

Biological control pre-release studies on the ecological impacts and biotic interactions of *Robinia pseudoacacia* L. (Black locust) with indigenous fauna and flora in South Africa

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Yours faithfully

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Abstract

The growing invasion of Afriomontane grassland ecosystems of South Africa by *Robinia pseudoacacia*, a tree from North America is threatening this fragile and fast disappearing biome. Despite numerous studies on the species in the northern hemisphere, a serious lack of information in the southern hemisphere was noted. With the species targeted for biological control in South Africa, there is a need to understand its impacts in a local context and set baseline indicators for future evaluation of management interventions. Thus, multi-scaled studies to determine and quantify the impacts of this IAP were undertaken in the eastern Free State Province of South Africa. These included interactions between the invasive alien tree, biotic and abiotic components of the ecosystem, and implications of control on human wellbeing. *Robinia pseudoacacia* functional traits such as phenology, canopy cover, tree diameter and population demographics such as population density were measured including how these influence abiotic components of the ecosystem such as temperature and light availability (microclimatic conditions). Furthermore, the cascading effects of environmental modification on native vegetation and grassland arthropods as well as interruption of key ecosystem services such as rangeland condition and pollination in agro-ecological ecosystems were evaluated. Contributions of the invasive alien tree to livelihoods of invaded communities were also assessed in order to avoid any conflict of interest that may arise from its management.

Results showed that invasion by *R. pseudoacacia* significantly transforms understory microclimatic conditions. Temperatures were at least 2°C lower under *R. pseudoacacia* stands as compared to uninvaded grassland while light availability differed by at least 1200 lumens·ft². Grass communities subsequently differed by 96% between invaded plots and adjacent uninvaded grassland. Species richness was significantly lower in the understory where there was a dominance of exotic grasses. Furthermore, these habitat conditions differentially affect

grassland arthropod assemblages, with grasshoppers in the family Acrididae, the most common grassland taxa to be excluded from invaded sites. Apart from the absence of their main host plants, invasion mediated differences in microenvironments appeared to be the main driver of this shift in arthropod assemblages.

This study also provided evidence that important ecosystem services such as grazing and pollination, are affected by *R.pseudoacacia* invasion. Apart from the reduction in available rangeland, changes in grass communities also significantly lowers the grazing capacity of rangelands. Differences in grass species composition between invaded and uninvaded rangeland resulted in a reduction in range condition especially under smaller and dense *R. pseudoacacia* stands. Furthermore, results from paired flower visitation rates in an agro-ecological system showed that *R. pseudoacacia* shares pollinators with apples. Since the two plants develop flowers during the same period, there is competition for key pollinators such as *Apis mellifera*. Hence, successful pollination of apples is compromised with a potential reduction in fruit yield and quality.

A survey conducted in this study showed that while the tree is currently being utilised by peri-urban dwellers, it is not the preferred species for several functions. Several respondents cited harvesting difficulties (due to thorns) and poor firewood qualities (production of bad smoke) as the major reasons for the underutilisation of *R. pseudoacacia*. Moreover, its contribution to household incomes is very insignificant while several negative impacts such as reduction in building space and uncontrollable spreading were noted.

Information generated from this study shows that *R. pseudoacacia* is negatively affecting grassland ecosystems and subsequent ecosystem services. Therefore, there is a need to find a sustainable management solution for the species. Considering the limited success achieved by current management efforts, biological control is expected to be the major technique

implemented for the species. Several impacts quantified in this study can be used to prioritise the species for management as well as a baseline for evaluation of future management interventions.

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General Introduction

1.1 Biological invasions

Globalization brought and continues to bring numerous societal benefits, among them increased trade and improved access to food for deficient areas (Hynes, 2014). This movement has resulted in the exchange of biological material between regions (Le Maitre *et al.*, 2004). This exchange can be either deliberate, for example, the introduction of edible species, ornamental plants, and plantation crops or accidental where weeds and pests are transported as 'hitchhikers' (Mack, 2003). While not all introduced species turn out to be invasive, those that do have an impact on ecosystems (Pysek & Richardson, 2008). There is evidence that they threaten native biological diversity, ecosystem function and human wellbeing (Le Maitre *et al.*, 2004; Moore, 2005; Van Wilgen *et al.*, 2014; Shackleton *et al.*, 2019) as well as having significant economic implications (Diagne *et al.*, 2020). Bioregions are reportedly losing their ecological distinctiveness due to a reduction in biodiversity and there are fears of homogenization of global ecosystems as a result of biological invasions (Olden *et al.*, 2011).

An understanding of the drivers of invasion such as propagule pressure and disturbance has grown substantially (Richardson *et al.*, 2007; van Wilgen *et al.*, 2020). At local scales, anthropogenic activities and climate change are some of the factors aiding the establishment and success of invasive alien species (Mack *et al.*, 2000; Le Maitre, 2004; Moore, 2005; Richardson & Pysek, 2008). However, some invaders do not necessarily need disturbance in order to establish in new ranges. For example, privet (*Ligustrum robustum* subsp. *walker* (Roxb.) Blume: Oleaceae), described as an 'ideal' invader in the Indian Ocean Islands of Reunion and Mauritius largely thrives in undisturbed habitats (Lavergne *et al.*, 1999). This explains the importance of understanding invader traits in order to anticipate their success, spread and impacts in invaded environments.

While invasive alien species are generally defined in terms of negative impacts on native biodiversity, it is difficult to generalize between species and regions (Chytry *et al.*, 2005). There is evidence that impacts of invasive alien species vary across spatial scales where they depend on the interaction of the species and the environment. For instance, South Africa has diverse environmental conditions in its nine terrestrial biomes hence the impacts of invasive alien species vary across biomes. For example, a biome scale assessment of the impacts of invasive alien species on biodiversity was found to be more severe in the fynbos than other biomes assessed (van Wilgen *et al.*, 2008a).

1.2 Invasive Alien Plants

Plants constitute a significant proportion of invasive alien species and have been widely studied (Moran *et al.*, 2013). Invasive alien plants (IAPs) have been reported to adversely affect plant and animal communities (Sitzia *et al.*, 2012), reduce water availability (Le Maitre *et al.*, 2016; Preston *et al.*, 2018) and alter soil chemical properties (Ehrenfeld, 2003) among other vital ecosystem components. In addition, they also pose a threat to agricultural ecosystems, as they compete with crops, potentially reducing food quality and quantity (Westbrooks, 1998). Although woody plants constitute a small proportion of introduced plants, they have the largest spatial coverage with the worst ecological impacts while presenting more conflict of interest than any other taxa (van Wilgen *et al.*, 2008b). Of the 100 worst invasive taxa listed globally by the Invasive Species Specialist Group (ISSG) an unequal percentage are invasive tree species (Lowe, 2000). According to Tyler *et al.* (2020), woody invasive species have in recent years been outcompeting other invasive species in several countries in Europe, this is also apparent in South Africa (Van Wilgen *et al.*, 2020).

