NS* $P > 0.05$
NP = Near Pristine, HI = Heavily Invaded, R = Restored riparian habitats

Table **A3.3**: Summary of ANOVA statistics comparing leaves of three host tree species at sites that are similar in their invasion treatment damaged by folivorous insects and diseased by fungal pathogens on leaves.

Species name	Invasion treatments	Damage /disease severity categories	Folivory	Fungal disease
Brabejum stellatifolium	NP	0%	4.37*	4.37*
		1–11%	1.54 *NS*	1.95 *NS*
		12–49%	0.32 *NS*	2.11 *NS*
		50–100%	1.19 *NS*	N/A
	HI	0%	19.68***	19.68***
		1–11%	4.34*	7.59**
		12–49%	1.29 *NS*	11.83**
		50–100%	2.82 *NS*	2.67 *NS*
	R	0%	13.44***	13.44***
		1–11%	0.08 *NS*	1.80 *NS*
		12–49%	0.55 *NS*	1.24 *NS*
		50–100%	0.12 *NS*	0.08 *NS*
Metrosideros angustifolia	NP	0%	87.02***	87.02***
		1–11%	7.41**	8.36**
		12–49%	N/A	N/A
		50–100%	N/A	N/A
	HI	0%	15.22***	15.22***
		1–11%	18.95***	4.24*
		12–49%	1.79 *NS*	3.36 *NS*
		50-100%	1.00 *NS*	N/A
	R	0%	10.04**	10.04**
		1–11%	15.22***	37.33***
		12–49%	0.18 *NS*	41.41***
		50–100%	19.48***	1.00 *NS*
Acacia mearnsii	NP	0%	4.09*	4.09*
		1–11%	17.91***	1.38 *NS*
		12–49%	N/A	0.51 *NS*
		50–100%	N/A	N/A
	HI	0%	2.61 *NS*	2.61 *NS*
		1–11%	17.64***	5.29*
		12–49%	6.88*	3.52 *NS*
		50–100%	N/A	4.82*
	R	0%	31.52***	31.52***
		1–11%	50.31***	11.88**
		12–49%	N/A	2.50 *NS*
		50–100%	N/A	N/A

Figures represent F- values

Significance: * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$; *NS* $P > 0.05$

NP = Near Pristine, HI = Heavily Invaded, R = Restored riparian habitats

Table **A3.4**: Size-classes (diameter (cm) measured at breast height) of tree species in near pristine, heavily invaded and restored riparian sites. Coefficients that are statistically significant relationship are indicated in **bold**.

Species name	Invasion treatments	F-value	*P*-value
Brabejum stellatifolium	Near pristine	2.35	0.14
	Heavily Invaded	1.71	0.22
	Restored	0.08	0.93
Metrosideros angustifolia	Near pristine	2.73	0.11
	Heavily Invaded	2.77	0.10
	Restored	1.02	0.39
Acacia mearnsii	Near pristine	0.85	0.45
	Heavily Invaded	**14.86**	**0.0006**
	Restored	1.18	0.34

CHAPTER 4

Invasive plant management and folivore damage on riparian trees: influence of altered arthropod communities and leaf nutrient content.

ABSTRACT

Predator densities and/or plant nutritional status can affect folivore densities, but these effects have not yet been assessed in an invasive species management context. This study investigated the influence of: (i) altered predatory arthropod numbers and community composition, and (ii) altered plant total leaf nitrogen and phosphorus levels, using two native tree taxa, *Brabejum stellatifolium* and *Metrosideros angustifolia* and one invasive tree *Acacia mearnsii* as subjects in riparian sites, with differing invasive tree treatments (treatments: near pristine, heavily invaded by *A. mearnsii*, and restored). Species richness, abundance and composition of folivorous and predatory arthropods were compared between sites within each invasion treatment and also between treatments. Folivore and predator abundance and species richness on all tree species was significantly influenced by invasion treatments of sites. Assemblage composition of folivores on each tree species differed between sites within each invasion treatment. Leaf nitrogen levels, but not leaf phosphorus levels, varied significantly among invasive treatments. For *B. stellatifolium*, leaf nitrogen levels were highest at restored sites where folivorous arthropods were abundant and the number of predators was intermediate. For this species, increased folivore abundance was significantly and positively correlated to levels of leaf damage. For *A. mearnsii*, folivorous insect abundance positively correlated to leaf nitrogen levels but not to damage levels. Altered arthropod communities did not explain observed patterns of leaf damage on this species. There was no relationship between leaf damage and nitrogen levels or altered arthropod communities on *M. angustifolia*. These results suggest that leaf nitrogen content is important for folivore food plant selection in *B. stellatifolium* and *A. mearnsii*, but leaf phosphorus content and the presence of predatory arthropods were of limited effect. Hence, individual plant species should be treated as separate entities when assessing plant health in a restoration scenario.

Keywords: Nitrogen, Phosphorus, Plant-animal interactions, Invasion treatment, Riparian sites.

4.1. INTRODUCTION

The importance of plant traits on forage selection by folivores is well established (Ohgushi, 2005; Tanentzap et al., 2011; Hare, 2012; Züst et al., 2012). Collectively, these studies demonstrate that leaf nutrients experienced by both terrestrial (Mattson, 1980; White, 1984; Peeters, 2002a, 2000b) and aquatic (Elser et al., 2000a) folivorous insects are dependent largely on their feeding styles, behavioural responses to differing plant nutritional quality (Peeters, 2002a, 2000b), abundance and richness of predators (e.g. spiders and mirid bugs) (Schmitz et al., 2000; Halaj and Wise, 2001) and habitat conditions (Münzbergová and Skuhrovec, 2013). Peeters (2002a) studied the role of feeding styles in dictating what nutrient folivores will eat, and reported positive correlations between leaf nitrogen contents and densities of sap-sucking insects, whereas leaf chewing insect densities respond negatively to leaf structural traits like leaf fibre and lignin. This implies that fibre, cellulose and lignin contribute more to leaf preference of chewers than leaf nutrient levels do. When plant quality is low insects with sucking mouthparts may respond by emigrating to better quality host plants or by increasing ingestion rates (Huberty and Denno, 2006a, 2006b).

Besides plant traits plant-folivore interactions may also be influenced by abundance and richness of predators (e.g. spiders and mirid bugs) (Schmitz et al., 2000; Halaj and Wise, 2001). Predatory arthropod activities may be enhanced, suppressed or unaffected by an increase in prey species richness or abundance (Sih et al., 1998). The actual role of predaceous arthropods in regulating the structure and population size of folivorous insects had been debated (e.g. Landis et al., 2000; Halaj and Wise, 2001; Schmitz, 2007). The mechanisms underlying effects of predators on folivores have been attributed to the spatial extent of prey's movement within or between different micro-sites (Landis et al., 2000; Schmitz, 2007) and the strategy of predators in locating and capturing prey (Sih et al., 1998). The 'green world hypothesis' has also been proposed to explain the effects of predaceous arthropods on folivores (Slobodkin et al., 1967). This hypothesis argues that, in case whereby richness and abundance of predator are altered probably due to changes in vegetation structure (as they rely of vegetation structure for web-building); they can suppress folivore populations to densities at which plants experience negligible levels of folivory (Halaj and Wise, 2001).

In riparian sites, dense stands of invasive trees, like Australian *Acacia* spp., can alter environmental conditions (e.g. microclimate conditions) (Yelenik et al., 2004, 2007), and soil micro-organisms (Slabbert et al., 2014) of the sites they invade. Thus, in such sites, habitat conditions may be important determinants of the interactions of plants with their folivorous insects (Cobb et al., 1997; Stenberg et al., 2008; Trotter et al., 2008; Abela-Hofbauerova et al., 2011; Cuevas-Reyes et al., 2011; Münzbergová and Skuhrovec, 2013). Changes in patterns of microclimatic conditions may accompany alterations in, (i) visual orientation of some insect species toward necessary resources (Stireman et al., 2005), (ii) the development of folivore and predator populations through survival and performance (e.g., progeny production may increase with longer warm periods) (Stireman et al., 2005), and (ii) leaf physiology and development (Closa et al., 2010). Changes in soil micro-organisms may affect nutrients (e.g. nitrogen and phosphorus) transfer in the soil and thus influence their availability to plants (Richardson, 2001). The resulting changes in plant nutritional quality may directly and indirectly affect both survivorship of and the amount of defoliation by folivorous insects, (Casotti and Bradley, 1991; Elser et al., 2000a; Ricklefs, 2008).

Studies that investigate the relationship between plant health and the associated fauna within riparian zones have tended to concentrate on soil properties and chemistry (van der Waal, 2009; Naudé, 2012; Slabbert et al., 2014), or patch size (Herrera and Dudley, 2003). Longcore (2003) attempted to integrate several plant measurements into an index of habitat complexity and successfully related this measurement to the distribution of arthropods, although the selection parameters were unclear. However, no previous studies have explored the relative importance of leaf nutrients and predator densities for plant-folivore interactions within trees of riparian sites in an invasion scenario.

The abundance and species richness of folivorous insects were determined on two native trees *B. stellatifolium*, (L.), and *M. angustifolia* (L.) and the invasive tree *A. mearnsii* (DeWild (L.)) growing in near pristine, heavily invaded and restored riparian sites. In this chapter the following questions were addressed: (i) are there differences in abundance and richness of folivorous insects between host trees among sites? (ii) If so, are these differences reflected in differences in levels of folivore damage? (iii) Do leaf nutrients differ among the host trees under different invasive management histories, and (iv) how do leaf nutrients and predator communities relate to folivorous insect abundance?

4.2. METHODS

4.2.1. Study site and plant species

Study sites were selected in the Molenaars, Dwars and Wit river catchments (see figure **3.1** in Chapter 3 for site location). Nine study sites in these three catchments were identified: three near pristine sites (NP) (reference sites), three heavily invaded (HI) sites (predominantly by *A. mearnsii*); and three restored sites (R) (formerly invaded sites that had been cleared more than *ca*. 7 years prior to this study). These are draining catchments with predominantly sandstone geology, except at Molenaars which has a mixture of sandstone and igneous rocks. Sites were situated in the Mountain Stream Transitional zone, and foothill reaches of the rivers. The dominance of sedimentary Table Mountain Group sandstones affects soil texture, resulting in coarsely-textured soils (Naudé, 2012). Riparian soils in the upper reaches of the Dwars are mainly shallow lithosol soils derived from weathered Cape Granite or a mixture of Table Mountain Group sandstones and Cape Granite (Heydorn and Grindley, 1982). Sites are characterised by seasonal Mediterranean-type climate with cool rainy winters (June-August) and hot dry summers (December-February) (Deacon et al., 1992). Site selection was based on the presence of the selected suite of native and invasive species, treatment of invasive alien plants and history of restoration activities.

These riparian ecosystems are largely covered by Fynbos vegetation (dominated by the families Ericaceae, Proteaceae and Restionaceae) (Mucina and Rutherford, 2006) including a variety of tree taxa that often form forest pockets (Goldblatt and Manning, 2000). Two species native to Fynbos riparian zones were studied: *Brabejum stellatifolium* (Proteaceae) and *Metrosideros angustifolia* (Myrtaceae). These evergreen tree species are the only indigenous members of their respective genera in South Africa and are broadly distributed throughout riparian zones of the Cape Floristic Region (CFR) (Galatowitsch and Richardson, 2005). Their folivorous insect communities and leaf nutrients were contrasted with that of the invasive tree species *Acacia mearnsii* (Fabaceae) with all three tree species co-occurring and competing in some degraded riparian zones.

4.2.2. Leaf collection and nutrient analysis

In the present study, the term ‘leaf’ denotes the primary photosynthetic organ of the plant. Leaf nutrients were measured for *B. stellatifolium*, *M. angustifolia*, and *A. mearnsii*. Only mature leaf samples were taken, and leaves were considered mature when fully expanded. These were collected from five replicate trees of each tree species from each of the nine sites selected. Samples from each tree individual were removed separately with scissors and placed in plastic re-sealable bags with moistened paper towelling. Hands were gloved to avoid contamination of leaf material. Samples were placed in insulated containers with ice, and were transferred to the Institute for Plant Production in Western Cape Department of Agriculture for processing.

Leaves were then dried, ground in a Wiley mill (Thomas Scientific, Swedesboro, New Jersey, USA) and analysed for foliar phosphorus (expressed as percentage of dry mass) using a CE Instruments NC2100 CN Analyser (CE Elantech, Incorporated, Lakewood, New Jersey, USA). Total nitrogen (expressed as percentage of dry mass) was determined by the semimicro Kjeldahl method using a Tecator Kjeltec Auto 1030 Analyser.

4.2.3. Arthropod collection

Foliage active arthropods were sampled using a modified petrol-driven suction apparatus (Stewart and Wright, 1995) with a 15 cm diameter nozzle fitted with a net at the opening. Samples were collected 1-2 weeks during summer, autumn, winter and spring, 2011-2012 from the host trees (in nine selected riparian sites) used for collection of leaf samples for nutrients content analysis. Crowns of five individuals per tree species were vacuumed with seventy pokes per individual tree (Roets and Pryke, 2013). Samples were transferred to plastic bags, and were stored at -20°C until further processing. Specimens were sorted and preserved in 70% ethanol for later identification. All arthropods collected were assigned to morphospecies (Oliver and Beattie, 1996b), and then identified to taxonomic order. All seasonal samplings were combined to give accurate annual presence of arthropods.

4.2.4. Feeding guilds

The guild concept used here follows Williams, (1993). Guilds were based on insect morphology, feeding method and target-tissues reported in Siemann, (1998). Larval Lepidoptera, Hemiptera and Coleoptera (members of folivorous insects group) were chosen as constituent groups for folivorous insects. For predators, Araneae, Mantodea, and Coleoptera were chosen (members of predaceous arthropods group). Folivorous insects were further categorised as either leaf chewers or suckers. Leaf chewers included those insects with biting mouthparts whereas leaf suckers included insects with tubular mouthparts that can pierce plant cells and suck their contents. Leaf suckers also included those insects that probably use salivary enzymes for penetration of leaf epidermal and mesophyll layers to reach vascular tissues e.g. Hemiptera (Peeters, 2002a).

4.2.5. Statistical analyses

Differences in numbers of arthropods across riparian sites within three invasion treatments were tested using Generalised Linear Mixed Models (GLMM). Separate analyses were run for sites within each invasion treatment. Specifically, numbers of arthropods were fitted to Poisson distributions with a log-link function using generalised estimating equations (Allison, 1999; O'Hara, 2009; Zuur et al., 2010). The Poisson distribution type was chosen to minimize the deviance statistic (Johnson et al., 2006). These GLMMs were performed using Proc Genmod of SAS 9.1 (SAS Institute Inc., Cary, USA). Subsequently, levels of leaf damage caused by folivorous insects were correlated (using Pearson correlations) with their abundance (using data from Chapter 3).

Effects of invasion treatment on community composition of folivorous insects (leaf suckers and chewers) and predators were tested across sites within three different invasion treatments and among sites within each invasion treatment using Permutational Multivariate Analysis of Variance analyses (PERMANOVA+) in PRIMER 6 (PRIMER-E, 2008). The F and p- values (as well as t values for pairwise differences) for similarity of predator communities, folivorous communities and the two different folivore functional groups (leaf chewers and suckers) were compared among sites that differ with regards to their invasion treatments for the host tree using 9,999 permutations (Roets and Pryke, 2013). Similarity matrices were assembled using Bray-Curtis similarity measures with data fourth-root transformed to reduce the weight of common species (Anderson 2001). Results were visually represented by performing cluster analysis (group average method) on the Bray–Curtis similarity matrix. Cluster memberships of sites with greater than 40% similarity were presented on a non-metric Multi-Dimensional Scaling (nMDS) plot.

Mean leaf nutrient levels (as percentage) were compared for each plant species across sites within three different invasion treatments and within sites in each invasion treatment using analysis of variance (ANOVA) followed by Tukey's multiple comparison tests. Thereafter folivorous insect numbers were correlated (Pearson correlations) with leaf nutrient levels and numbers of predators to identify whether any of these three could explain observed differences in folivorous insect numbers. All correlations and ANOVA analyses were performed using STATISTICA version 11 (Statsoft Corporation Tulsa, U.S.A). Unadjusted $P < 0.05$ values are reported.

4.3. RESULTS

4.3.1. Effect of invasion treatment on numbers of folivorous insects and predators

The folivorous insect fauna from the sampled trees comprised of 28353 individuals belonging to 266 morphospecies. For *B. stellatifolium* and *A. mearnsii*, higher numbers of folivorous insects (and leaf chewers) were collected in the restored sites (12,440 individuals and 247 morphospecies) than were collected in near pristine or heavily invaded sites. *Metrosideros angustifolia* had more folivorous insects in near pristine sites than in restored and heavily invaded sites (Table **4.1**). On *B. stellatifolium*, these patterns were largely driven by a marked dominance of two weevil species namely *Setapion provincial* and *Setapion quantillum* (Balfour-Browne, 1944) which represented 59% of chewing insects collected. Abundant leaf suckers were collected in near pristine sites for *B. stellatifolium* and *M. angustifolia* but not for *Acacia mearnsii*, which had higher numbers in restored sites (Table **4.1**). Among the sucking insects, no particular species was dominant. More predators were collected in near pristine, heavily invaded and restored sites for *B. stellatifolium*, *M. angustifolia* and *A. mearnsii* respectively (Table **4.1**). Comparisons of sites within each invasion treatment revealed significant differences of abundance of all folivores, leaf chewers, leaf suckers and predators among sites for all host trees (Table **A4.2**).