In South Africa, woody IAPs are estimated to cover more than 10 million hectares, translating to over 8% of the total land area (Van Wilgen *et al.*, 2001; Nyoka, 2003). In addition, South Africa has more invasive trees per square kilometre than any other country (Henderson, 2001). For example, *Eucalyptus camaldulensis* Dehnh (Myrtaceae) reportedly covers over 500 000 ha in South Africa alone (Hirsch *et al.*, 2020). While efforts to curtail further spread are underway, the list of invading trees and areas under invasion continues to change due to new introductions, spread of existing species and control efforts (Henderson & Wilson, 2007). These invasive trees are distributed across all the nine biomes of South Africa, although some such as the fynbos and grassland biome have been more affected than others.

1.3 Invasion of grassland ecosystems

Grassland ecosystems are the largest worldwide representing 40.5% of terrestrial land area (Suttie *et al.*, 2005). These assume different names such as prairies, steppes, savannahs, veldts or veld, rangelands, pampas, llanos and cerrados, depending on geographical region (WWF, <https://www.worldwildlife.org/habitats/grasslands>). In South Africa, grasslands make up 30% of the total land surface area, making them the second largest biome in the country (SANBI, 2013) (Fig. 1.1). They provide several key ecosystem services, among them grazing for both domestic and wild animals. In South Africa, grasslands support 60% of commercial agriculture, of which 44% is range-based cattle production (SANBI, 2013; O'Connor & van Wilgen, 2020). Further, services such as thatching grass and traditional medicines which are key to livelihoods in rural populations in South Africa, are also obtained from this biome (Kobisi *et al.*, 2019). Moreover, montane grasslands of South Africa are the major source of the country's fresh water as most rivers have their heads in this biome (Taylor *et al.*, 2016).

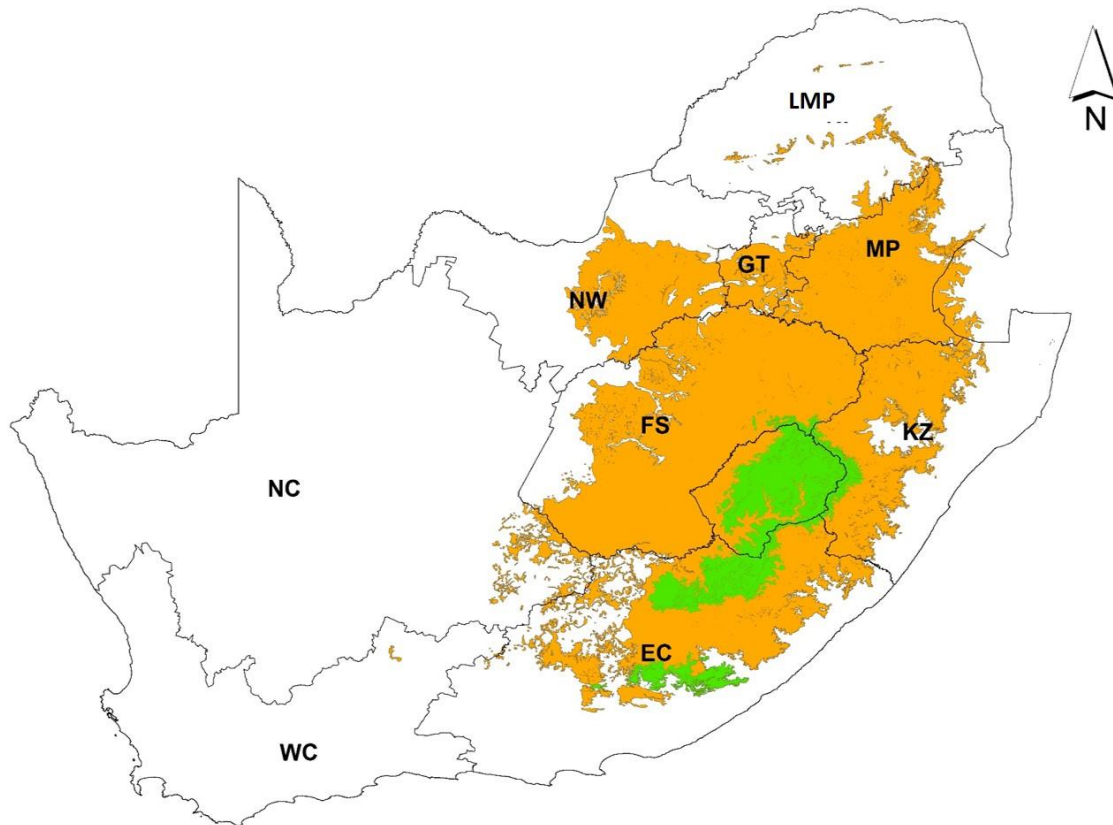


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The Grassland biome of South Africa is dominated by herbaceous, short and simple structured vegetation and is regarded as a biodiversity hotspot with several endemic plant species (Mucina *et al.*, 2006; Clark *et al.*, 2011). However, grasslands, including the South African grassland biome, are increasingly becoming fragmented by among other factors such as anthropogenic disturbances, global change and woody plant invasions (Archer *et al.*, 2017). In South Africa, this biome is under threat from several disturbances such as habitat transformation from agriculture, mining, plantations, changing fire regimes, human habitation as well as invasive alien plants (Henderson & Wilson, 2007; SANBI, 2013; Martin, 2019). Thus, grasslands are

109 losing their native diversity and associated services such as pollination services and grazing
 110 capacity (Hansen *et al.*, 2018; Yapi *et al.*, 2018; O’connor & van Wilgen, 2020).

111 South African grasslands have been invaded by more than 320 invasive alien plants dominated
 112 by Fabaceae, Rosaceae, Salicaceae, Myrtaceae and Cactaceae families (Henderson, 2001,
 113 2007). Woody alien plants in particular are successful invaders of grasslands as they use their
 114 aerial dominance to alter microhabitats, leading to displacement of native grassland vegetation
 115 (Hejda *et al.*, 2009). While some IAPs in the montane grasslands have a control programme
 116 against them (Klein, 2011), several do not, despite having evident impacts on the environment.
 117 The majority of those species which currently do not have co-ordinated control programmes,
 118 originate in the northern hemisphere. Examples include *Salix* spp ; *Poplar* spp, *Pyracantha*
 119 *angustifolia*, Marsh (Rosaceae), *Robinia pseudoacacia* L. (Fabaceae), *Rubus* spp., *Rosa*
 120 *rubiginosa* L. (Rosaceae) and *Gleditsia triacanthos* L. (Fabaceae) (Fig. 1.2) (Martin, 2021).
 121 However, some of these species such as Australian *Acacias* and *Eucalyptus* species have been
 122 on the priority list of the South African government for some time due to their current or
 123 anticipated impacts (Van Wilgen *et al.*, 2008b).



Figure 1.2: A variety of northern temperate weeds invading the grassland biome in eastern Free State Province, South Africa- *Poplar*, *Pine* and *Pyracantha* species. Photo: Martin G, 2020.

1.4 Biotic and abiotic interactions

Invasive alien plants can modify ecological communities and ultimately ecosystem function (Van Wilgen *et al.*, 2008a; Clusella-Trullas & Garcia 2017). For example, Livingstone *et al.* (2020) showed that the invasive vine, *Vincetoxicum rossicum* (Kleopow) Barbar. (Apocynaceae), reduced plant community diversity and altered trophic interactions. While, these findings are not unique to this study as there is abundant literature on the impacts of IAPs on community structure and function (Hejda *et al.*, 2009; Lazzaro *et al.*, 2019; Pysek *et al.*, 2012), the manner in which they occur is highly dependent on local environmental conditions

and the invading species' functional traits (Milanovic *et al.*, 2020). These traits include size, fitness, high growth rates, high reproductive capacity among others (van Kleunen *et al.*, 2010). The mechanism by which IAPs alter both above- and below-ground biophysical components of the ecosystem determines the nature and direction of responses by ecosystems. For instance, microclimates are a key determinant of ecological niches for various plant and animal assemblages and the resultant interactions (Vallés *et al.*, 2011). However, mechanisms such as shading and, shifts in temperature and light regimes modify habitat structure and physiological requirements of dependent organisms. For example, in the Nama-Karoo biome of South Africa, Yelenik *et al.* (2004) found a reduction in the abundance of native grasses due to the shading effect of *Acacia saligna* (Labill.) H.L.Wendl. (Fabaceae) whilst promoting the weedy, shade tolerant *Erharta calycina* Sm. (Poaceae). Furthermore, some IAPs have been found to alter soil physicochemical properties through mechanisms such as biological nitrogen fixation and allelopathy, an effect more pronounced for leguminous trees from the Fabaceae family (Ehrenfeld, 2003; Marchante *et al.*, 2008). Several IAPs also alter the water budget, encouraging the proliferation of species which are adapted for water scarce environments (Gorgens & van Wilgen 2004). Alteration in fire regimes has also been associated with alien plants and has the effect of destroying seedbanks for native vegetation (Brooks *et al.*, 2014).

1.5 Impacts on ecosystem services

Key ecosystem services such as grazing and water provision across all biomes in South Africa are likely to be impacted by invasive alien plants by up to 71% in the future (van Wilgen *et al.*, 2008a). Despite widespread assessments of environmental impacts of invasive alien plants and services derived therein, agroecological systems have received less attention (Pratt *et al.*, 2017). In South Africa, particularly the grasslands of eastern Free State Province, there is a mixture of range-based livestock production, cropping and horticulture (Hensley *et al.*, 2006).

Thus they form an important agro-ecological system. Key ecosystem service requirements for this system include pollination for crops as well as grazing for both domestic and wild fauna. However, the introduction of novel resources in the form of invasive alien plants potentially affects the provision of ecosystem services. While, Hansen *et al.* (2018) reported a reduction in pollination of native grassland vegetation due to invasion mediated fragmentation, there is little information on how cropping systems are affected. However, judging from the increasing hire of bee colonies for pollination services there is a decline in natural pollinators or the available pool is overstretched. Similarly, the capacity of grasslands to provide grazing has reportedly been affected by Australian *Acacia* and *Prosopis* species (Ndhlovu *et al.*, 2011; Yapi *et al.*, 2018). While these two ecosystem services are easy to quantify due to their economic significance, the same is not true for other services such as cultural. However, this does not signify their triviality, hence the need for a holistic approach in quantifying the impacts of invasive alien plants on ecosystem structure and function.

1.6 Economic impacts of invasive alien plants

Economic impacts of IAPs arise from their direct effects on ecosystems leading to losses in critical services such as pollination, grazing and water provisioning among others. Pimentel *et al.* (2005), estimated the costs of invasion by more than 25 000 plants at US\$35 billion annually in the United States. In South Africa, various sector specific losses have been documented for specific species. For example, Humphrey *et al.* (2019), quantified potential losses in the livestock sector due to invasion of grasslands by *Robinia pseudoacacia* L. (Fabaceae) at ~ US\$39 million. These losses were attributed to reduced grazing as the cover of the IAP increases. Prior to biological control of the invasive aquatic weed, *Azolla filiculoides* Lam. (Azollaceae), economic impacts were estimated at US\$58 million (de Wit *et al.*, 2001). These were as a result of disrupted irrigation and reduced access to water for livestock. Economic

impacts of invasion not only arise from environmental damage, but also from costs of managing invasions. For example, South Africa spent approximately US\$100 million to manage invasive alien plants between 1995 and 2000 (de Wit *et al.*, 2001) while the costs of managing cacti were estimated at US\$45 million prior to the introduction of biological control agents. Similarly, the economic costs of controlling four aquatic weeds, *Pista stratiotes* L. (Araceae), *Salvinia molesta* D.S. Mitch. (Salviniaceae), *A. filiculoides* and *Myriophyllum aquaticum* (Vell.) Verdc. (Haloragaceae) were estimated at R1 billion if mechanical and chemical means were to be applied (Maluleke *et al.*, 2020). These are funds that could have been used elsewhere to improve the livelihoods of people.