Near pristine sites had significantly higher species richness of folivorous insects and predators than heavily invaded sites for all tree taxa combined. All host tree species had their lowest species richness in heavily invaded sites except for *M. angustifolia* where restored sites had lowest species richness of all folivores and leaf chewers (Table **4.1**). When arthropod species richness was analysed within each host tree species between sites with similar invasion treatment, there was considerable variation in species richness of all folivores, leaf chewers, leaf suckers and predators, but the pattern of strength of variation between sites differed (Table **A4.2**).

Table **4.1**: Summary of Generalised Linear Models (with Poison Distribution and log-link functions) on means for differences in abundance and species richness of arthropod trophic groups from the three focal tree species found in riparian sites that differ in their invasion treatment.

		Tree species		
Dependent variable	Overall	*B. stellatifolium*	*M. angustifolia*	*A. mearnsii*
Abundance				
All folivores	R > NP > HI	R > NP > HI	NP > HI > R	R > NP > HI
Leaf chewers	R > HI > NP	R > NP > HI	NP = HI > R	R = NP > HI
Leaf suckers	NP > R > HI	NP > R > HI	NP > HI > R	R > NP > HI
Predators	NP > R > HI	NP > R > HI	HI = R > NP	R = HI ≥ NP
Species richness				
All folivores	NP > R = HI	NP > R > HI	NP = HI > R	NP = R = HI
Leaf chewers	NP > R = HI	NP > R = HI	HI = NP ≥ R	NP = R = HI
Leaf suckers	NP > R = HI	NP > R = HI	NP = R = HI	R = NP = HI
Predators	NP > R > HI	NP > R > HI	NP = R ≥ HI	NP = R = HI

Sites are ordered with those with the highest means on the left and the lowest on the right. Data are combined totals for the four seasons of the study

NP = Near Pristine, HI = Heavily Invaded, R = Restored riparian habitat types; = signifies no significant differences, > signifies that sites to the left are significantly more species-rich/abundant; ≥ signifies that the first habitat is significantly more species-rich/ abundant than the last habitat.

4.3.2. Effect of invasion treatment on community composition of folivorous insects and predators

Community composition of all folivores and leaf suckers (all tree taxa combined) (as determined by PERMANOVA) varied among sites in all three invasion treatments but none of the pairwise comparisons were significant (Table 4.2). For host trees separately, only community composition of leaf suckers on *B. stellatifolium* varied significantly, but pairwise comparisons were not significant (Table 4.2). Comparisons of arthropod communities on each host tree separately from sites of similar invasion treatment revealed significant differences in folivore, leaf chewer, leaf sucker and predator composition between sites (Table 4.3 and Table A4.3). nMDS plots showed that, for folivore communities from all three host tree species combined, restored and near pristine sites grouped together (Fig. 4.1a). Invasion treatment affected groupings of arthropods collected from *B. stellatifolium*; heavily invaded sites and restored sites were similar and generally grouped together, whereas both were dissimilar to near pristine sites (Fig. **4.1b**). For *M. angustifolia*, near pristine and restored sites were similar but did not necessarily group together (Fig. **4.1c**). Arthropod communities associated with *A. mearnsii*, had intermixed sites (Fig. **4.1d**).

Table **4.2**: Permutation multivariate analysis (PERMANOVA) to determine similarity of folivorous insects and predators assemblages among riparian sites as well as selected tree species**.**

	Inv	NP versus HI	NP versus R	HI versus R
All folivores	1.56**	1.29	1.22	1.24
Leaf chewers	0.71	0.82	0.90	0.79
Leaf suckers	1.44*	1.36	1.18	1.05
All predators	1.01	1.19	1.03	0.78
B. stellatifolium				
All folivores	1.03	0.19	0.28	0.89
Leaf chewers	0.71	0.82	0.90	0.79
Leaf suckers	1.34*	1.26	1.16	1.05
All predators	1.03	1.16	1.01	0.86
M. angustifolia				
All folivores	0.92	1.03	0.95	0.89
Leaf chewers	0.78	0.93	0.91	0.79
Leaf suckers	1.10	1.15	0.98	1.02
All predators	0.95	1.07	0.93	0.91
A. mearnsii				
All folivores	0.89	0.88	0.99	0.95
Leaf chewers	0.68	0.72	0.79	0.94
Leaf suckers	1.13	1.04	1.21	0.93
All predators	0.92	1.03	0.95	0.89

Figures represent F-values, number of permutations for each analysis = 9,999

The insects were collected from riparian zones with different invasion treatments coded as ‘Inv’, NP = Near Pristine, HI = Heavily Invaded, R = Restored riparian sites, * $P < 0.05$, ** $P < 0.01$

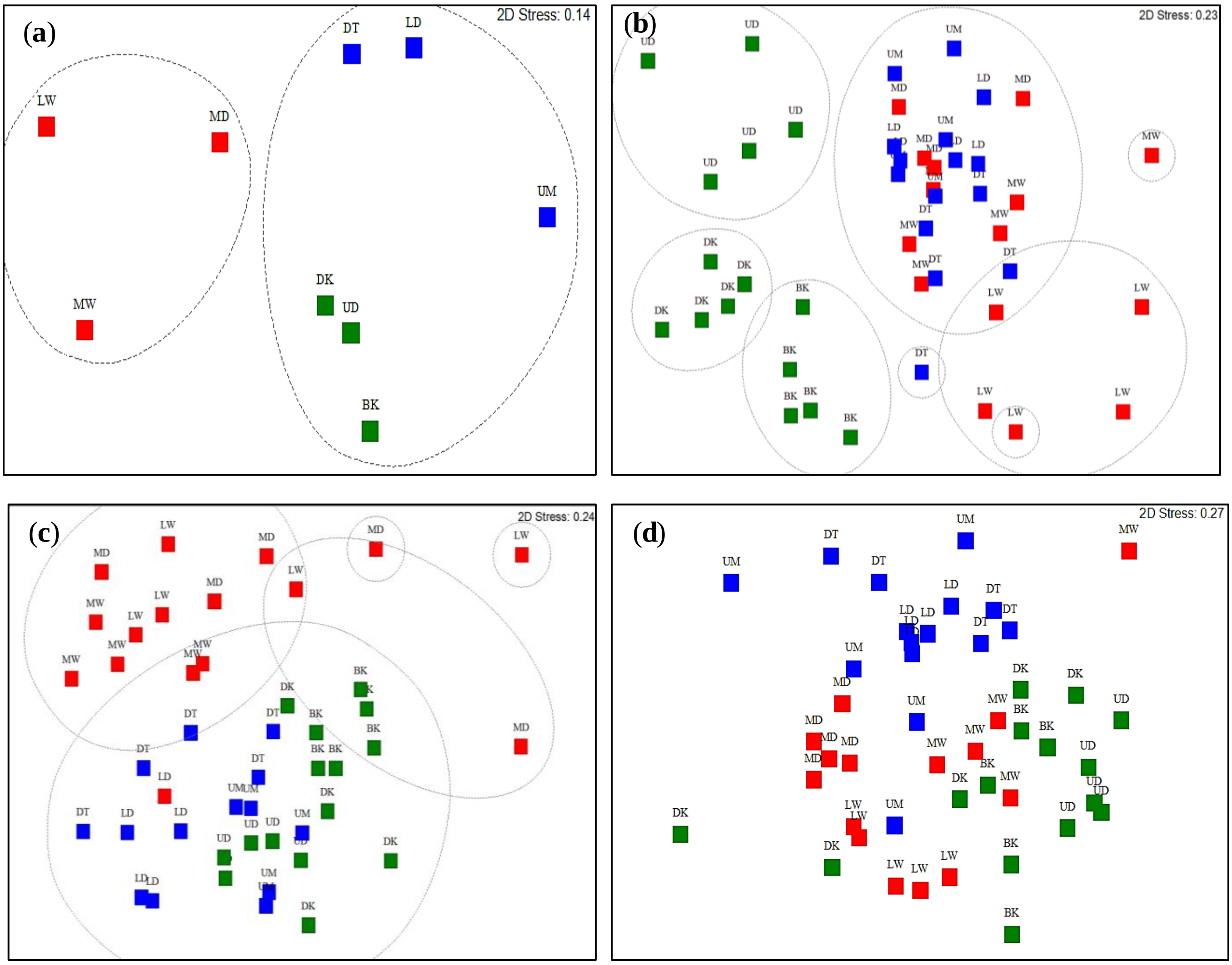

Figure **4.1**: non-metric Multidimensional Scaling (nMDS) plots of folivore assemblages from near pristine (green), heavily invaded (red), and restored (blue) riparian sites for (**a**) all folivores from all host trees combined, folivores collected from (**b**), *B. stellatifolium* (**c**) *M. angustifolia* and (**d**) *A. mearnsii*. The ellipses around the points in (**a**), (**b**) and (**c**) represent groupings which were 40% similar independently determined by cluster analysis (group average method). Riparian sites: UD = Upper Dwars, DK = Du toits Kloof, BK = Bains Kloof, MD = Mid Dwars, LW = Lower Wit, MW = Mid Wit, LD = Lower Dwars, DT = Du Toit, and UM = Upper Molenaars rivers.

Table **4.3**: Main test results of Permutation multivariate analysis (PERMANOVA) to determine similarity of folivorous insect and predator communities between riparian sites that are similar in invasion treatment for three tree species.

Dependent variable	Inv	Tree species *B. stellatifolium*	 *M. angustifolia*	 *A. mearnsii*
All folivores	NP	3.33***	3.30***	2.26***
	HI	3.21***	1.99***	3.29***
	R	3.32***	3.73***	3.56***
Leaf chewers	NP	4.68***	3.51***	2.33***
	HI	3.88***	1.94**	2.74***
	R	3.98***	4.40***	3.63***
Leaf suckers	NP	1.94***	3.19***	1.59**
	HI	1.78**	2.01**	3.24***
	R	2.02**	2.86***	2.90***
Predators	NP	2.94***	2.38***	1.96***
	HI	3.21***	3.79***	4.28***
	R	1.68***	3.30***	2.31***

Figures represent F-values, number of permutations for each analysis = 9,999.
The insects were collected from riparian zones with different invasion treatments coded as 'Inv', NP = Near Pristine, HI = Heavily Invaded, R = Restored riparian sites, * $P < 0.05$, ** $P < 0.01$, *** $P \leq 0.001$

4.3.3. Effect of altered folivorous insect numbers on leaf damage

Folivore damage levels on *B. stellatifolium* increased significantly with an increase in total folivore and leaf sucker abundance. For *M. angustifolia*, and *A. mearnsii* there was no relationship between the levels of folivore damage and total folivore, chewer and sucker abundance (Table **4.4**). When comparing sites within each invasion treatment, only the abundance of combined folivores and leaf suckers on *A. mearnsii* individuals in heavily invaded sites significantly and positively correlated to number of leaves damaged by folivorous insects (Table **A4.4**).

Table **4.4**: Correlation statistics for the interactions between folivorous insect damage on *B. stellatifolium*, *M. angustifolia* and *A. mearnsii* in relation to abundance of leaf suckers, leaf chewers and all folivorous insects combined. Coefficients corresponding to a statistically significant relationship are indicated in **bold**. (Leaf damage data were obtained from Chapter 3).

	# of leaves damaged by folivorous insects	
Arthropod Abundance	r^2	*p*-values
B. stellatifolium		
All folivores	**0.56**	0.02
Leaf suckers	**0.45**	0.05
Leaf chewers	0.42	0.06
M. angustifolia		
All folivores	0.07	0.51
Leaf suckers	0.13	0.34
Leaf chewers	0.02	0.72
A. mearnsii		
All folivores	0.01	0.85
Leaf suckers	0.02	0.69
Leaf chewers	0.31	0.12

4.3.4. Effect of invasion treatment of riparian sites on leaf nutrient levels

Leaf phosphorus (P) content (%) did not vary among sites for any plant species tested (Table 4.5, Fig. 4.2). However, comparison of sites with similar invasion treatment revealed that P content in *B. stellatifolium* leaves was significantly different from site to site, with restored sites showing stronger differences ($P < 0.001$) than near pristine and heavily invaded sites (Table 4.6). Leaf P content also differed significantly for *M. angustifolia* in heavily invaded sites and *A. mearnsii* in restored sites (Table 4.6).

Leaf nitrogen (N) varied significantly among sites for *B. stellatifolium* and *A. mearnsii* (Table 4.5). For *B. stellatifolium*, levels of leaf nitrogen were significantly higher in restored sites compared to heavily invaded and near pristine sites (Table 4.5, Fig. 4.2). Significantly higher levels of N for *Acacia mearnsii* were found in heavily invaded sites as compared to near pristine and restored sites (Table 4.5, Fig. 4.2). Among sites with similar invasion treatment, only the leaves of *A. mearnsii* in restored sites had a significantly different N content (Table 4.6).

Table **4.5**: Comparison between mean leaf nutrient levels (main ANOVA results) for three riparian tree species at sites that differ in their invasion treatments.

	SS	MS	*F*
Nitrogen			
B. stellatifolium	24.02	12.01	68.12***
M. angustifolia	1.19	0.59	1.65
A.mearnsii	3.44	1.7	8.39***
Phosphorus			
B. stellatifolium	0.01	0.004	2.30
M. angustifolia	0.004	0.002	2.71
A. mearnsii	0.01	0.004	3.04

Figures represent F- values
Significance: $* P \leq 0.05$; $** P \leq 0.01$; $*** P \leq 0.001$

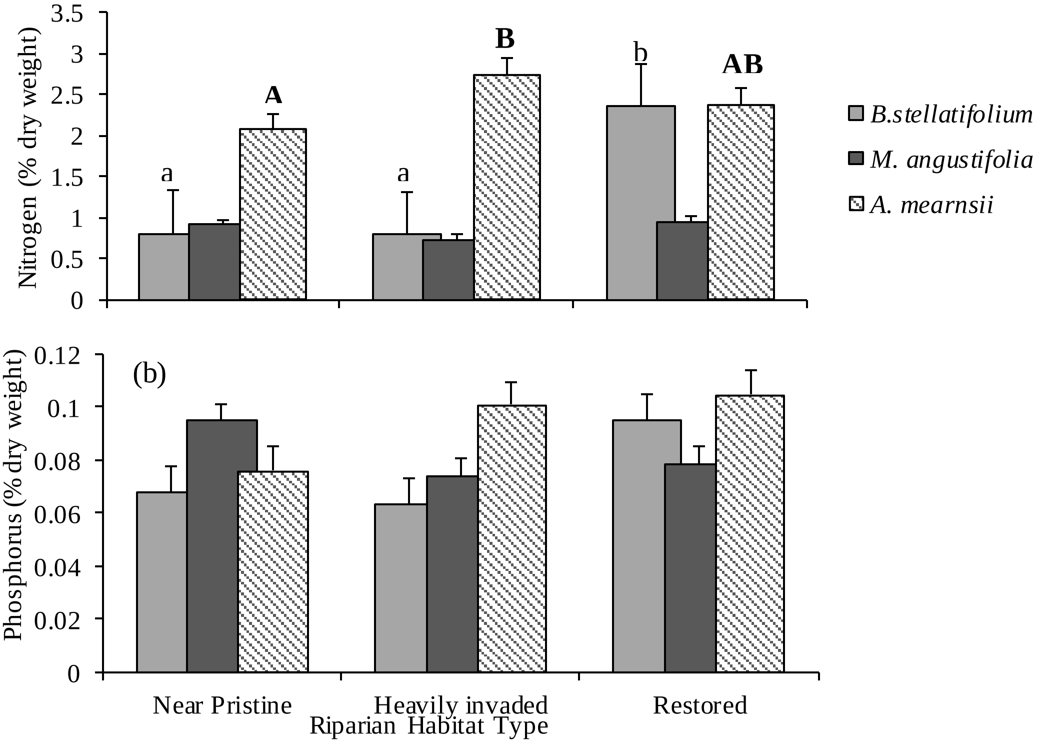

Figure **4.2**: Mean ± SE of (a) foliar Nitrogen content and (b) Phosphorus content measured as percentage of dry mass of three tree species used in the study found in riparian sites with invasion treatment. Significant differences in damage caused are indicated above bars by different lower case letters for *B. stellatifolium*, differences in the number of asterisks for *M. angustifolia* and different uppercase letters for *A. mearnsii*.

Table **4.6**: Results of one-way ANOVA test of effects of invasion treatment of riparian sites on mean leaf nutrient levels for three riparian tree species.

Tree species	Invasion treatment	Nitrogen	Phosphorus
Brabejum stellatifolium	Near pristine	0.22	7.22**
	Heavily Invaded	2.14	8.50**
	Restored	0.72	26.66***
Metrosideros angustifolia	Near pristine	0.03	2.79
	Heavily Invaded	0.52	15.6***
	Restored	0.83	0.35
Acacia mearnsii	Near pristine	0.37	0.03
	Heavily Invaded	1.32	3.27
	Restored	11.98***	10.01**

Figures represent F- values

Significance: * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$

4.3.4. Effects of predator abundance and leaf nutrient levels on folivorous insect abundance

The relationship between leaf nutrients, predators and folivorous insect abundance was investigated using linear regression. Of all the parameters recorded, nitrogen displayed significant positive correlations with all folivorous insects for *B. stellatifolium* and *A. mearnsii* (Table 4.7). Significant positive correlations were also apparent between nitrogen and leaf chewers for *B. stellatifolium*, and between predators and leaf chewers for *M. angustifolia* (Table 4.7).