1.7 Invasive alien trees and rural livelihoods.

Rural inhabitants in most developing countries including the grasslands of South Africa rely on ecosystems and the services they provide for their livelihoods (Adams *et al.*, 2018; Wisely *et al.*, 2018). For instance, grassland inhabitants obtain some of their house construction materials (thatching grass), sweeping brooms and medicines from the diverse plants of this ecosystem. However, the introduction of alien plants affects the relationship between society and ecosystems. While there is evidence of negative impacts of these alien species on ecosystems and societies in general, there are some species which are being used in invaded communities (Shackleton *et al.*, 2015). Control of such species often results in conflicts as they variably impact on different sections of the society. Examples of such tree species include *Eucalyptus globulus* Labill. (Myrtaceae), *Acacia mearnsii* De Wild. (Fabaceae), *A. saligna*, and *Pinus pinaster* Aiton (Pinaceae) which were largely introduced as plantation trees for timber. It has also been argued that perceptions of invasive alien species are based on prejudices as focus is directed more towards negative rather than positive impacts (Bonanno, 2016). Seastedt (2014) postulated that some invasive plant species are gaining recognition in the

mitigation of global environmental change. There is also the realization that it might not be possible to restore native vegetation (Ruwanza & Tshililo, 2019) and invasive plants may provide some of the services derived from natives (Walther *et al.*, 2009; Erviner *et al.*, 2012; Seastedt, 2014). In some instances, some invasive alien plants offset their impact through provision of other services beneficial to society. Given these conflicting views from local to scientific communities on the role of certain invasive alien species, there is a need to harmonize divergent views in the design of IAP control programmes and attempt to find a balance between the negative and positive impacts.

1.8 Management of invasive alien plants

Knowledge of invasive alien species and their impacts has grown exponentially over the last decades which has culminated in improved management strategies which are often reliant on understanding the ecology of the invading species (Ramírez-Albores, 2019). Local ecological interactions play a role in the responses of different species to management (Blackburn *et al.*, 2011; Bartz & Kowarik, 2019; Robertson *et al.*, 2020). Environmental variability on a spatial scale also shapes the response of alien plants to control (Vicente *et al.*, 2019). Management of biological invasions need to have both ecological and social considerations if they are to be successful. In addition, there are growing demands for quantifiable success indicators for management initiatives.

1.8.1 Mechanical Control

Invasive alien plants can be controlled manually by felling, ring barking or hand pulling. These measures constitute mechanical control. In South Africa, mechanical control forms the backbone of the Working for Water, a programme aimed at clearing invasive alien plants while providing employment to marginalised communities (Turpie *et al.*, 2008). Mechanical clearing has further been incorporated into wide-scale expanded public works programmes awarded to

organized entities. However, regrowth is always a challenge hence the need for continuous follow-ups. Furthermore, the costs of clearing are very high and implementation in difficult terrain is often a challenge.

1.8.2 Chemical Control

Herbicides may be applied against targeted plants. These can be used singly or as part of an integrated approach. In many terrestrial plant control programmes, herbicides are applied as a follow-up to mechanical control to prevent coppicing. While wide coverage might be achieved through aerial applications, there are concerns over non-target effects. Moreover, where there is targeted application, areas covered are minimal and access is usually a concern. Efficacy is also dependent on the ability of the applicator to detect even the smallest plants. For example, juvenile *Opuntia stricta* Haworth. (Cactaceae) plants were often missed during chemical control in Kruger National Park (Lotter & Hoffmann, 1998). Thus, the demand for reapplication drives costs high. In recent years, there has been a drive towards improved environmental safety. Hence, biological control is one of the options which has been highlighted globally and in South Africa as a cost effective, safe and sustainable management option.

1.8.3 Biological Control

Biological control against invasive alien plants involves the use of introduced, highly selective herbivorous arthropods and/or pathogens (Zachariades *et al.*, 2017). This is typical of classical biological control of pests as the natural enemies (biological control agents) are sourced from the origin of the invasive plants and have co-evolved with their host (Cock *et al.*, 2015). For IAPs, biological control has been adopted by 85 countries and the general consensus is that it has minimal impact on the environment and relatively lower costs compared with other methods such as chemical and mechanical control (Winston *et al.*, 2014; Hill *et al.*, 2020). In

South Africa, 87 agents have been released against 66 IAPs (Zachariades *et al.*, 2017). In some instances, more than one agent has been released for a single IAP. South Africa has had significant success in managing invasive tree species using integrated control measures.

1.8.4 Control of invasive trees in South Africa

Despite the difficulty of controlling invasive alien trees, notable successes have been reported for several species in South Africa (Marais *et al.*, 2004; Moran *et al.*, 2013; Hill *et al.*, 2020). While huge sums of money have been spent on mechanical and chemical control in the past, the majority of successful programmes integrate biological control (Zachariades *et al.*, 2017). Examples include the successful control of *Sesbania punicea* (Cav.) Benth. (Fabaceae) in South Africa using three biological control agents (Hoffmann & Moran, 1991). These authors reported that the three agents, *Trichapion lativentre* Beguin- Billcorcq (Coleoptera) (florivorous), *Rhyssomatus marginatus* (Coleoptera) Fahraeus (seed feeder) and *Neodiplogrammus quadrivittatus* (Coleoptera) Olivier (stem borer) managed to reduce the growth, phenology and fecundity of the host, *S. punicea* leading to a reduction in population densities in as little as two years. Another example is the control of Australian acacias using a combination of flower galling wasps and seed attacking agents (*Melenterius* species) that have successfully reduced the reproductive capacity of the target species (Richardson *et al.*, 2015; Impson *et al.*, 2021). This strategy to induce reproductive sterility has been applied mainly on species with the potential to generate conflicts where halting further landscape spread rather than population reduction is desired (Impson *et al.*, 2011).

1.9 Evaluation of IAP control programmes with a biological control component

Many attempts have been made to assess the success of invasive alien control programmes (Morin *et al.*, 2009). However, approaches tend to vary from one region to the other. In South Africa, notable attempts have been made to evaluate the control of invasive alien plants in the

Cape Floristic Region (CFR) (van Wilgen *et al.*, 2020). The most common measure of success in all these attempts was the reduction in the cover of alien plants as an indicator of success. Hoffmann (1995) categorised success of biological control programmes on a qualitative scale based on costs and the need for further interventions. However, this system has since been revised and an improved evaluation system has been conceptualized and is now being implemented (Hoffmann *et al.*, 2019; Moran *et al.*, 2021). This approach defines parameters and categories of success based on plant population dynamics measurable approximately 10 years post agent release. In general, including Hoffmann *et al.* (2019) the key consideration in the control of IAPs is the return to ecosystem functioning. To measure success at ecosystem level, Paterson *et al.* (2010) established native diversity linked benchmarks for the control of *Pereskia aculeata* Miller (Cactaceae) in South Africa. Similar parameters were used to evaluate biological control of two aquatic weeds *P. stratiotes* and *S. molesta* (Coetzee *et al.*, 2020; Motitsoe *et al.*, 2020). In both cases, the authors found significant recovery of macroinvertebrate communities following the release and action of biological control agents. Thus, this highlights the importance of both pre-release and post-release studies especially for biological control programmes (McFadyen, 1998).