Table **4.7**: Correlations between mean levels of leaf nutrients, predator numbers and mean abundance of folivorous insects.

	Nitrogen	Phosphorus	Predators
B. stellatifolium			
All folivores	0.63***	0.03	0.005
Leaf suckers	0.14	0.17	0.006
Leaf chewers	0.64***	0.02	0.01
M. angustifolia			
All folivores	0.05	0.13	0.20
Leaf suckers	0.03	0.02	0.08
Leaf chewers	0.06	0.37	0.12**
A. mearnsii			
All folivores	0.69**	-0.19	-0.27
Leaf suckers	0.28	0.11	0.39
Leaf chewers	0.14	-0.23	0.36

Significance: * $P < 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$

Between sites with similar invasion treatments, the Nitrogen (N) content in near pristine sites, was only significantly, positively correlated to the abundance of all folivores of *B. stellatifolium*, while in restored sites, leaf chewers of *A. mearnsii* correlated to N content. In heavily invaded sites, all folivores, leaf chewers and leaf suckers on *A. mearnsii* and leaf suckers on *B. stellatifolium* were significantly and positively correlated to leaf nitrogen content (Table **4.8**). Leaf Phosphorus (P) content in near pristine sites was only positively correlated with leaf suckers for *B. stellatifolium* and *A. mearnsii*, whereas in heavily invaded sites and restored sites, P was correlated with all folivores and leaf chewers on *M. angustifolia* (Table **4.8**). Leaf chewers for *A. mearnsii* in heavily invaded sites were also significantly correlated with P (Table **4.8**). There was no significant relationship between abundance of predators and that of all folivores, leaf chewers and leaf suckers for all host trees in near pristine and restored sites ($P > 0.05$). In heavily invaded sites, all folivores and leaf chewers for *M. angustifolia* were significantly and negatively correlated to abundance of predators (Table **4.8**).

Table **4.8**: Correlations between mean levels of leaf nutrients, predator numbers and mean abundance of folivorous insects for three riparian tree species at riparian sites with different invasion treatments.

		Nitrogen			Phosphorus			Predators		
Invasion treatment		*Bs*	*Ma*	*Am*	*Bs*	*Ma*	*Am*	*Bs*	*Ma*	*Am*
Near pristine	All folivores	0.26*	0.00	0.00	0.00	0.05	0.22	0.09	0.22	0.09
	Leaf chewers	0.34	0.06	0.11	0.21	0.05	0.09	0.06	0.22	0.03
	Leaf suckers	0.00	0.00	0.06	0.48**	0.04	0.45**	0.03	0.18	0.05
Heavily invaded	All folivores	0.16	0.09	0.65***	0.06	0.31*	0.21	0.21	-0.61***	0.00
	Leaf chewers	0.11	0.19	0.60***	0.06	0.40**	0.20	0.19	-0.67***	0.00
	Leaf suckers	0.58***	0.01	0.71***	0.02	0.02	0.21	0.17	0.11	0.00
Restored	All folivores	0.04	0.23	0.02	0.04	0.36*	0.04	0.07	0.01	0.00
	Leaf chewers	0.04	0.16	0.46**	0.06	0.38*	0.39*	0.13	0.02	0.03
	Leaf suckers	0.04	0.18	0.00	0.01	0.09	0.01	0.16	0.00	0.00

Figures represent F- values
Significance: * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.
Bs = Brabejum stellatifolium
Ma = Metrosideros angustifolia
Am = Acacia mearnsii

4.4. DISCUSSION

The trophic structure of arthropod assemblages can differ under different degrees of plant invasion, particularly for folivores and predators (Gratton and Denno, 2005; Mgobozi et al., 2008; Simao et al., 2010). The three invasion treatments of riparian sites represented in this study, differed in species richness and abundances of most functional groups. Like in other studies, (e.g. Simao et al., 2010), here dense stands of *A. mearnsii* reduced arthropod abundance and species richness substantially. Simao et al., (2010) experimentally manipulated the presence of an invader, whereas the current study investigated invasion in natural riparian ecosystems. Therefore, the negative effects of invasive plants on native biodiversity are not confined to natural occurring invaded ecosystems only but even to experimentally manipulated terrestrial ecosystems. Reductions in folivore richness and abundance in this study could be attributed to many factors including underlying reductions in native plant diversity due to IAPs (Litt et al., 2014), changes in plant nutritional quality (Graves and Shapiro, 2003) and reduction in predators (Simao et al., 2010). The results suggested that reductions in folivore species richness and abundance were most likely influenced by plant nutritional quality (e.g. see Peeters, 2002a) and predators (e.g. see Sobek et al., 2009). Similarly, reductions in predator richness and abundance in response to invasion could be due to habitat alterations limiting predator access to suitable web-building locations (e.g. through changes in plant structural diversity) in invaded sites (Halaj et al., 2001; Borges and Brown, 2001) or to reductions in food availability (e.g. Mooney et al., 2010). Thus in this study the reduced number of predators at invaded sites is perhaps a result of both less abundant food resources in the form of folivorous arthropods or the creation of less diverse habitat conditions due to formation of monotypic stands. The low abundance and species richness of predators in heavily invaded sites, indicates that predation, at least on folivores, is of limited importance in the regulation of folivore abundance.

Restoration efforts seemed to positively impact the recovery of folivorous insects. Such impact was evident from the increased abundance and richness of folivorous insects sampled in restored sites compared with those sampled in near pristine sites. This observation is consistent with other studies that found that arthropod species richness in restored areas were similar to that in natural Fynbos (e.g. Magoba and Samways, 2012). Increased abundance and richness of folivorous insects following restoration efforts, was unlikely to be due to absence of apparent top-down control, as predators also increased in abundance and richness in restored sites. The reason for this increased abundance and richness is unclear, although increases in native plant species richness may have been a factor (Chapter 3).

In this study, invasive plant treatment influenced community composition of folivorous insects. The community composition of folivorous insects in restored sites was comparable to that in near pristine sites, whereas heavily invaded sites supported folivorous insect species that otherwise might not occur in near pristine or restored sites. Patterns of community composition also differed among host tree species. For *B. stellatifolium*, near pristine site communities were clearly separate from those of restored and heavily invaded sites, which were closely related. This indicates that several folivorous species associated with *B. stellatifolium* are specialised to diverse riparian habitats and do not occur in open (restored) and less diverse (heavily invaded) sites. For *M. angustifolia*, near pristine sites clustered closely with restored sites. Arthropod communities of all sampling sites were intermixed for *A. mearnsii*. This indicates that, whatever the invasion treatment, arthropod communities associated with *A. mearnsii* remain similar. When sites under each invasion treatment were compared, the composition of all considered functional groups varied significantly among study sites. This provided strong evidence that each and every site has unique catchment signature despite overtly similar invasion treatment, a result similar to the findings of King and Schael (2001).

Restored sites had a folivore-dominated trophic structure, as folivores were more abundant in restored sites than they were in near pristine sites. The abundant folivores in restored sites were *Setapion provincial* and *Setapion quantillum* (exclusively feeding on *B. stellatifolium*). This may indicate why the levels of damage by folivores, particularly on *B. stellatifolium*, were higher in restored sites (as shown in Chapter 3). For *M. angustifolia* and *A. mearnsii* no such shift in trophic structure was evident, despite high levels of folivory in heavily invaded (for *A. mearnsii*) and restored (*M. angustifolia*) sites (as seen in Chapter 3).

Leaf folivory varied considerably among host trees with differing invasion treatments (as shown in Chapter 3). One would assume that this variation in leaf damage among host trees among sites was due to site differences in the abundance of leaf feeders. When all invasion treatments were considered, increased folivore abundance was significantly correlated to increased levels of leaf damage only for *B. stellatifolium*. The lack of significant correlations for other host trees suggest that factors other than folivore abundance such as nutrient availability and predator abundance are responsible for the observed high levels of folivory in restored and heavily invaded sites.

Recorded levels of foliar nitrogen of the host trees fell within the range of values reported for other Mediterranean trees (Fife et al., 2008) and for other evergreen trees such as Australian

Acacia spp. (Peeters, 2002a). Likewise, leaf phosphorus levels were within the lower and upper ranges of reported values for these hosts (Morrow, 1983; Fife et al., 2008). Foliar nutrient content data confirmed that *B. stellatifolium* had greater levels of foliar nitrogen at restored sites as did *Acacia mearnsii*. When sites within each invasion treatment were compared, only nitrogen content for *A. mearnsii* at restored sites differed significantly. Phosphorus levels did not vary significantly for any plant species tested across all invasion treatments, but did change significantly when sites per invasion treatment were compared for most host trees. The possible mechanisms underlying these variations in leaf nutrient content could not be ascertained in this study.

Many reports have shown that folivores numbers are limited by leaf nutrient content (Schade et al., 2003; Huberty and Denno, 2006b) and presence of predators (Schmitz et al., 2000; Halaj and Wise, 2001). The fact that plant species richness did not affect levels of leaf damage by folivores and fungal pathogens (Chapter 3), suggests that higher nitrogen content in heavily invaded and restored sites for *A. mearnsii* and *B. stellatifolium* might explain higher folivore damage in those sites (as shown in Chapter 3). Of the covariates tested, leaf nitrogen was the only factor that was both positively correlated with all folivores and leaf chewers for *B. stellatifolium* and all folivores for *A. mearnsii*. This result suggests that nitrogen content is an important driver of arthropod dynamics for *B. stellatifolium* and *A. mearnsii* rather than phosphorus content and predator presence. The lack of relationship between leaf phosphorus content and levels of folivory was unexpected since phosphorus is one of the fundamental requirements for proper cellular functioning and growth rate of consumers (Elser et al., 2000a; Ricklefs, 2008). The absence of strong top-down effects imposed by predators on total folivorous insect abundances on host trees concurred with some previous studies (Bock et al., 1992; Ritchie, 2000; Cornelissen and Stiling, 2006). Cornelissen and Stiling, (2006) suggested that the lack of folivorous insect responses to top-down manipulations may be explained by differences in site microclimate and, probably, sample size. However, because covariate models do not imply causality, it is possible that other factors in addition to leaf nutrients and presence of predators do impact folivore abundances.

Considering individual folivore functional groups, results indicated that nitrogen was the most important factor influencing leaf chewers associated with *B. stellatifolium*. Similarly, Boavida and Neuenschwander, (1995b) and Awmack and Leather, (2002), also noted that leaf chewer numbers correlate well with leaf nitrogen levels. Predator abundance was closely, positively, correlated with leaf chewers for *M. angustifolia* only, but its biological significance is uncertain.

The effects of bottom-up forces over top-down forces in host trees found in heavily invaded and restored sites may partly be because exotic *Acacia* spp. enrich Fynbos riparian ecosystems with nutrients (Naudé, 2012). Evidence to date suggests that increased soil nutrients often improve host quality (e.g.Wolf, 2008). Increases in host nutritional quality may enhance the overall abundance of folivorous insects and, as a consequence, could result in increased predation (Cornelissen and Stiling, 2006). Other proposed factors are those relating to: (i) defence characteristics of leaves of host plant (e.g. Mooney and Gulmon 1982; Moore et al., 1988; Dudt and Shure, 1994; Awmack and Leather, 2002; Peeters, 2002a), (ii) host plant size (Castagneyrol et al., 2013), (iii) host apparency (meaning likelihood of an individual plant being identified and attacked by folivores), and (iv) host acceptance (e.g. leaf toughness) (Gruner et al., (2005; Castagneyrol et al., 2013). Host apparency may be of particular interest to explore in future given the evident canopy and structural changes brought about by IAPs to ecosystems, and likewise the subsequent removal of IAPs by Working for Water (Samways et al., 2011). This highlights the importance of researching a wide range of plant traits when studying overall responses of folivory.

In conclusion, this study showed that IAPs can alter the trophic structure of riparian sites and removal of IAPs can promote the re-establishment of the associated arthropod communities. The study also revealed that at least for the factors considered, bottom-up influences on insect folivore abundance may be more important than top-down effects in strongly disturbed environments such as the riparian sites studied. Only the folivore community of *M. angustifolia* was correlated to densities of predators stressing the need to assess numerous factors when studying trophic interactions. Clearly, the regulation of folivore communities involves many complex interactions (see, Dudt and Shure (1994); Ritchie, 2000; Castagneyrol et al., (2013). Future studies that explore mechanisms of interaction among folivore numbers, leaf structural traits, plant resources, and predators should provide further important insights

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APPENDIX **4**

Table **A4.1**: Numbers of folivorous insect and predatory arthropods collected in each site.

	Near pristine			Heavily invaded			Restored		
	UD	DK	BK	MD	LW	MW	DT	UM	LD
Abundance									
All folivores	3195	2651	4145	3255	1044	1623	2457	5895	4088
Leaf chewers	1519	1762	1819	2244	484	900	1637	4568	1912
Leaf suckers	1676	889	2326	1011	560	723	820	1327	2176
Predators	732	504	922	673	327	432	664	400	778
Species richness									
All folivores	149	159	149	139	96	92	125	93	134
Leaf chewers	62	78	70	76	43	47	56	48	67
Leaf suckers	87	81	79	63	53	45	69	45	67
Predators	96	97	82	53	42	47	55	79	67

Riparian sites: UD = Upper Dwars, DK = Du toits Kloof, BK = Bains Kloof, MD = Mid Dwars, LW = Lower Wit, MW = Mid Wit, LD = Lower Dwars, DT = Du Toit, and UM = Upper Molenaars rivers

Table **A4.2**: Summary results for Generalised linear models (Poisson distribution and log-link function) (for each host plant separately) on comparisons of species richness and abundance data of all folivores, leaf chewers, leaf suckers and predators among sites within each invasion treatment.

Dependent variable	Inv	*B. stellatifolium*	*M. angustifolia*	*A. mearnsii*
Abundance				
All folivores	NP	UD ≥ DK = BK	BK > UD > DK	BK > DK > UD
	HI	MD > MW > LW	MD > MW > LW	MD > LW = MW
	R	UM >. LD > DT	UM > DT > LD	LD > DT > UM
Leaf chewers	NP	DK = BK ≥ UD	BK > UD > DK	BK > DK > UD
	HI	MD > MW > LW	MD > MW > LW	MD > LW > MW
	R	UM > LD > DT	UM > DT > LD	LD > DT > UM
Leaf suckers	NP	UD > DK = BK	BK > UD > DK	BK > DK = UD
	HI	MD > MW > LW	MD = MW = LW	MD > LW = MW
	R	UM > LD > DT	UM > DT > LD	LD > DT > UM
Predators	NP	BK > UD > DK	UD > BK = DK	UD = BK = DK
	HI	MD = LW = MW	MD > MW > LW	MD > MW = LW
	R	DT = LD = UM	LD > UM > DT	LD > DT > UM
Species richness				
All folivores	NP	UD ≥ DK = BK	UD = DK = BK	DK ≥ BK = UD
	HI	MD = LW = MW	MD = MW ≥ LW	MD > LW = MW
	R	LD = UM = DT	UM = LD = DT	LD > DT > UM
Leaf chewers	NP	DK > UD = BK	UD = DK = BK	BK = DK > UD
	HI	MD = LW = MW	MD > MW = LW	MD > LW = MW
	R	UM = LD = DT	LD = UM ≥ DT	LD = DT > UM
Leaf suckers	NP	UD = DK = BK	UD = BK ≥ DK	DK = BK = UD
	HI	MW = MD = LW	MD = MW = LW	MD > LW = MW
	R	LD = UM = DT	UM = DT = LD	LD > DT > UM
Predators	NP	DK = UD > BK	UD = BK = DK	DK = UD = BK
	HI	MD = MW = LW	MD = MW > LW	MD > MW = LW
	R	DT = LD = UM	UM = LD = DT	LD ≥ DT = UM

Sites are ordered with those with the highest means on the left and the lowest on the right. The arthropods were collected from riparian zones with different invasion treatments coded as ‘Inv’, NP = Near Pristine, HI = Heavily Invaded, R = Restored riparian sites,
Riparian sites: UD = Upper Dwars, DK = Du toits Kloof, BK = Bains Kloof, MD = Mid Dwars, LW = Lower Wit, MW = Mid Wit, LD = Lower Dwars, DT = Du Toit, and UM = Upper Molenaars rivers; = signifies no significant differences, > signifies that sites to the left are significantly more species-rich/abundant; ≥ signifies that the first site is significantly more species-rich/ abundant than the last site.

Table **A4.3**: Pairwise test results of Permutation multivariate analysis (PERMANOVA) to determine similarity of folivorous insect and predator communities among riparian sites similar in terms of invasion treatment for three host tree species.

Dependent variable	Near pristine sites			Heavily invaded sites			Restored sites		
	UD vs DK	UD vs BK	DK vs BK	MD vs LW	MD vs MW	LW vs MW	DT vs UM	DT vs LD	UM vs LD
B. stellatifolium									
All folivores	2.01**	1.7**5	1.72**	2.02**	1.72**	1.62**	1.91**	1.58**	2.01**
Leaf chewers	2.33**	2.12**	1.99**	2.19**	2.07**	1.67**	2.34**	1.64**	2.02**
Leaf suckers	1.51**	1.27	1.41*	1.47**	0.99	1.48**	1.27*	1.31*	1.79*
Predators	1.81**	1.62**	1.72**	1.75**	1.57**	2.04**	1.18	1.23*	1.47**
M. angustifolia									
All folivores	1.81**	1.89**	1.76**	1.21	1.56*	1.49**	2.06**	1.70**	2.05**
Leaf chewers	1.83**	2.18**	1.62**	1.29	1.37*	1.54*	2.37**	1.73**	2.22**
Leaf suckers	1.79**	1.63**	1.92**	1.09	1.73**	1.44*	1.69*	1.65*	1.73**
Predators	1.49**	1.73**	1.41*	2.12**	1.76**	1.96**	1.72**	1.96 **	1.79 **
A. mearnsii									
All folivores	1.53**	1.48*	1.49**	1.76**	1.97**	1.72**	1.61**	1.98**	2.09**
Leaf chewers	1.32	1.54**	1.74**	1.78**	1.81**	1.42**	1.58*	2.07**	2.08**
Leaf suckers	1.43*	1.14	1.19	1.50*	1.92**	1.97**	1.47*	1.67**	2.06**
Predators	1.79**	1.31*	1.12	2.38**	1.48*	2.22**	1.75**	1.43*	1.36*

Figures represent t-values, number of permutations for each analysis = 9,999:

Significance: * $P < 0.05$, ** $P < 0.01$, *** $P \leq 0.001$

vs = versus

Riparian sites: UD = Upper Dwars, DK = Du toits Kloof, BK = Bains Kloof, MD = Mid Dwars, LW = Lower Wit, MW = Mid Wit, LD = Lower Dwars, DT = Du Toit, and UM = Upper Molenaars rivers.

Table A**4.4**: Results of correlations between number of leaves damaged by folivorous insects and abundance of folivorous insects (total, suckers and chewers) for three riparian tree species on riparian sites with similar invasion treatment.

		# of leaves damaged by folivorous insects		
Invasion treatment		*Brabejum stellatifolium*	*Metrosideros angustifolia*	*Acacia mearnsii*
Near pristine	All herbivores	0.00	0.03	0.17
	Leaf chewers	0.02	0.02	0.18
	Leaf suckers	0.03	0.02	0.07
Heavily Invaded	All herbivores	0.01	0.19	0.81***
	Leaf chewers	0.01	0.14	0.22
	Leaf suckers	0.03	0.11	0.83***
Restored	All herbivores	0.21	0.00	0.01
	Leaf chewers	0.11	0.01	0.00
	Leaf suckers	0.12	0.13	0.02

Significance: *** $P \leq 0.001$

CHAPTER 5

Physiological responses to leaf folivory and fungal diseases in a native riparian tree, *Brabejum stellatifolium*.

ABSTRACT

The canopies of many tree species sustain a large diversity of folivorous arthropods and fungal pathogens and these organisms are thought to have a major influence on overall tree and stand productivity. Leaf fungal diseases caused by *Phyllosticta owaniana* and *Periconiella velutina*, commonly found on the native riparian tree *Brabejum stellatifolium* (wild almond), like other pathogenic fungi are likely to negatively affect their host plant species by reducing a plant's photosynthetic efficiency. In addition to these two fungal pathogens, the weevils *Setapion provincial* and *Setapion quantillum* (Coleoptera: Apionidae) are abundant in wild almond canopies. Despite their pervasive occurrence, the impacts of these arthropods and fungal pathogens on host tree leaf physiology have not been examined. Using a suite of methods including measuring photosynthetic rates and completing visual analyses of leaves, the physiological response of wild almond leaves to fungal pathogens and folivore damage in riparian habitats of the Western Cape Province, South Africa was assessed. Leaf nitrogen (N) and phosphorus (P) and leaf water content were also determined. Large declines in photosynthetic rates and other physiological parameters were associated with increasing damage severity caused by weevils and fungal pathogens in leaves of *B. stellatifolium*. Nitrogen and phosphorus content was generally negatively associated with leaf fungal disease severity. Water and phosphorus content were negatively correlated with increased weevil damage levels, while N content was positively correlated with it. Leaves infected by *Periconiella veluntina* showed increased water content with damage severity, whereas water content declined as *P. owaniana* damage increased. These results showed that not only leaf area is reduced by folivory and fungal pathogens but normal plant metabolic functioning is affected through various mechanisms. This study provides valuable information about why *B. stellatifolium* and other native trees are likely to recover slowly in restored sites where they are severely damaged by both folivores and fungal pathogens.

Keywords: Insect-folivory, Fungal pathogens, *Brabejum stellatifolium*, Gas exchange, Water content, Nitrogen, Phosphorus.

5.1. INTRODUCTION

Folivorous arthropods and fungal pathogens cause damage to photosynthetic tissue, thereby affecting plant growth and, ultimately, the primary production of plant communities (Hood et al., 1990; Faeth, 1992); Aldea et al., 2006b; Yang et al., 2007; Nabity et al., 2009). Insect folivory has been shown to reduce net primary production in forests, grasslands and global agricultural output by an average of 15, 24 and 14%, respectively, and these values can exceed 70% during outbreaks (Cyr and Pace, 1993; Oerke and Dehne, 1997). A number of researchers have explored how fungal pathogens and folivores reduce net photosynthesis either through direct observation and measurement (Welter 1989; Trumble et al., 1993; El-Omari et al., 2001; Thomson et al., 2003; Bechtold et al., 2005; King and Caylor, 2010) or through modelling (Rouse, 1988; Johnson and Teng, 1990; Pinnschmidt et al., 1995; Lopes and Berger, 2001). The results from these studies show that the magnitude of effects of insect folivores and fungal pathogens on photosynthesis are highly variable and that it depends largely on the type of feeding damage (e.g. piercing or chewing damage), type of fungal disease (e.g. chlorotic or necrotic leaf lesions), or the mode of defence deployed by the plant (Cooke and Whipps, 1980; Peterson and Higley, 2001; Aldea et al., 2005; Nabity et al., 2009). However, a review by Nabity et al., (2009) showed that the impact of folivory on photosynthetic rate is generally negative regardless of feeding mode.

The direct loss of foliage due to folivore activities leads to a reduction of photosynthetic capacity (Nabity et al., 2009). A growing body of evidence suggests that the underlying mechanisms leading to a reduction in leaf photosynthetic capacity are complex, ranging from disruptions in fluid or nutrient transport (Sack and Holbrook, 2006), induced reductions in metabolic processes (e.g. Zanger et al., 2002) to triggering defence-related responses (Kessler and Baldwin, 2002). For instance, in the leaves of *Quercus velutina* Lam. and *Cercis canadensis* L. the deleterious action of folivores and fungal pathogens affected electron transport in the chloroplasts of the remaining tissues, thereby reducing photosynthetic rate (Aldea et al., 2005). This comes because of changes in the primary photosynthetic reactions (discussed by Velikova et al., 2010) which can be manifested as: (i) reduced carbon assimilation rates and photosystem II quantum operating efficiency and in damaged leaves (Zanger et al., 2002) because of triggered production of defence compounds to dissuade folivorous insect and fungal pathogen attack (Bi and Felton, 1995; Thordal-Christensen et al., 1997; León et al., 2001; Bown et al., 2002), (ii) the emissions of volatile organic compounds (VOC) (Dicke and Hilker, 2003) which can affect defence mechanisms of plants (Dicke and

Hilker, 2003), and (iii) carbon and nitrogen can be diverted away from primary metabolism like production of photosynthetic enzymes (Agrawal et al., 2000) to induced production of defence compounds (Aldea et al., 2006b). The diversion of carbon and nitrogen away from primary metabolism may be exacerbated when it is necessary for a plant to produce defence compounds against damage caused, by folivorous insects and fungal pathogens simultaneously (Faeth, 1992; Saikkonen et al., 2001). This may lead to leaf abscission (Hoad et al., 1998; Manter and Kavanagh, 2003) instead of offering cross-protection (Aldea et al., 2006b). Apart from plant defence, variability in the impact of insect folivores and fungal pathogens on photosynthesis can be influenced by plant nutritional quality and water availability (Mattson, 1980; Casotti and Bradley, 1991; Snoeijers et al., 2000; Robert et al., 2002).

Nutrients like nitrogen are essential for growth, survival and reproduction of folivores (Mattson 1980; Ayres et al., 2000; Bentz and Townsend, 2001), and performance and growth of fungal pathogens on or within plants (Franzluebbers and Hill, 2005; Larkin et al., 2012). On entering a plant, nitrogen (N) available to a fungal pathogen will depend on the tissue being colonised and nitrogen content of that invaded tissue (Walters and Bingham, 2007). Mode of nutrition may also affect available nitrogen, with hemibiotrophic fungal pathogens having access to a wider range of N sources, than biotrophic fungal pathogens, which are limited to N available in the extrahaustorial matrix of the apoplast (Wood et al., 1988; Olufemi et al., 2002). So what is the effect of N on fungal pathogens and folivory in plants? Available data indicates that an increase in fungal disease and insect folivory severity with an increase in leaf N content (Von Tiedemann, 1996; Jensen and Munk, 1997; Robert et al., 2005). The effects of phosphorus (P) on the impacts of fungal disease and folivory are not well understood, as studies are contradictory. Sweeney et al. (2000) showed that P had a moderately suppressive effect on wheat leaf rust. Develash and Sugha (1997) found that increased P content increased the severity of downy mildew disease on onion, although such increase was relatively low when compared with increasing N content. However, general trends relating to fungal pathogen damage and insect folivory on foliar nutrients, and their effect on plant functioning are equivocal as there are few systematic studies which compare these two damage types (e.g. Ayres et al., 2000; Awmack and Leather, 2002; Huberty and Denno, 2006b).

In this study, physiological parameters and leaf nutrients in wild almond leaves with varying levels of fungal pathogen and folivore damage were investigated. This was done to determine why some native plants in restored riparian zones, like *M. angustifolia* and *B. stellatifolium*, recover slowly after removal of invasive alien plants such as *A. mearnsii* (as shown in Chapter 3). The following questions on the interaction between fungal pathogens, folivorous insects and their host *Brabejum stellatifolium* L. were addressed: (i) What are the impacts of the fungal pathogens *Phyllosticta owaniana* (G. Winter) and *Periconiella velutina* (G. Winter) and the weevils *Setapion provincial* (Balfour-Browne) and *Setapion quantillum* (Balfour-Browne) on gas exchange in leaves? (ii) How do gas-exchange parameters, nutrients and water content relate to damage severity?

5.2. METHODS

5.2.1. Host plant, fungal pathogens and folivorous insects

Brabejum stellatifolium is an evergreen riparian tree that is abundant in riparian zones of the western part of the Fynbos biome, and it tolerates a variety of soils and habitats (Galatowitsch and Richardson, 2005). This species is a target species for ecological restoration of Fynbos riparian zones (Galatowitsch and Richardson, 2005). Assessment of leaf damage (see Chapter 3) confirmed that wild almond leaves are commonly infected by *Phyllosticta* spp. (leaf spots) and *Periconiella* spp. (black sooty mould) and heavily damaged by weevils (*Setapion provincial* and *Setapion quantillum*) throughout riparian zones in the Western Cape Province.

Phyllosticta and *Periconiella* are large genera of ascomycete fungi, and numerous species cause disease on a variety of host plants (Stone et al., 2004). *Phyllosticta* spp. occur in most temperate and Mediterranean habitats, and the diseases they cause are largely characterised by chlorotic to necrotic localised lesions (Sinclair et al., 1987). *Periconiella* spp. (black sooty mould) is described as nonparasitic, saprophytic, and superficial on plants (Reynolds, 1999; Jouraevaa et al., 2006), and are found on abaxial leaf surfaces giving the leaf a black colour. The *Phyllosticta* and *Periconiella* species that infect wild almond at Western Cape riparian sites were identified as *Phyllosticta owaniana* and *Periconiella velutina* (Doidge and Bottomley, 1931; Swart et al., 1998; Arzanlou et al., 2007). Lesions of *P. owaniana* typically are between 2 and 10 mm in diameter and are angular to nearly round. The fungal pathogen produce dark-brown spores on the upper surfaces of the lesions and produce conidia on dark reddish brown stromata. Black mould is a dark-pigmented fungus that covers the leaves.

The two species of weevils most abundant in South-Western Cape (*Setapion provincial* and *Setapion quantillum*) are host specific to *B. stellatifolium* (Rourke, 1971; Procheş, 2007).

They are members of an African endemic group of Apionidae called Tanaonimi (Procheş, 2007). *Setapion provincial* is seasonal whereas *S. quantillum* is likely to be seen throughout the year (Procheş, 2007). Despite occurring in large numbers, into the hundreds of millions (see Procheş, 2007), no study has been undertaken quantifying leaf physiological responses to their damage. Both species create numerous very small openings in the cuticle of the leaf (Fig **A5.1**).

Before starting the measurements of leaf physiological parameters, visual assessment of the presence of folivory damage caused by *P. owaniana* and *P. velutina* diseases was conducted on each of ten trees (diameter at breast height between 10–28 cm) in February 2010 in riparian habitats. From each individual tree, ten fully expanded leaves were selected to study their physiology. Each leaf was visually analysed for damage severity (intact = 0%; 1 = > 0–20%; 2 = 21–40%; 3 = 41–60% and 4 = 61–100% of leaf area damaged (LAD)) modified from Benítez-Malvido (1999), García–Guzmán and Dirzo (2001), García-Guzmán and Dirzo (2004) and Eichhorn et al. (2010). Damage severity was defined as the percentage of leaf area visibly infected by a fungal pathogen or area damaged by feeding activities of arthropods.

After measuring photosynthesis (see below) all experimental leaves were removed from the trees (with their petioles intact) and immediately transported to the laboratory, where their outlines were traced on a transparency and they were photographed. Images were then used to determine the percentage of leaf tissue consumed by weevils and infected by fungi using Digimizer image analysis software version 4.2.2 (MedCalc Software, Ostend, Belgium).

5.2.2. Physiological parameters

Net photosynthesis measurements: All experiments were conducted during midday in summer, when air temperatures were >30ºC. Obtaining photosynthesis measurements under natural environmental conditions is important to determine the status of radiation-use efficiency (Bradford et al., 2005). A portable photosynthesis system, LCpro (ADC, Hoddesdon, UK) was used to collect photosynthetic measurements from individual leaves (matured and fully expanded), still attached to the tree. On each individual tree ten damaged leaves were randomly selected, and paired with three comparable, but undamaged leaves which served as a control. Using the LCpro, two to four photosynthetic rate (A_{max}), measurements per leaf (sun-exposed) were collected, and the values averaged to yield a single value for each leaf for data analysis. Measurements were carried out from 12:00 to 14:00 when photosynthetic photon flux density (PPFD) levels are usually at their highest (Tang et al., 1988) so that all *B. stellatifolium* leaves were light saturated. The stomatal conductance (g_s), transpiration rate (E) and leaf temperature (Tleaf in °C) variables were measured concurrently with photosynthetic rates by appropriate sensors installed within the leaf chamber.

Leaf water content: Separate set of leaves (apart from the ones used for photosynthesis measurements) were collected and weighed to determine fresh weight (FW). They were then dried in the oven for 48 h at 60°C to determine dry weight (DW). Damage severity was determined as outlined above for leaves used in photosynthesis measurements. Water content was calculated using the equation:

$$((FW - DW)/FW) * 100 \qquad \text{(Eq. 1)}$$

Leaf nutrient content analysis: Nutrient analyses were performed on dried leaf tissue samples used for leaf water content determination by the Institute for Plant Production in Western Cape Department of Agriculture. Nitrogen concentrations were determined by the semimicro Kjeldahl method using a Tecator Kjeltec Auto 1030 Analyser after saturating samples in concentrated sulphuric acid with a selenium catalyst. Phosphorus was measured using an Inductively Coupled Plasma Spectrometer.

5.2.3. Data analyses

A one-way analysis of variance (ANOVA) followed by a post-hoc Tukey multiple comparison test was used to examine differences in leaf gas-exchange processes and nutrients (separately) between the control and various damage severity classes in mature leaves. Correlation analysis was used to examine the relationships between increases in damage severity and increases in A_{max}, g_s, E, Tleaf and leaf nutrients for each damage type. Pearson correlation coefficients were calculated to examine the strength of these relationships. All statistical analyses were performed in STATISTICA version 11 (Statsoft Corporation Tulsa, U.S.A).

5.3. RESULTS

5.3.1. Black mould (*Periconiella velutina)*–photosynthesis and gas exchange relationships

There was significant negative correlation between the amount of leaf area covered by black mould and photosynthetic rate (A_{max}: $r = -0.84$, $F_{(1,\ 17)} = 17.77$, $P < 0.0001$; Fig. **5.1a**). *Brabejum stellatifolium* leaves with low damage levels had significantly lower mean photosynthesis values compared to undamaged leaves (Table 5.1). As damage increased, a significant decrease in A_{max} was observed for severely damaged areas (Table 5.1).

Other physiological processes were also affected by black mould disease. In our study, a small increase in damaged leaf area resulted in large and significant reduction in stomatal conductance (g_s: r = -0.82, $F_{(1,\ 17)}$ = 10.87, P < 0.0001; Fig. 5.1b), as indicated by decreasing mean values at increasing damage intervals (Table 5.1). However, no significant difference was observed between the control and the first damage level. Stomatal conductance has a close relationship with leaf transpiration since water exits the leaf through the stomata. In our study, transpiration and damage intensity (damaged leaf area) were significantly and negatively correlated (E: r = -0.58, $F_{(1,\ 17)}$ = 1.96, P = 0.03; Fig. 5.1c) and varied over the four damage intervals (Table 5.1). Leaf respiration was lower in those intervals where stomatal conductance was lower (Table 5.1). Overall, the transpiration levels of leaves covered with black mould were low (<2 mmol m^{-2} s^{-1}).