The restoration of ecosystem structure and function following invasive alien plants control is often a complex process. In many instances passive restoration has failed because of a number of factors. Some IAPs have legacy effects such as nitrogen fixation which results in a lag phase between effective population control and ecosystem recovery. For example, while Ndhlovu *et al.* (2011) believed natural restoration was taking place following the removal of *Prosopis* from rangelands, they acknowledged the absence of a representative pre-intervention site to benchmark their findings. Clearing invasive alien plants may also create a niche for secondary invasion thus compromising ecosystem restoration (Ruwanza, 2019). In addition, several invaders have assumed provisioning services to invaded communities hence the need to strike

a balance between control and these services. Cock *et al.* (2015), differentiated between biological control success and impact with the former dealing with processes of agent establishment and action on target pests and the later dealing with societal and environmental benefits ensuing from control of the pest (Fig. 1.3). However, the underlying requirement for all approaches is the need for quantifiable and comprehensive baseline data to remove ambiguity in post-control evaluations (Blossey *et al.*, 2018). Not only is this data critical in the evaluation of success, but it also aids the selection of agents which with a likelihood of success (McFadyen, 1998).

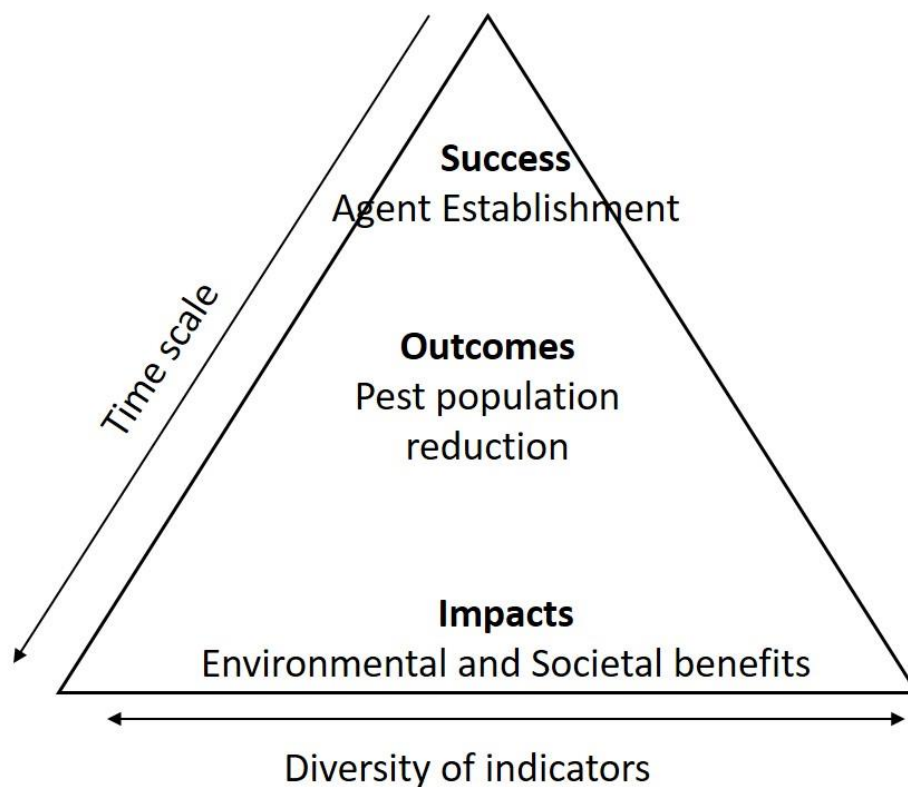


Figure 1.3: Stages in the assessment of the success of invasive alien plants using biological control agents. (redrawn from Cock *et al.*, 2015).

1.10 Study species: *Robinia pseudoacacia* (Black locust)

Robinia pseudoacacia L. (Black locust), a tree species native to North America, is one of the most widely distributed species outside its native range as it covers greater parts of Europe, Asia, temperate South America, Africa, Australia and New Zealand (Sitzia *et al.*, 2016; Vítková *et al.*, 2017). Its deliberate introduction and propagation was driven by various societal needs including timber production, land reclamation, as an ornamental plant as well as apiculture (Cierjacks *et al.*, 2013; Vítková *et al.*, 2017). Although *R. pseudoacacia* is regarded as a pioneer species in abandoned agricultural fields and pastures (Czarapata, 2005; Von Holle & Motzkin, 2007), it can spread to undisturbed sites such as forests, woodlands, riparian zones, thickets and grasslands replacing native vegetation (Rehounkova & Prach, 2008). Its ability to propagate rapidly through root suckers and abundant seed banks (Call & Nilsen, 2003) renders it highly invasive. Vegetative regeneration has been identified as key to *R. pseudoacacia* establishment, spread, and persistence (Stone, 2009). In South Africa, *R. pseudoacacia* is considered a category 1b invasive species which is prohibited but exempted if in possession or under control (DEA, 2016). Records from the Global Biodiversity Information Facility (GBIF) show presence in 32 other countries including South Africa (Fig. 1.3). According to Redei *et al.* (2017), *R. pseudoacacia* covers approximately 4 million hectares globally, 63% of which are in Europe. There is a strong possibility that the current distribution is wider than reported as in some cases information is scanty especially in Africa as limitations have been noted on invasive species studies on both taxonomic and geographic scales (Nel *et al.*, 2004; Pyšek & Richardson, 2010; Martin, 2019).