There were significant differences among leaf temperature values for leaf damage categories (Tleaf: r = 0.72, $F_{(1,\ 17)}$ = 4.59, P < 0.0001; Fig. 5.1d). The observed mould-induced decline in A_{max} may also be partially due to a temperature effect, since the abaxial surface temperature of black mould diseased leaves of the highest disease level was on average 4°C warmer than that of a mould-free leaf (Table 5.1).

Table **5.1**: Effects of black mould on mean photosynthetic rate (A_{max}), stomatal conductance (g_s), leaf temperature (Tleaf) and leaf transpiration (E) of *B. stellatifolium* leaves. Means ± SE with the same letter are not significantly different, different letters denote means that are statistically different (P < 0.05) as determined by one-way ANOVA and Tukey's HSD test.

Damage category	A_{max}	g_s	Tleaf	E
Control	8.99 (0.45)^{a}	0.22 (0.01)^{a}	25.35 (0.69)^{a}	1.71 (0.15)^{a}
>0 – 20%	4.78 (0.48)^{b}	0.23 (0.01)^{a}	27.00 (2.66)^{b}	1.39 (0.08)^{ab}
21 – 40%	2.39 (0.31)^{c}	0.12 (0.01)^{b}	30.25 (0.64)^{bc}	1.13 (0.04)^{bc}
41 – 60%	1.39 (0.26)^{c}	0.10 (0.01)^{b}	31.94 (0.77)^{c}	1.01 (0.06)^{c}

Although water content of wild almond leaves increased with increasing levels of black mould leaf coverage, the increase was not significant ($F_{(1,\ 11)} = 1.69$, $P = 0.2$; Fig. 5.2). Leaf N content ranged from 0.2 to 1.6% dry weight (DW) and leaf P content from 0.04 to 0.16% DW. There was a significant, negative correlation between nitrogen and levels of disease severity (r = -0.61, $F_{(1,\ 11)} = 4.04$, $P = 0.005$; Fig. 5.3a) and with P (r = -0.27, $F_{(1,\ 11)} = 2.17$, $P = 0.25$; Fig. 5.3b).

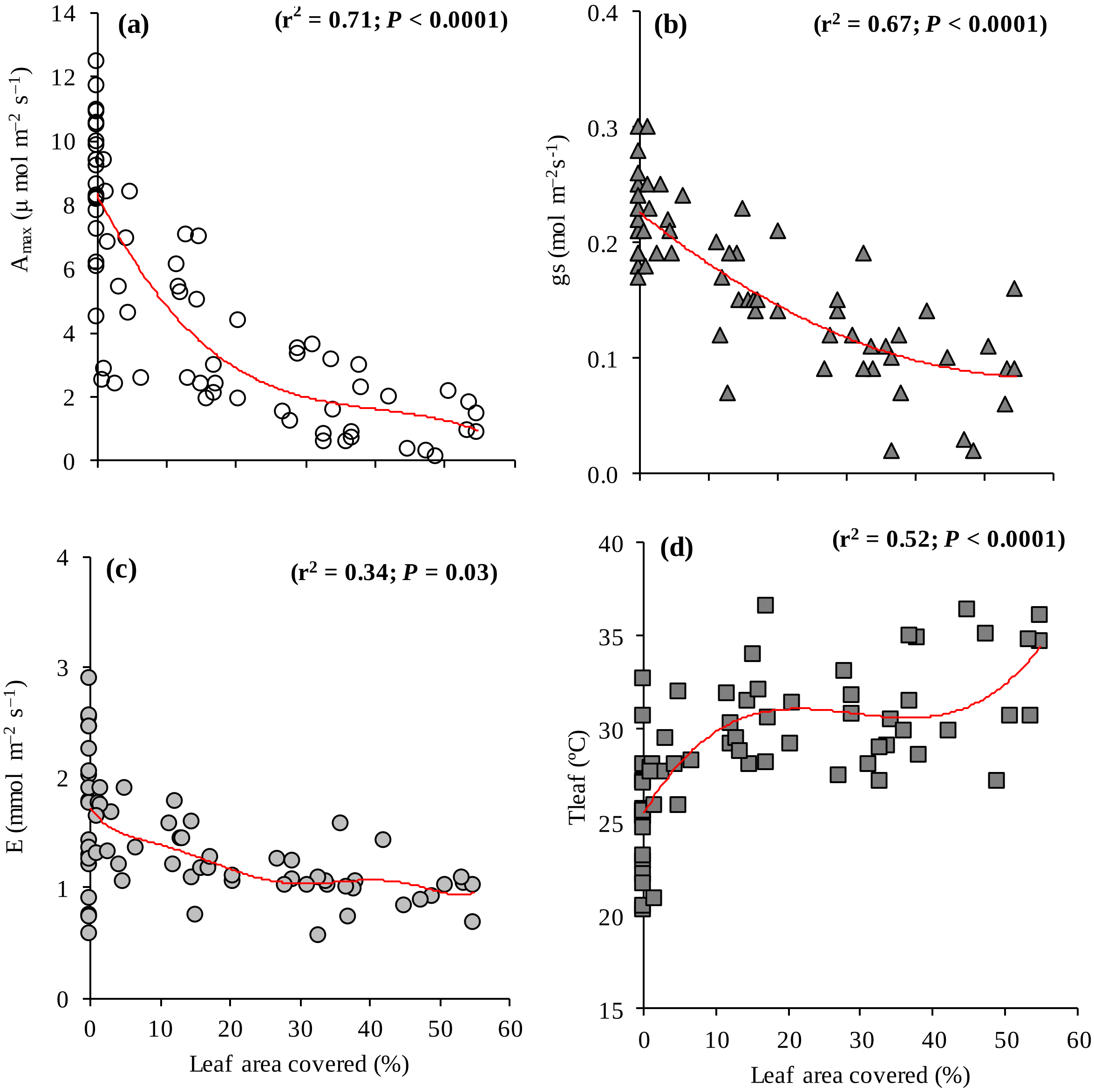

Figure **5.1**: Photosynthetic rate (A_{max}: **a**), net stomatal conductance (g_s: **b**), transpiration (E: **c**) and leaf temperature (°C) (Tleaf: **d**) in relation to percentage of leaves in the crowns of *B. stellatifolium* trees infected by black mould. r = Pearson correlation coefficients. All the trends were non-linear; polynomials provided the best fit.

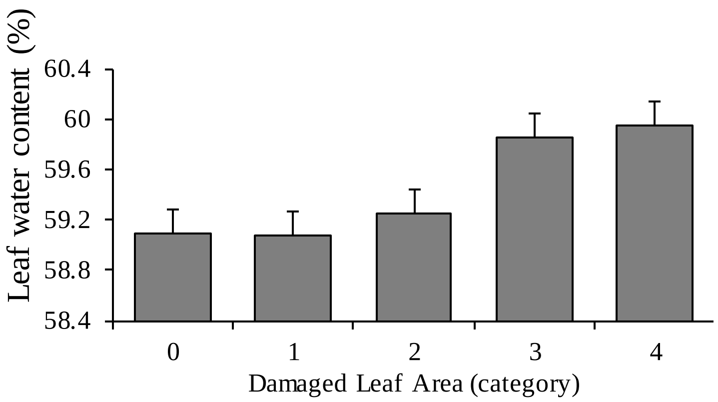

Figure **5.2**: Mean ± standard error of leaf water content in healthy leaves and in leaves damaged by black mould, with different categories of leaf area damaged (0 = intact; 1 = 1–5%; 2 = 6–11%; 3 = 12–24%; 4 = 25–49%). ANOVA followed by Tukey's multiple comparison test revealed no significant differences (*P* > 0.05) between the different categories (n = 20).

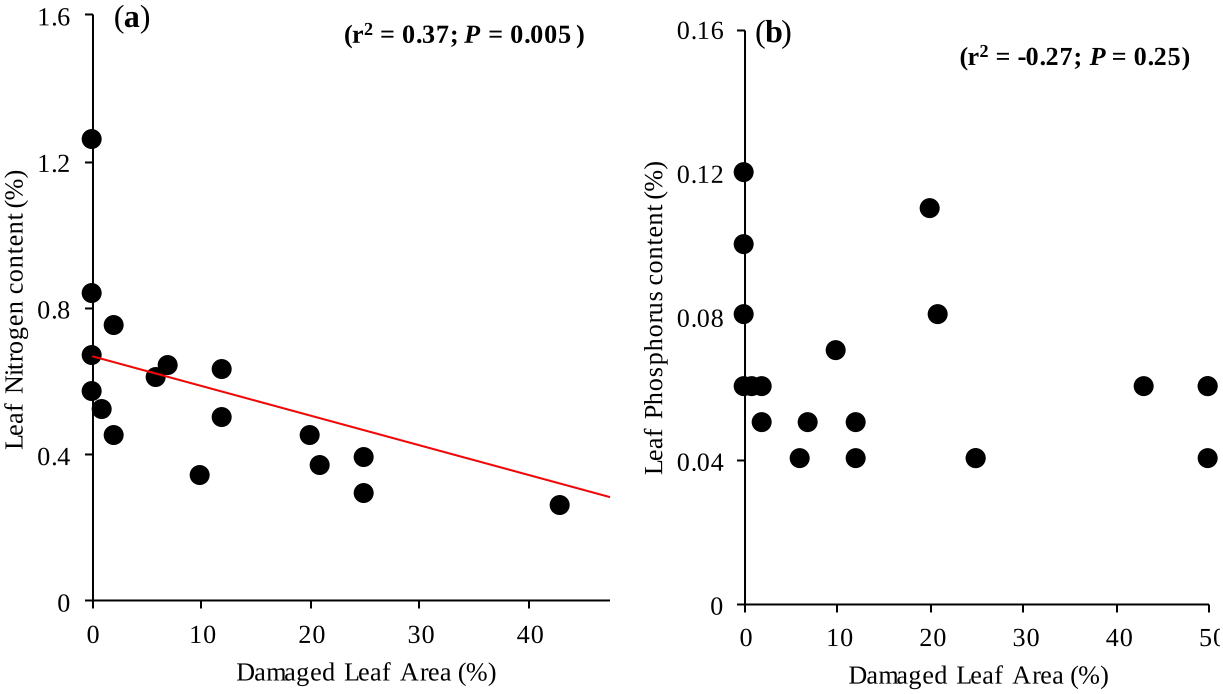

Figure **5.3**: Correlation between black mould severities, and (**a**) plant N and (**b**) P content (n = 20). The line represents linear regression analyses. r^2 = Pearson correlation coefficients.

5.3.2. Leaf spot *(Phyllosticta owaniana)* –photosynthesis and gas exchange relationships

Phyllosticta owaniana leaf disease had a significant negative effect on photosynthetic rate of wild almond leaves. As disease severity increased, A_{max} decreased significantly (A_{max}: r = -0.76, $F_{(1,\ 15)}$ = 7.18, P = 0.000001*;* Table **5.2**; Fig. **5.4a**). A_{max} was reduced by approximately one half at 20% disease severity, and values approached zero for leaves with >50% necrotic leaf area. Leaf stomatal conductance was strongly and negatively related to disease severity (g_s: r = -0.81, $F_{(1,\ 15)}$ = 12.47, P < 0.0001; Fig. **5.4b**). Stomatal conductance stabilised at low levels for disease severity values between 21 and 80% of the lesioned leaf area (Fig. **5.4b**, Table **5.2** There was a positive correlation between A_{max} and g_s (r = 0.58, P = 0.00001; results not shown). Leaf transpiration (E: r = -0.72, $F_{(1,\ 15)}$ = 2.55, P = 0.01; Table **5.2** ; Fig. **5.4c**,) and leaf temperature (Tleaf: r = -0.89, $F_{(1,\ 15)}$ = 12.88, P < 0.0001; Table **5.2**; Fig. **5.4d**,) were also negatively correlated with the necrotic area.

Table **5.2**: The effects of leaf spot on photosynthetic rate (A_{max}), stomatal conductance (G_s), leaf temperature (Tleaf) and leaf transpiration (E) of *B. stellatifolium* leaves. Means ± (SE) in the same column with the same letter are not significantly different, different letters denote means that are statistically different (P < 0.05) as determined by one-way ANOVA and Tukey's HSD test.

Damage category	A_{max}	G_s	Tleaf	E
Control	8.99 (0.45)^{a}	0.22 (0.01)^{a}	25.35 (0.69)^{a}	1.71 (0.15)^{a}
> 0 – 20%	7.69 (0.48)^{a}	0.13 (0.01)^{b}	22.08 (3.50)^{b}	1.57 (0.09)^{ab}
21 – 40%	4.81 (0.75)^{b}	0.08 (0.01)^{b}	17.81 (0.68)^{c}	1.01 (0.05)^{bc}
41 – 60%	3.18 (0.58)^{b}	0.08 (0.03)^{b}	13.53 (0.90)^{cd}	0.79 (0.13)^{bc}
61 – 80%	2.91 (0.31)^{b}	0.09 (0.07)^{b}	11.30 (0.23)^{d}	0.41 (0.15)^{c}

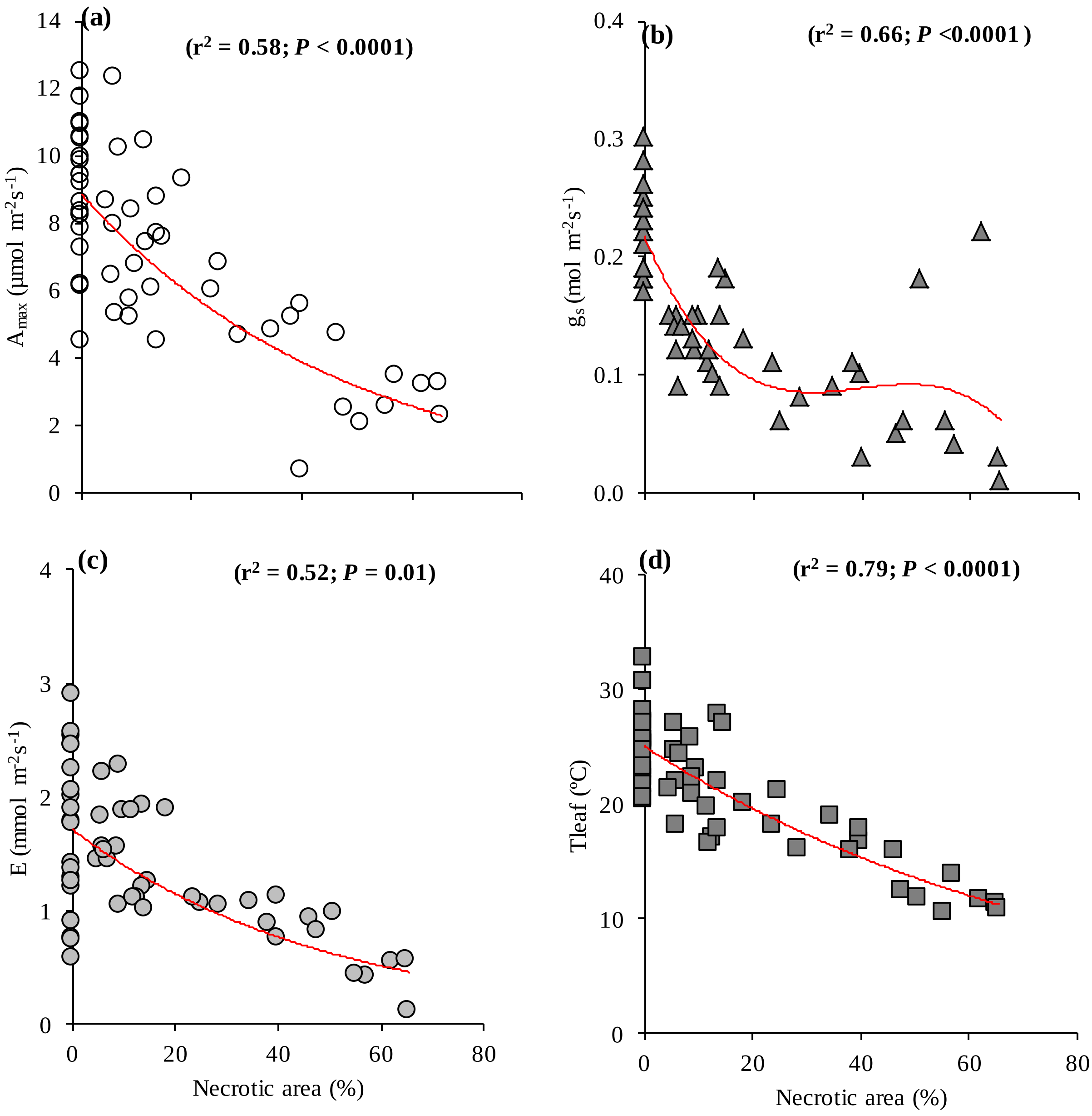

Figure **5.4**: Effect of leaf area damaged by Leaf spot on photosynthetic rate (A_{max}: **a**), net stomatal conductance (g_s: **b**), and transpiration (E: **c**) and leaf temperature (Tleaf: **d**) of leaves in the crowns of wild almond trees infected by leaf spot. r = Pearson correlation coefficients. All the trends were non-linear but polynomials which were the best fit.

Contrary to the effect of black mould leaf coverage (Fig. 5.2), there was a significant decrease in water content in leaves affected by leaf spot with increasing levels of fungal disease ($F_{(1,\ 15)}$ = 3.66, *P* = 0.02; Fig. 5.5). Leaf spot severity correlated significantly and negatively with N (r = -0.85, $F_{(1,\ 15)}$ = 9.74 , *P* = 0.0004; Fig. 5.6a).

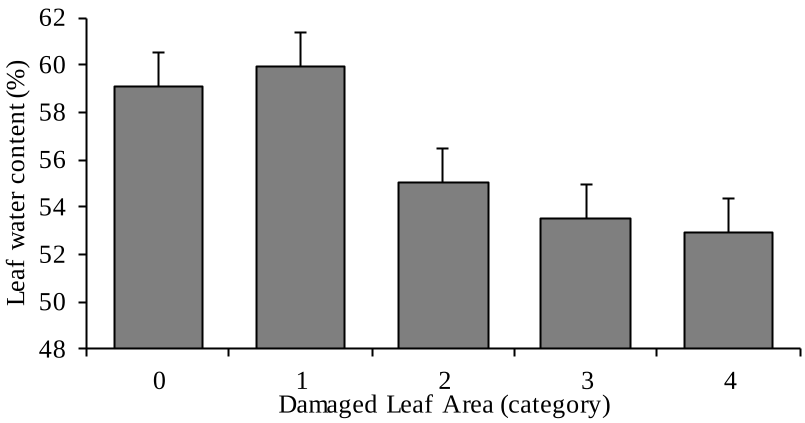

Figure **5.5**: Mean ± standard error of mean (SE) of leaf water content in healthy leaves and in leaves damaged by leaf spot, with different percentages of leaf area damaged (0 = intact; 1 = 1–5%; 2 = 6–11%; 3 = 12–24%; 4 = 25–49%). Tukey's multiple comparison test revealed no significant differences between the different categories (*P* > 0.05) (n = 20).

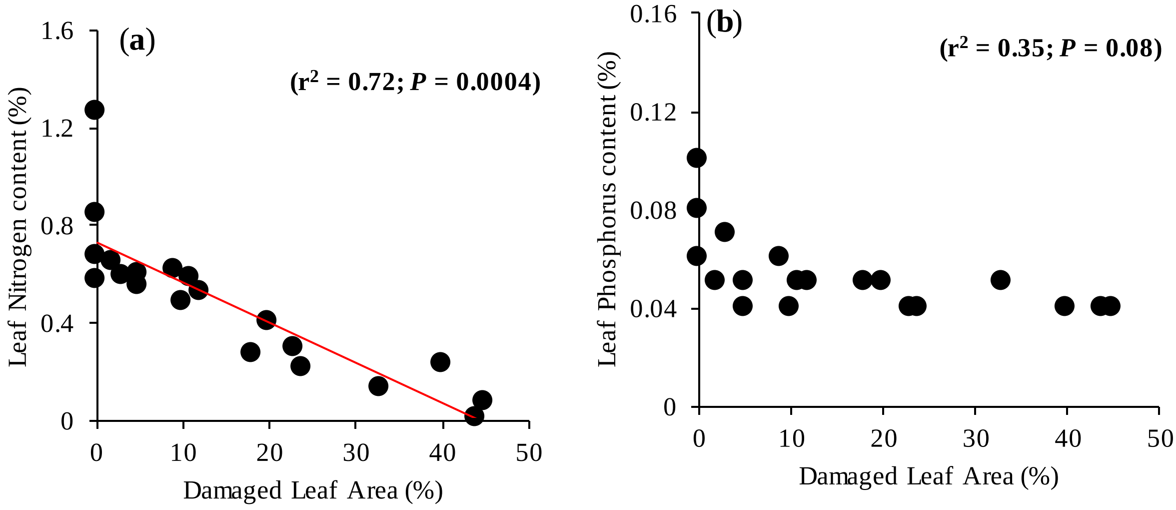

Figure **5.6**: Correlation between leaf spot severities, and (**a**) plant N and (**b**) P content (n = 20). The line represents a linear regression analyses. r^2 = Pearson correlation coefficients.

5.3.3. Weevil-folivory–photosynthesis and gas exchange relationship

The effect of *Setapion provincial* and *Setapion quantillum* mesophyll-feeding leaf weevils on the photosynthetic rate of wild almond leaves resulted in significant non-linear negative correlations (r = -0.91, $F_{(1,\ 9)}$ = 24.20, *P* < 0.0001; Fig. 5.7a). Photosynthetic rate was significantly reduced in leaves even at low levels of weevils' folivory (Table 5.3). As infestation increased an abrupt and significant decrease in photosynthetic rate occurred (Table 5.3).

Stomatal conductance of damaged leaves increased significantly with increasing damage severity (g_s: r = 0.83, $F_{(1, 9)} = 11.77$, $P < 0.0001$; Fig. 5.7b). There was a significant effect of folivory severity on leaves with both transpiration rates (E: r = 0.78, $F_{(1, 9)} = 11.85$, $P < 0.0001$; Table 5.3; Fig. 5.7c) and leaf temperature (Tleaf: r = 0.79, $F_{(1, 9)} = 10.39$, $P < 0.0001$; Table 5.3; Fig. 5.7d) being higher in damaged leaves than they were in healthy leaves. Both transpiration and leaf temperature showed a significant jump at the highest damage classes over the less severe damage classes (Table 5.3).

Table **5.3**: Effects of folivorous insects (the weevils *Setapion provincial* and *Setapion quantillum*) on photosynthetic rate (A_{max}), stomatal conductance (g_s), leaf temperature (Tleaf) and leaf respiration (E) of *B. stellatifolium* leaves. Means ± (SE) in each column with the same letter are not significantly different, different letters denote means that are statistically different ($P < 0.05$) as determined by one-way ANOVA and Tukey's HSD test.

Damage category	A_{max}	g_s	Tleaf	E
Control	8.99 (0.45)^{a}	0.22 (0.01)^{bc}	25.35 (0.69)^{a}	1.71 (0.15)^{a}
>0– 20%	5.69 (0.59)^{b}	0.12 (0.004)^{a}	27.61 (0.66)^{a}	2.20 (0.16)^{a}
21 – 40%	3.11 (0.33)^{c}	0.18 (0.01)^{d}	27.97 (0.86)^{a}	2.13 (0.25)^{a}
41 – 60%	2.38 (0.31)^{c}	0.19 (0.01)^{cd}	29.15 (0.81)^{a}	2.26 (0.26)^{a}
61 – 80%	1.73 (0.28)^{c}	0.22 (0.01)^{bcd}	34.45 (1.02)^{b}	3.63 (0.31)^{b}
81 – 100%	0.57 (0.16)^{c}	0.26 (0.01)^{b}	35.33 (0.23)^{b}	4.68 (0.11)^{b}

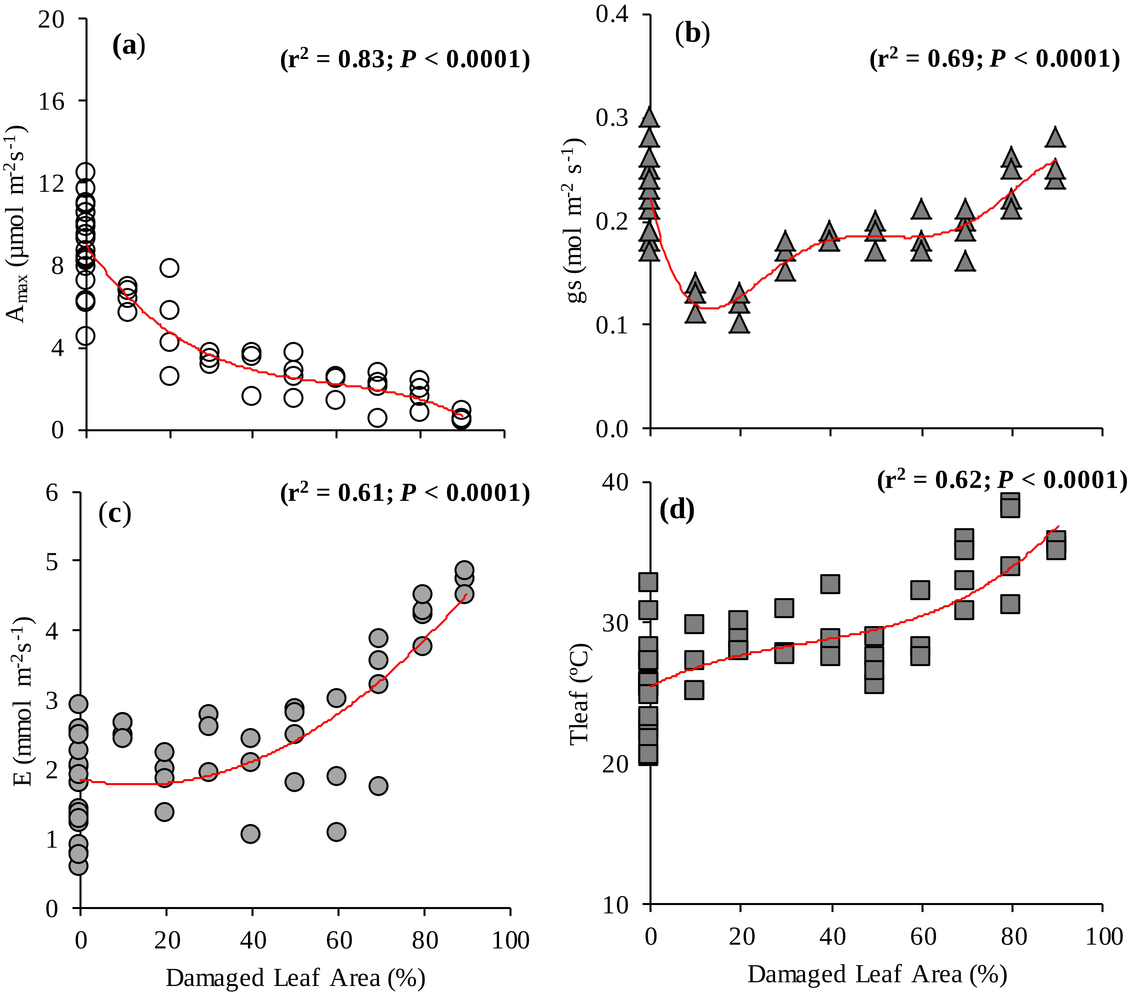

Figure **5.7**: Photosynthetic rate (A_{max}; **a**), net stomatal conductance (g_s; **b**), and transpiration (E; **c**) and leaf temperature (Tleaf; **d**) of leaves in the crowns of Wild almond trees damaged by folivorous insects (the weevils *Setapion provincial* and *Setapion quantillum*). r = Pearson correlation coefficients. All the trends were non-linear.

Water content of wild almond leaves showed no change with increasing levels of folivory ($F_{(1,\ 13)}$ = 1.27, P = 0.3; Fig. 5.8) when compared with their respective controls. No relationship was observed between weevil damage and leaf N content (r = 0.31, $F_{(1,\ 13)}$ = 1.01, P = 0.18; Fig. 5.9a) and P (r = -0.22, $F_{(1,\ 13)}$ = 1.36, P = 0.36; Fig. 5.9b). All the trends were linear.

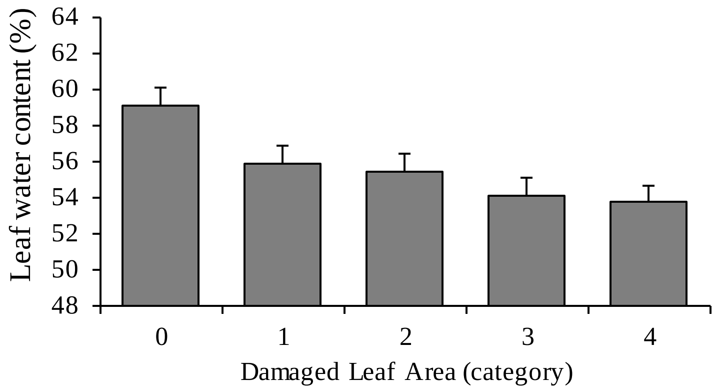

Figure **5.8**: Mean ± standard error of mean (SE) of leaf water content in healthy leaves and in leaves damaged by folivorous insects (the weevils *Setapion provincial* and *Setapion quantillum*), with different percentages of leaf area damaged (0 = intact; 1 = 1–5%; 2 = 6–11%; 3 = 12–24%; 4 = 25–49%) and statistical output for the ANOVA, n = 20. Tukey's multiple comparison test revealed no significant differences ($P > 0.05$) between the different categories.

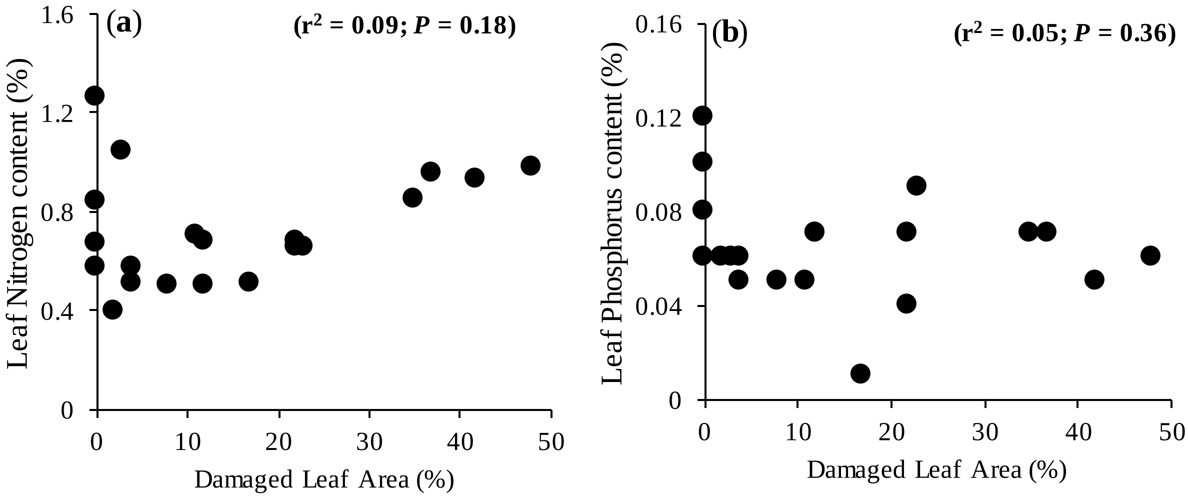

Figure **5.9**: Correlation between damaged leaf area by weevil and (**a**) plant N and (**b**) P content (n = 20). The line represents a linear regression analysis. r^2 = Pearson correlation coefficients.

5.4. DISCUSSION

5.4.1. Fungal pathogens

In this study, fungal pathogens caused steep reductions in the photosynthetic rate of non-diseased leaf tissue with an increase in disease severity, which agrees with results of previous studies (e.g. Oliveira et al., 2012). In the current study, when disease severity reached 40% of leaf area, photosynthetic rate was reduced three-fold. This implies that minor increase in infections of fungal pathogens at restored sites (where *B. stellatifolium* was severely damaged as compared to near pristine and heavily invaded sites (Chapter 3)) has a dramatic, negative impact on plant health as measured by photosynthetic rate. The reduction in photosynthetic rate of infected leaves has shown to be dependent on stomatal functioning (Wong et al., 1979; Farquhar and Sharkey 1982; Lopes and Berger, 2001; Aldea et al., 2005). Previous studies suggest that fungal pathogens can either reduce (Prats et al., 2007) or increase (Lopes and Berger, 2001; Oerke et al., 2006; Allègre et al., 2007) stomatal conductance. In this study, stomatal conductance of wild almond leaves decreased sharply with increases in fungal disease severity. The heavy coverage of black mould on the abaxial leaf surface has been shown to cover the stomata (see Filho and Paiva, 2006; Santos et al., 2013). The main result of this interference is that the normal gas exchange processes of diseased parts of the leaves are severely affected (Santos et al., 2013) with negative consequences for production. This indicates that *B. stellatifolium* is, when under heavy black mould coverage and severe damage by leaf spot, severely stressed as its normal physiological metabolism is affected.

Stomatal conductance has been shown to relate linearly with transpiration rate (Lopes and Berger, 2001). In wild almond leaves, the decrease in stomatal conductance mirrored a decrease in transpiration, thereby lowering concentration of water vapour and hence affecting photosynthetic rate. Other than this, no other underlying mechanisms that cause reduction of transpiration in diseased wild almond leaves were found here, nor could they be inferred from the measurements taken. However, Grimmer et al., (2012) in their review suggested that the decrease in transpiration may be related to the method in which fungal pathogens infect leaves. For example, fungi causing necrotic lesions like *Phyllosticta owaniana* produce spore-bearing structures that can use the stomatal pores as entry route or erupt through the epidermis, breaking open the cuticle (Grimmer et al., 2012). It has also been shown that fungal pathogens associated with a reduction in transpiration are associated with hypertrophy of chlorenchyma, reduction in air spaces, or obstruction of conductive tissue and stomata (Ellis et al., 1981).

Apart from the mentioned variables, the observed disease-induced decline in photosynthetic rate may also be partially due to a temperature effect (Wood et al., 1988; Hüve et al., 2011; Archontoulis et al., 2012). High temperature exacerbates reductions in photosynthetic rate (Wood et al., 1988; Olufemi et al., 2002; Hüve et al., 2011). However, this may be applicable only to black mould damage. Wood et al., (1988) showed that an increase of about 4°C is enough to contribute to suppression of photosynthetic rate. As leaf temperatures of wild almond increased by about 4°C in most diseased leaves compared to the non-diseased controls, it is likely that leaf temperature may partially be responsible for the decline in photosynthetic rate, but not in the case of leaf spot, where temperature declined with a decline in photosynthetic rate. This implies that although the two fungi have similar major impacts on photosynthesis, the mechanisms behind these impacts differ. The heating effect by black mould coverage may be partially due to the natural absorption of radiant energy by black objects as well as because the mould acts as a barrier to air movement with a loss of control over transpiration-induced cooling. Changes in membrane semi-permeability brought about by fungal pathogens that cause physical lesions on the leaf surface like leaf spot often result in decreases in leaf temperature due to disrupting leaf water status (e.g. Oerke et al., 2006).