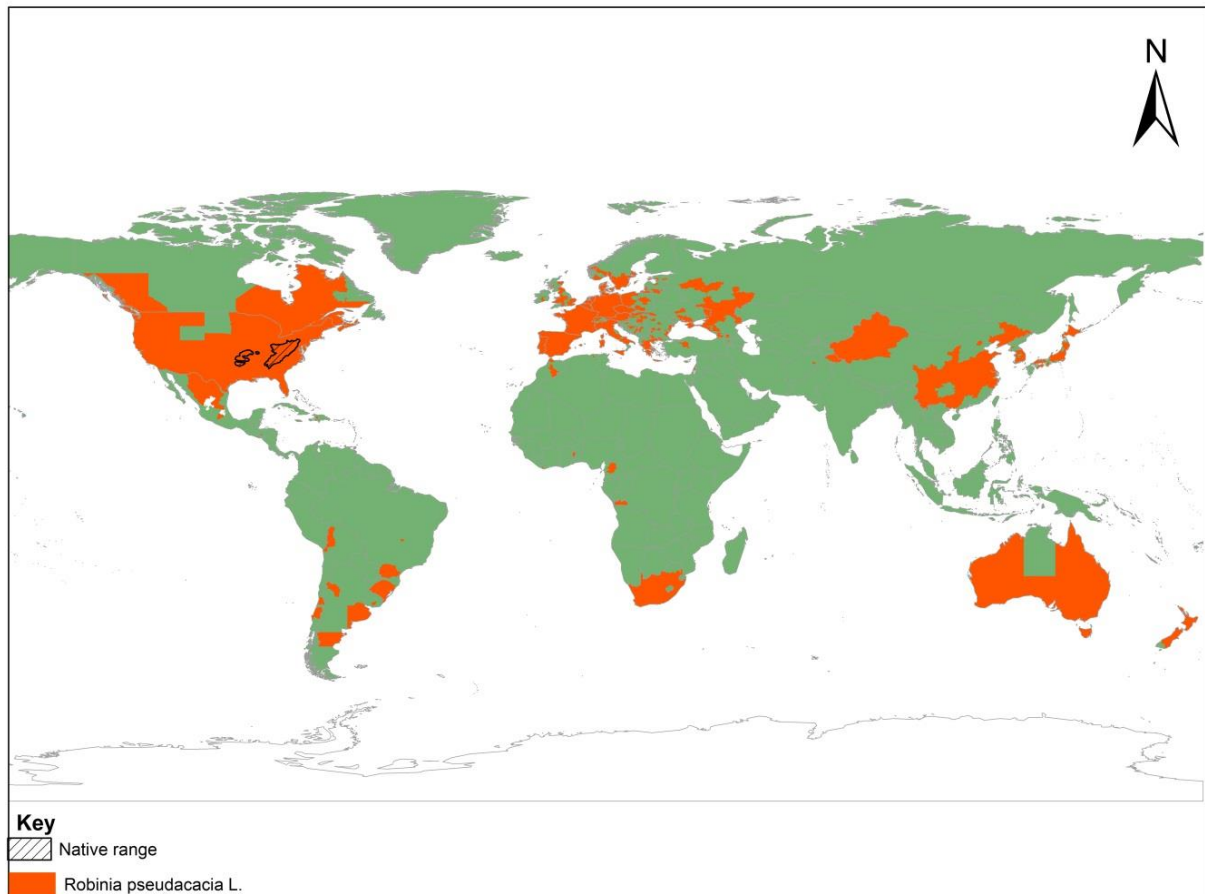


Figure 1.4: Distribution of *Robinia pseudoacacia* L. (Fabaceae) based on Global Biodiversity Information Facility (GBIF) 2019 records.

In South Africa, all the nine provinces have been invaded by *R. pseudoacacia* and its detrimental effects are becoming evident (Fig 1.4 & 1.5) (Henderson & Wilson, 2007). Despite extensive studies on *R. pseudoacacia* in the northern hemisphere, a review by Martin (2019) showed that its impacts on native biodiversity and ecosystem functioning in South Africa and the southern hemisphere in general are not well documented. In addition, both its positive and negative contributions to livelihoods of poor rural populations of the country are unknown. Localized efforts to control this invasive alien tree using mechanical and chemical methods have resulted in intense coppicing and root sprouting (Fig. 1.6) as also reported around the world (Jackson & Strait, 1987; Vitkova *et al.*, 2017). Martin (2019) highlighted a number of chemical options available for the control of *R. pseudoacacia*. While variable levels of success

were recorded, the major concern is regeneration of stands especially from root suckers. Besides the limited success achieved with mechanical and chemical methods, their labour intensive nature and occurrence of the tree in terrain which is difficult to access makes them less desirable. Given its detrimental impacts and management difficulties, *R. pseudoacacia* has been prioritized for biological control in South Africa under the recently developed Biological Control Target Selection (BCTS) system (Canavan *et al.*, 2021; Martin, 2019).



Figure 1.5: Aerial photography showing reduction in grassland (circled areas) due to *Robinia pseudoacacia* invasion over a 50-year period in Clarens, eastern Free State Province, South Africa. Imagery Source: National Geo-Spatial Information (NGI), Department of Rural Development and Land Reform, South Africa.

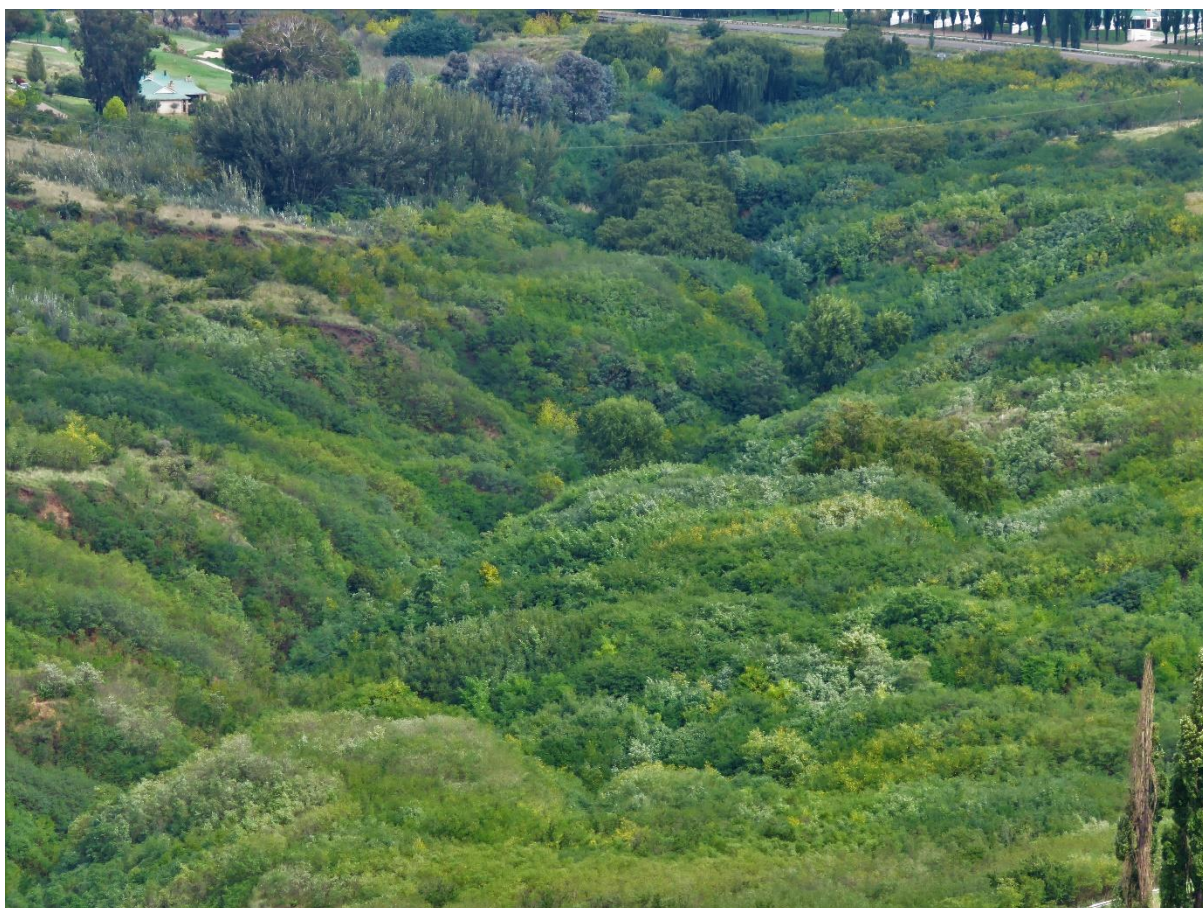


Figure 1.6: A *Robinia pseudoacacia* infestation near Clarens town in eastern Free State South Africa. Note the density of the infestation. *Photo: Martin G.*

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369 **Figure 1.7:** *Robinia pseudoacacia* resprouting (Top and Bottom) following mechanical
370 clearance followed by herbicide application in Clarens, eastern Free State, South Africa.
371 Photos: Martin G, 2019.

1.11 Aims

The aim of this thesis was to establish impacts of *R. pseudoacacia* on grassland ecosystems which might act as baseline data for future management of the species in South Africa (Fig. 1.8).

The first objective was to ascertain the canopy traits of *R. pseudoacacia* in South African grasslands and related changes in understory microclimates. The effect of these changes on endemic grassland arthropod community assembly were further evaluated. These three components constitute Chapter 2 and establish the functional traits that need to be monitored in order to conserve the functional diversity of grasslands. In addition, they give an indication of plant-based measurements of successful control of *R. pseudoacacia*.

The impacts of *R. pseudoacacia* invasion on biodiversity were further followed up in Chapters 3 and 4 where effects on key ecosystem services were explored. Grazing is a key grassland ecosystem service which is dependent on the diversity, quality and cover of grasses. Thus any reduction in the grass diversity can potentially affect economic livestock production. Similarly, pollination is important in maintaining natural plant diversity as well as improving yields in agroecosystems. In view of the increasing expenses on hired bee colonies for pollination services by farmers, the return to natural pollination is therefore desirable. These two ecosystem level parameters form the long term success indicators of control measures.

Some invasive alien species have been integrated into the livelihoods of local communities especially in developing countries. This necessitates the evaluation of interactions between the invasive alien tree and invaded communities. Information on how communities are using the resource, its perceived values and negative impacts inform management goals and strategies. These aspects are explored in Chapter 5 where potential valuable contributions of *R. pseudoacacia* to vulnerable communities in South Africa are assessed.

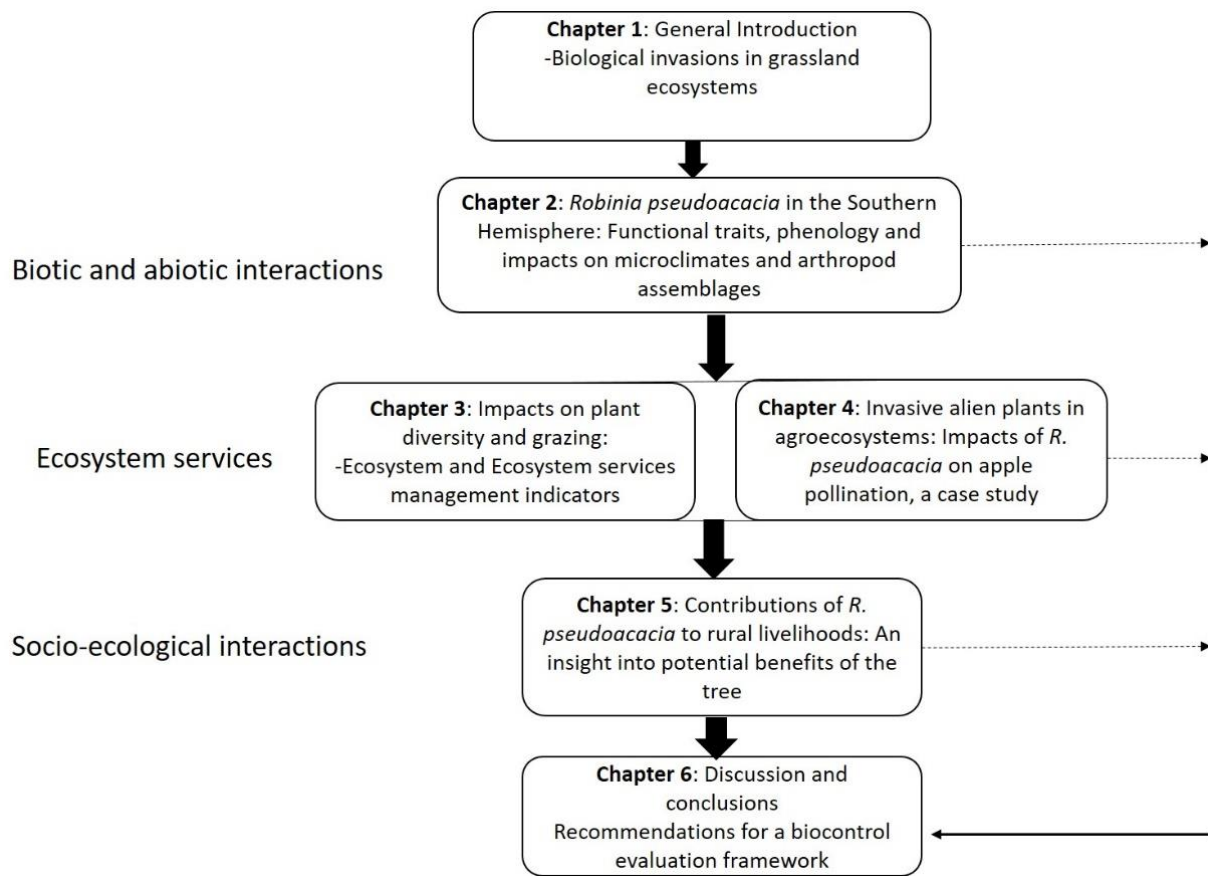


Figure 1.8: Thematic areas addressed by the thesis and how they contribute to the broad aim of establishing a biological control agent post-release evaluation framework.