This study also showed that leaf spot disease, but not black mould, affected nutrient absorption and/or translocation, as leaf water content decreased significantly with increases in disease severity. It is possible that the observed decreases in water content under increased leaf spot reduce leaf tolerance to desiccation induced by disease damage. The lack of relationship between black mould and leaf water content was not expected since accumulation of a biotrophic fungal pathogen mat has potential to reduce penetration of sunlight and stomatal opening, thus suppressing water loss (see Wood et al., 1988; Filho and Paiva, 2006).

Since plants use defences such as increasing the level of leaf tannins and lignins to dissuade biotic stressors (Casotti and Bradley, 1991), a significant decrease in the levels of N could possibly represent a reduction in plant defences against fungal pathogens. Alternatively, plants may translocate N to leaves that are less affected or to healthy leaves, such as was found by Munns (2002). Either one of these responses would result in a decline in photosynthetic rate as nitrogen is essential to photosynthesis. This highlighted that fitness of severely damaged native trees in invaded and restored sites is likely to be affected (Chapter 3). The lack of a response of leaf water and leaf P content to black mould infections and leaf P content for leaf spot suggest that factors other than P and leaf water content have an effect on the amount of leaf material diseased or that they are not related to severity of fungal pathogens. This warrants further research.

5.4.2. Insect Folivory

Insect folivory has been shown to either increase (Welter, 1989) or decrease (e.g. see Zangerl et al., 2002; Heng-Moss et al., 2006) photosynthetic rate. In this study, damage from the mesophyll-feeding leaf weevils *Setapion provincial* and *S. quantillum* caused large reductions in photosynthetic rate. The presence of even a few scars representing <0.5% of leaf area was enough to significantly reduce photosynthetic rate, which implies that at a tree level, even light insect damage can reduce production. Thus, this implies weevil damage can have a potentially large impact on the health of *B. stellatifolium* in restored and invaded sites (see Chapter 3).The decrease in photosynthetic rate due to folivory is usually attributed to decrease in stomatal conductance to compensate for non-stomatal water loss around folivory damage (Labandeira et al., 1994; Aldea et al., 2005). Contrary to this, stomatal conductance of damaged leaves increased with increasing damage severity in this study. This is, however, consistent with results of previous studies investigating effects of chewing insects on plant physiology (Aldea et al., 2005; Neves, 2006; Marlin et al., 2013). The increased stomatal conductance is likely because the weevils considered in this study create numerous very small openings in the cuticle (Fig **A5.1**), which explains the increase in stomatal conductance, and, in turn, the observed increase in transpiration. Increased evaporative water loss implies leaf cooling, which was not found. Instead, leaf temperature increased, which is consistent with Peterson et al., (1998). This increase in leaf temperature suggests some dehydration of the tissue along the edges of the chew-damage, an observation previously reported by Aldea et al. (2005), which could result in a loss of temperature regulation through transpirational cooling (Peterson et al., 2004).

Other possible reasons for reduced photosynthetic rate due to folivory, though not quantified in this study, include alterations in chlorophyll content after folivory damage (Herde et al., 1999), production of reactive oxygen species (Bi and Felton, 1995; Bown et al., 2002) and increases in the production of defence compounds, some of which are strongly autotoxic and directly inhibit photosynthesis (Zangerl et al., 2002; Gog et al., 2005).

Although increases in folivory severity have been related to increases in leaf water content in other studies (Ricklefs, 2008; Münzbergová, and Skuhrovec, 2013), this was not true of this study. This implies that other factors besides leaf water content are responsible for reaction of *B. stellatifolium* to folivores. Leaf P and N content are well-acknowledged fundamental requirements for proper cellular functioning and growth of consumers (Richardson et al., 2002; Elser et al., 2000a; Ricklefs, 2008). Leaf N content ranged from 0.2 to 1.6% dry weight and leaf P content from 0.04 to 0.16% DW, levels that are consistent with plants that grow naturally (Center et al., 1999). Huberty and Denno (2006b) found leaf N and P content increased with increasing folivores performance. Based on this evidence, the expectation was that a significant correlation exists between N and folivory severity, however this was not found. Thus the observed increase in N in leaves of *B. stellatifolium* in restored sites (Chapter 4) is unlikely to be due to folivory by the prominent leaf weevils *Setapion provincial* and *S. quantillum* (Chapter 3), but such plants may be susceptible to fungal infection and associated declines in productivity.

It was previously noted that native trees in restored sites continue to be damaged by folivorous insects and fungal pathogens resulting in damage levels in excess of that seen at invaded sites (Chapter 3). This study showed that even slight increases in severity of folivory and leaf fungal pathogens can have severe effects on tree metabolic functioning (e.g. decreased photosynthetic rate). While these mechanisms are unclear, they may partially explain the slow recovery of plant cover of native plants in restored sites.

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APPENDIX **5**

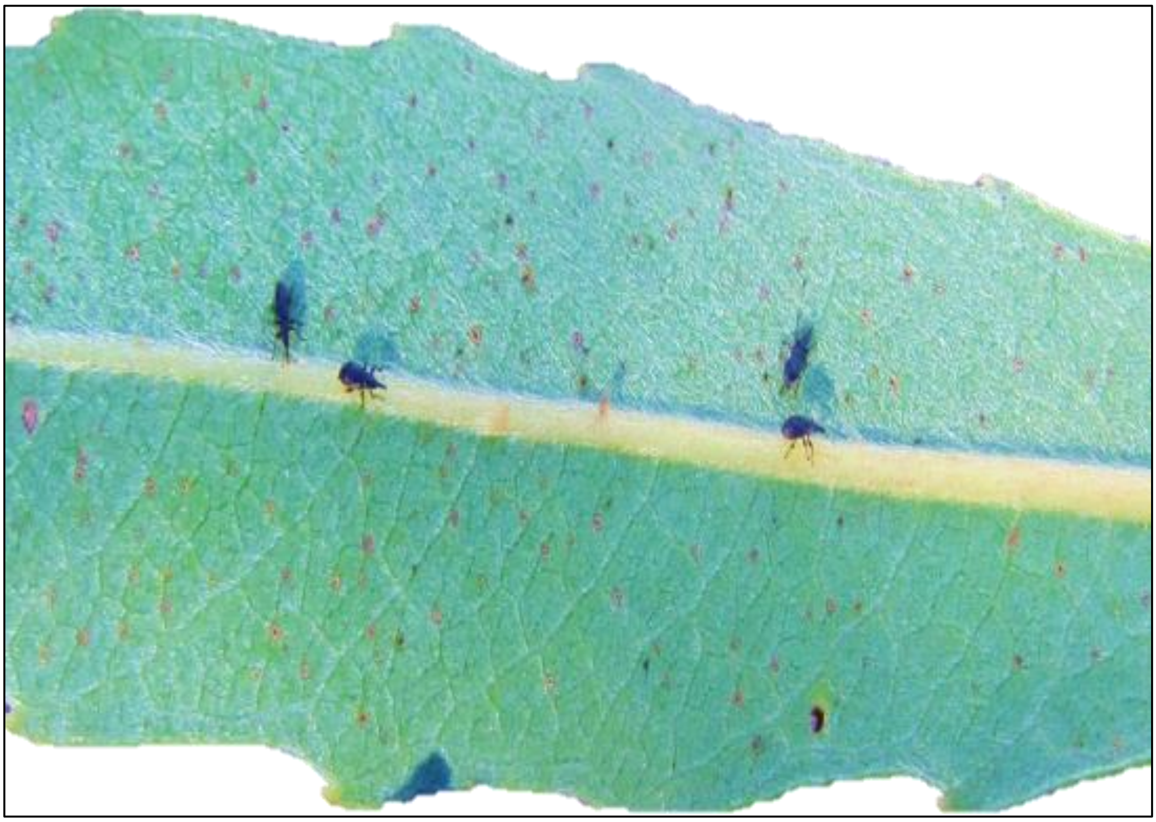

Figure **A5.1**: *Setapion provinciale* and *S. quantillum* feeding upon leaf lamina of *Brabejum stellatifolium* and occasionally damaging the mid-vein of the leaves.

CHAPTER 6

General conclusions, management recommendations and future research

Management of riparian habitats in South Africa, where Invasive Alien Plants (IAPs) have infested approximately 20 million hectares of land (van Wilgen et al., 2012) is crucial for the successful conservation of biodiversity and enhancement of ecosystem services such as generation of clean water. However, little is known about how riparian zones should be managed to optimize tree health within the riparian habitats. This dissertation highlights the potential effects of invasive alien plants on tree health in riparian zones by focusing on the role of folivory and tree fungal pathogens. This research is the first to investigate the role of folivorous insects and fungal disease on tree health in riparian zones in South Africa. Two common and widespread native trees, *Brabejum stellatifolium* and *Metrosideros angustifolia* together with one invasive alien tree, *Acacia mearnsii* were used as subjects. The results indicate that different invasion treatments of riparian zones have the potential to affect tree health by impacting on the diversity and abundance of damage causing organisms (e.g. arthropods). General conclusions for each chapter are as follows:

6.1. General conclusions

Chapter 2:

Heavily invaded sites tended to support fewer arthropod species than near pristine and restored sites. Presence of dense stands of IAPs did not seem to benefit arthropods β1-diversity either, as it resulted in homogenization and shifts in arthropod communities' composition. It has been previously stated that a decline in plant species richness in heavily invaded sites is assumed to be indicative of local extinction (Rutherford et al., 2012), a phenomenon known as "threshold of irreversibility" (as defined by Aronson et al., 1993). However in this study, arthropod alpha diversity in restored sites recovered, suggesting invasion effects were not irreversible. Alpha-diversity of some arthropod assemblages associated with restored sites were indistinguishable from arthropod assemblages in near pristine sites, a trend common in previous research that focused on arthropods and invasive plants (e.g. Magoba and Samways, 2011). The high arthropod species richness in near pristine sites highlighted the importance of valuing and reserving them within riparian landscapes for conservation of natural arthropod communities that are intolerant to IAP disturbance.

Community species composition of arthropods did not just differ among invasion treatments, but also showed differences among sites with similar invasion treatment, suggesting the presence of specific river signatures. This showed that the responses of sites to plant invasion are site-specific, and therefore it is important to consider rivers individually when conceptualising restoration and management programmes in order to obtain a high heterogeneity in plants and arthropod species in riparian ecosystems both locally and regionally.

Chapter 3:

Native tree species diversity and abundance in riparian zones that were previously invaded by IAPs together with their associated arthropods (Chapter 2) self-recovered after seven years of IAPs being removed. One would have expected their interaction (native trees and their associated arthropods) to return to their original state (similar to the near pristine sites). However, this seemed unrealistic as levels of folivory and leaf fungal pathogens on native trees (*B. stellatifolium* and *M. angustifolia*) were higher in restored sites as compared to near pristine sites, whereas for *A. mearnsii* leaf loss was higher in heavily invaded sites than in near pristine or restored sites. Such changes were assumed to be due to changes in host abundance and native species richness. Levels of leaf damage by folivorous insects for native trees and IAPs were only influenced by host abundance. Native tree species richness had no apparent influence on levels of damage by folivorous insects and fungal pathogens. This indicates that in the riparian zones studied, folivorous insects seem to be benefiting from reduced native tree host abundance and increased host abundance for IAPs, whereas native tree species richness does not seem to be a major determinant for overall folivory.

Chapter 4:

This study showed that IAPs can alter trophic structure of riparian sites and removal of IAPs can promote the re-establishment of the associated arthropod communities. For example, heavily invaded sites supported low numbers of predators as compared to near pristine and restored sites. This indicated that predation on folivores played a limited role in regulating folivore abundances.

Studies from a variety of habitats suggest that the impact of folivory and fungal disease on plant communities is a consequence of their influence upon the availability of resources such nutrients (e.g. Brown, 1994; Throop, and Lerdau, 2004). This was evident from this study as comparisons between foliar nutrient content data confirmed that *B. stellatifolium* at restored sites had greater levels of foliar nitrogen than *M. angustifolia*, as did *A. mearnsii* in heavily invaded sites. *Brabejum stellatifolium* and *A. mearnsii* experienced high damage levels in restored sites and heavily invaded sites, respectively (as seen in Chapter 3). Restored and heavily invaded sites have been shown to be nutrient rich as impacted by IAPs (e.g. Naudé, 2012). Correlative analyses suggested that leaf nitrogen content for *B. stellatifolium* and *A. mearnsii* is thus a possible driver of folivore choice in food plants, whereas the effects of leaf phosphorus content and the presence of predatory arthropods on folivore plant choice were limited. This is in agreement with the suggestion that bottom-up forces (e.g. nutrients like nitrogen) should be stronger than top down effects (e.g. predaceous arthropods) in regulating organism numbers in nutrient-rich habitats (restored and heavily invaded riparian habitats here) (Gratton and Denno, 2003).

Chapter 5:

In this study, folivory and fungal infections reduced photosynthesis through (i) leaf damage leading to a decreased stomatal conductance by fungal pathogens and an increase in stomatal conductance by folivory, and (ii) the contamination of leaf surfaces with black sooty mould which impedes stomatal conductance but also further reduces photosynthesis by impairing the light reaching the leaf surface (and perhaps changing leaf temperature). Leaf nitrogen content was also influenced by fungal disease severity. These results illustrate that the impact of fungal pathogens and folivorous insects do not only reduce leaf area (Chapter 3) but also impair normal physiological metabolism of the plant with consequent ill effects on health of their hosts. These results add valuable information in understanding the slow recovery of plant cover of native plants such as *B. stellatifolium* in restored sites.

6.2. Management recommendations

This study indicated that alien plant invasion affects arthropod assemblages. The observed decreases in arthropod abundance in heavily invaded sites indicate that they (i) have a negative effect on certain species of wildlife that depend on arthropods as their primary food resource (e.g. reptiles, grassland birds, and small mammals) (Litt and Steidl, 2010; Litt et al.,

2014) and (ii) a possibility that essential processes like nutrient cycling and pollination will be negatively affected (Williams, 1993). A decrease in arthropod orders like Hymenoptera and Lepidoptera (members of the pollinator group) can change native vegetation composition over the long term (Litt et al., 2014). Therefore, to maintain arthropod diversity and function in riparian zones, alien plant clearing efforts must be maintained or even increased in future.

This study has revealed a recovery in native tree species after removal of IAPs, and a corresponding recovery of richness in arthropods associated. The ability of arthropods to respond rapidly to a recovery in native tree species is not in doubt, so that continued clearing even without any other concomitant active restoration efforts being required is essential to improve the recovery of riparian flora and fauna (Groffman et al., 2006). Within riparian zones, it is also important to collect data on other taxonomic groups (e.g., mammals) to assess their ability to recover from IAP infestations as done here for arthropod assemblages. Therefore, a survey of the species of flora and fauna in riparian zones needs to be done prior to the eradication of IAPs. With this background data, rare and threatened species can be identified and plans made for their conservation (Magoba, 2012).

Because complete eradication of IAPs is often unlikely and impractical (van Wilgen et al., 2012), retaining and maintaining mosaics of near pristine riparian zones should be given high priority since it is from these areas that the recolonization of flora and fauna in restored sites can take place (e.g., Samways and Sharratt, 2010). This may also aid in quicker recovery of native arthropod communities especially those that respond to changes in vegetation structure like predaceous arthropods (Litt et al., 2014). Apart from maintaining near pristine riparian habitats, it is also crucial that future riparian zone restoration projects consider the effects of biotic connections from adjacent habitats (e.g. corridors or patches), and the landscape context (surrounding tree cover and distance from native, mature riparian vegetation) for the perpetuation of these riparian habitats on riparian zones.

All catchments considered in this study had specific signatures. Thus, this should be taken into account when attempting biodiversity restoration in riparian habitats. For the purpose of this study, the following factors are worth considering: (i) the plant invasion treatment of the habitat and (ii) ‘irreplaceability or uniqueness’ (Roux et al., 2002). The occurrence of unique species in near pristine sites in this study reflects the need to conserve such river types to help achieve conservation targets (Roux et al., 2002).

Here, habitat treatments affected the magnitude and direction of effects of folivorous insects and leaf fungal pathogens. Folivorous insects and fungal pathogens were predominant on host trees in restored and heavily invaded sites, more so than in near pristine sites. The reasons for this were not clear for this study, although the stress generated from clearing may have negatively affected remaining native trees in restored sites. It must, therefore be a primary objective to re-instate cover and maintain the natural disturbance regimes after alien clearing for the success of long-term restoration. One way to do this would be to actively re-establish native plants after clearing IAPs. Establishing native plantings may increase habitat heterogeneity for arthropods, and avoid infestations of folivorous insects of individual trees which may occur when densities of native plants are otherwise low.

Results from this study also showed that leaves of host trees in restored and heavily invaded riparian ecosystems are nitrogen-rich. *Acacia mearnsii* being a nitrogen-fixer, may have enriched soils with nutrients in restored and heavily invaded sites (where some hosts were nitrogen rich) (see van der Waal, 2009; Naudé, 2012). Changes in soil nutrients can modify plant quality, which in turn, can change insect abundance and subsequent damage levels of folivores as seen in this study. Clearly, with ever-increasing levels of plant invasion in South Africa (discussed by van Wilgen et al., 2012), a better understanding of the nature of the soil chemistry before and after removal of IAPs and of how plants are likely to respond to changed soil nutrients is essential. Understanding the physiological mechanisms by which host plants respond to present and future resource availability will benefit river managers and policy makers when (re)defining and planning restoration strategies. Specifically, plant- and leaf- level traits can indicate how to manipulate properties of plant and arthropod communities to optimize restoration of invaded ecosystems (Funk, 2013).

6.3. Future research

Results from this study contributed to our understanding of folivorous insects and fungal pathogens and their effects on tree health in riparian zones, and restored and heavily invaded sites in particular. The following areas need further investigation:

Chapter 2:

- Diversity partitioning might be better explained by vegetation characteristics not include here like vegetation height (Longcore, 2003) elevation (Pryke and Samways, 2012), connectivity of riparian terrestrial area, litter quality and changes to soil characteristics due to plant invasions (see Litt et al., 2014).

- Adjacent habitats potentially influence dispersal rates and patterns of arthropods. Dispersal limitation is often stated as the main cause for spatial patterns in diversity, more so for sessile plants than for mobile organisms, the latter being capable to choose their habitat and host trees actively (Loreau and Mouquet, 1999). However, this does not rule out the fact that the observed difference in β-diversity among host plant could be due to dispersal limitation, hence the need to investigate this further. Fleishman et al., (2003) found that dispersal limitation influences contribution of β-diversity to total diversity of the ecosytem.
- Arthropod samples were grouped based on their morphological characteristics, and insufficient information was available at a species level. Species-level information could provide information on host specialist and species-specific responses.
- Distinct catchment signatures were evident in the study: Future research could look into multiple factors that cause catchment differences in arthropod communities' spatial variation. Influential factors may include microclimatic conditions (Samways and Sharratt, 2010), fire history of each catchment and form and function of native vegetation communities (Litt et al., 2014).

Chapter 3:

- The lack of relationships between the factors assessed, and in particular native tree species richness and levels of leaf damage by folivorous insects and fungal pathogens, implies that differences found may be outside the limits of this study. Leaf physical (e.g. toughness or thickness of the leaves) and chemical traits (e.g. influential chemical traits such as defensive compounds like alkaloids and tannins) are known determinants of the level of leaf damage specific to each species (Peeters, 2002b). This necessitates further investigation, since these traits are likely to affect palatability of leaves and resistance to folivory, probably explaining why more than 60% of the folivory occurred in the nutrient-rich (restored and heavily invaded) sites.

Chapter 4:

- A study of soil chemistry may explain why some host plants in restored sites had leaves that were more nutrient-rich than those in the other riparian habitats, how widespread this phenomenon is, and how this drives the observed trends in insect assemblages.

- This study only used one sampling technique per four seasons; utilising a combination of various insect sampling methods and over extended time series could be valuable. van Hengstum (2014) illustrated that the choice of insect sampling techniques has a major influence on the outcome of the study, and that a combination of different methods is highly recommended as it gives best overview of community-wide changes. Sutherland, (2006) stated that folivores are adequately sampled with combination of direct searching and collecting (e.g. vacuum sampling and sweeping). Vacuum insect sampling collects fewer invertebrates than sweep netting does, and that is why it advisable to combine both (Sutherland, 2006).

Chapter 5:

- Plants employ various morphological, biochemical and molecular mechanisms to defend themselves against folivorous insects and fungal pathogen attacks (War et al., 2012). Understanding these for riparian Fynbos plants would contribute to our knowledge of plant-folivore and plant-fungal disease interactions. This understanding would give insights into how the employed plant defense mechanisms are likely to affect the metabolic costs of affected hosts, as well as the feeding, growth and survival of folivores and fungal pathogens.

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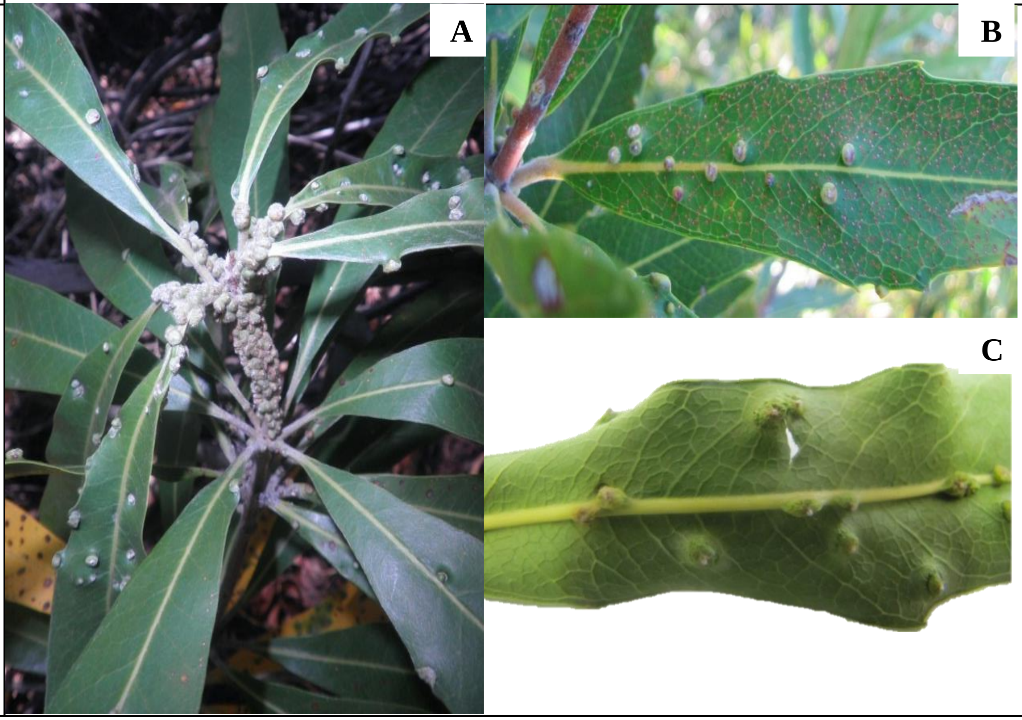
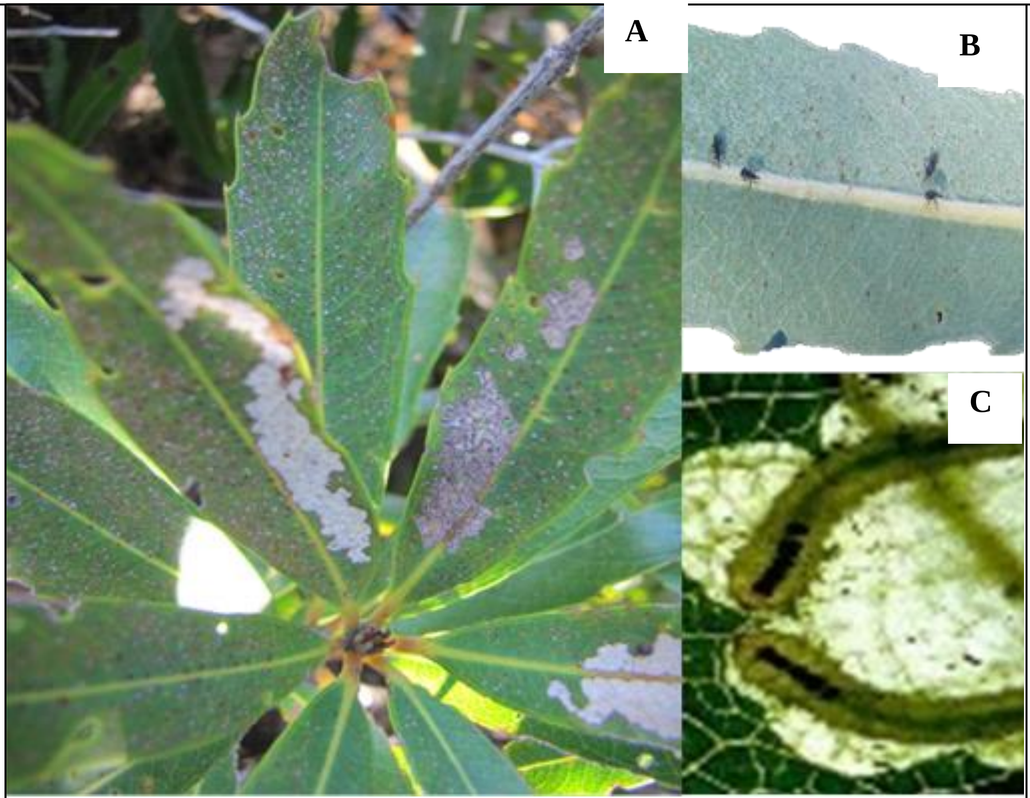
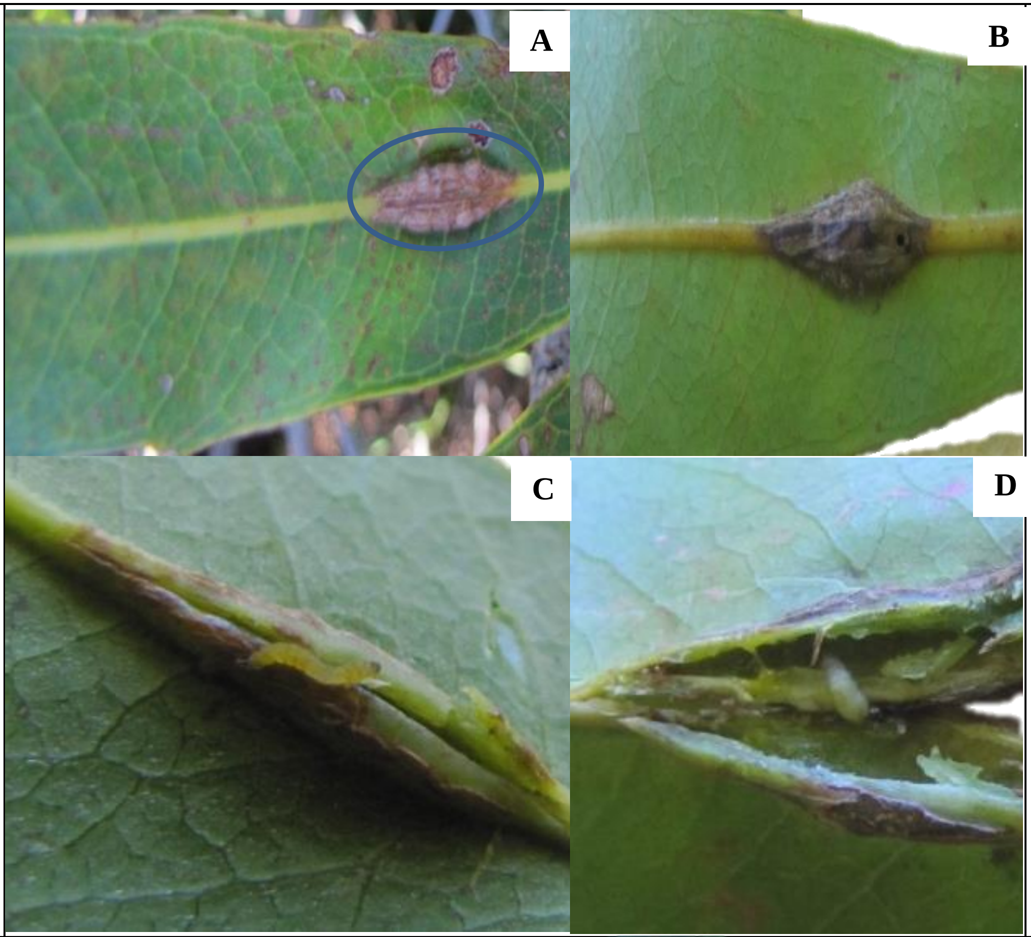
APPENDIX **6**

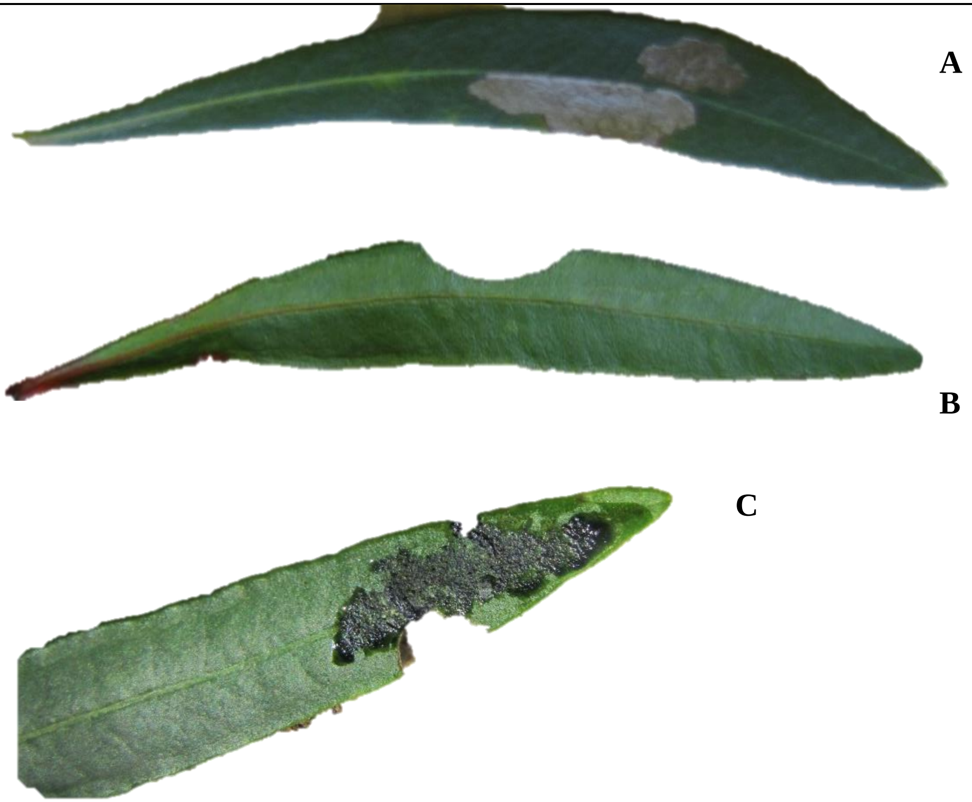
Table **A6.1**: Types of folivory and leaf fungal diseases observed on three focal host plants.

Host	Damage types	Description of damages
Brabejum stellatifolium		Healthy Leaf
		**Knobs:** These are domatia (little houses) caused by symbiotic mites (Figure A, B and C).

Brabejum stellatifolium		Grazing damage by weevils (Figure A and B) is very common in *B. stellatifolium*. The weevils (shown in Figure B) feed on leaf lamina and occasionally also on the mid-vein of leaves. In figure C, unidentified caterpillars extensively feed on the leaf lamina.

Brabejum stellatifolium		Abnormal swellings of plant material forming on petioles commonly known as galls. These chambered galls (Figure A and B) have significant layers of so-called nutritive tissue upon which the developing larvae (Figure C and D) and often feed.
Brabejum stellatifolium		Beetles graze small holes on leaf tissue most commonly toward the mid-vein and along leaf margins.

Brabejum stellatifolium		Unidentified larvae or caterpillars tunnel beneath the leaf surface and feed on leaf tissue from that location. The mines found in this study are commonly known as 'Worm-trail leaf mine'. This is a rare leaf mine that follows a long, winding path around and never widens substantially. They appeared to be transparent suggesting that the mine is not deep beneath the cuticle.
Meterosideros angustifolia		Healthy leaf
Meterosideros angustifolia		Leaf spotting caused by sap-feeding insects. In this study, the most common sap-feeding were diaspid scale insects on *M. angustifolia* and planthoppers in *A. mearnsii*.

Meterosideros angustifolia		In figure A: Unidentified caterpillars extensively feeding on the leaf lamina. In figure B and C: typical beetle feeding damage around margins of *M. angustifolia* leaves.

Acacia mearnsii		Damage caused by sap-feeding insects that commonly belong to the order Hemiptera. These insects caused hard to notice minor necrotic yellowing of the leaf tissue around the feeding site of planthopper. Chewing damage caused by grazing beetles at the margins of the *A. mearnsii* leaflets was also observed.

Table **A6.2**: Description of fungal disease symptoms and the isolated fungal morphotypes for each plant species.

Host	Disease name	Symptom description	Fungal morphotype
Brabejum stellatifolium	Leaf spot 	Irregular dark brown necrotic spots (1 -2 cm in diameter) that may combine.	Colony on culture PDA media matures slowly, reaching about 50 mm diameter after 20 days, circular, olivaceous greenish to olivaceous black, often with darker concentric zones; aerial mycelium sparse, and faintly more grey. **Fungus:** *Phyllosticta owaniana*
	Black mould 		Colonies on MEA slow-growing, reaching 4 mm diameter after 14 days at 24 °C, with entire margin; aerial mycelium rather compact, raised, and olivaceous-grey. **Fungus:** *Periconiella velutina*

Metrosideros angustifolia	Leaf spot 	Very small necrotic spots then enlarge rapidly up to 3cm in diameter and become brown. Black spore masses appear in the necrotic tissue.	Dark-green and septate mycelium in culture, with dark, scattered picnidia appear in the spots. **Fungus**: *Alternaria* spp.
Acacia mearnsii		Rust coloured necrotic spots, more or less round in shape.	The colonies are predominantly white while on medium they assumes a reddish mahogany hue.. In shape the colonies look flat, and round. **Fungus:** *Uromycladium alpinum*