CHAPTER 2

An assessment of the invasive alien tree, *Robinia pseudoacacia* canopy traits and its effect on grassland microclimates and subsequent arthropod assemblages*

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2.1 Introduction

Habitat transformation, climate change and invasive alien species are the major threats to ecosystem structure, integrity and function (Erviner *et al.*, 2012; Walther *et al.*, 2009). By interfering with coevolved interactions between biotic and abiotic components, invasive alien species modify ecosystems, to the detriment of their integrity and human wellbeing (Shackleton *et al.*, 2019; Walther, 2010). Impacts of invasive alien species on ecological interactions can be direct or indirect. For example, dietary shifts have been observed especially in pollinators following invasion by plants with showy inflorescences (Gibson *et al.*, 2013) or increased dominance of canopy dwelling arthropods when grasslands are invaded by woody trees (Andersen *et al.*, 2019). Of interest, is how invasive plants can alter ecosystem structure, community assembly and subsequent trophic interactions such as predation and competition.

Plants have the highest number of taxa invading terrestrial and aquatic environments and as such, many studies have been conducted to quantify their impacts (van Wilgen *et al.*, 2020). For example, there is ample evidence to show that invasive alien plants (IAPs) modify ecosystems through reduction of water availability and quality (Le Maitre *et al.*, 2002), change soil chemical composition (Marchante *et al.*, 2008) and reduce native plant and animal diversity (Hejda *et al.*, 2009). These impacts result from complex interaction between the invaded environment and IAP functional traits (Yelenik *et al.*, 2007). Thus, IAPs, through their functional traits, which include canopy characteristics, seed production rates, water use and biological nitrogen fixation can alter microclimates and microhabitats (van Kleunen *et al.*, 2010; Milanovic *et al.*, 2020). Furthermore, the release of root and leaf leachates may have inhibitory effect on the growth of competing plants (Thiébaud *et al.*, 2019). Alteration of the physical environment by changing thermal regimes and illumination due to the shading effect of IAPs may also result in the creation of novel environments in which natives fail to adapt or

compete (Lazzaro *et al.*, 2018). This can lead to a gradual change in plant succession trends, an effect that cascades through trophic levels (Schirmel *et al.*, 2016).

Grasslands are the largest biome covering over 40% of the global surface area, making them habitat to many endemic plant and animal species worldwide (Suttie *et al.*, 2005). Moreover, grasslands provide natural grazing as a service to both wildlife and range-based livestock production systems (Palmer & Ainslie, 2005; Humphrey *et al.*, 2019; O'Connor & van Wilgen, 2020). Unfortunately, this biome is rapidly being transformed due to natural and anthropogenic disturbances (van Wilgen *et al.*, 2008). For example, highland grasslands in South Africa are becoming fragmented due to agricultural activity as well as invasion by a number of alien plants (Wigley *et al.*, 2010; Carbutt, 2012). Woody plants, in particular, have been successful invaders in grasslands as they have superior traits such as high canopy cover which alter habitat conditions for native grassland flora (Hejda *et al.*, 2009; Chikowore *et al.*, 2021).

To fully understand the influence of invasive alien plant traits on invaded communities, it is necessary to study the ecology of the species in their native and invaded ranges. This includes knowledge of the growth season and characteristics of the plants. The growth season of plants is defined by their phenology, which in turn influences certain ecological processes (Richardson *et al.*, 2018). For instance, plant productivity, carbon storage, evapotranspiration and trophic interactions such as predation and pollination all depend on plant phenology (Brown *et al.*, 2016; Richardson *et al.*, 2019). Spatio-temporal variability in phenology also offers an opportunity to monitor forest health as it is linked to climatic variation and other stresses such as pest damage (Richardson *et al.*, 2009). For example, the impact of late spring frost on the deciduous tree, American buckeye, *Aesculus glabra* Wild. (Sapindaceae), induced stresses which persisted beyond the year of occurrence (Augsburger, 2011). Therefore, monitoring the timing and intensity of canopy greenness enables the tracking of critical plant growth phases and helps to predict key ecological processes.

Temperatures also have a direct impact on plant and animal growth processes (Sendall *et al.*, 2015). At the centre of the climate change debate temperature fluctuations are at the core. This is particularly important for endemic ectotherms, which rely on ambient temperature for their thermoregulation. Forest shelters modulate microclimates creating conditions that are unique and differentially suitable for other organisms. For instance, in a study to quantify the effect of canopy openness and seasonality on microclimates in France, Gaudio *et al.* (2017) found minimum temperatures to be higher and maximum temperatures to be lower under tree canopies than in open lands especially in summer. Microclimates also tend to vary significantly at very fine spatio-temporal scales (Pincebourde *et al.*, 2016). In grassland ecosystems, the introduction of trees creates wetter and cooler conditions, which are novel to resident biota (von Arx *et al.*, 2013). The growth of understory vegetation under these conditions will therefore depend on the incident solar radiation which influences temperatures. Moreover, invasive alien trees are associated with the homogenization of understory habitats, reducing food resources and habitats for specialist organisms (Andersen *et al.*, 2019; Kadlec *et al.*, 2018; Chikowore *et al.*, 2021).

For ectotherms, body temperature is dependent on the environment (Chown & Nicolson 2004; Chidawanyika *et al.*, 2017, 2020). Thus, invasion-mediated modification of vegetative structure can affect behavioural thermoregulation among ectotherms and ultimately their assemblages (Clusella-Trullas & Garcia, 2017). For example, Watling *et al.* (2011) reported that alteration of microclimates by the invasive alien shrub, *Lonicera maackii* (Rupr.) Maxim. (Caprifoliaceae) the Amur honeysuckle negatively impacted amphibian species richness and composition in Missouri, USA. This was despite the absence of a direct trophic linkage between the two taxa. Similarly, heterogeneity on alpine plant assembly was recently shown as a mediating factor among flower visitation rates (Ohler *et al.*, 2020), underlying the importance of vegetative structure on arthropod assemblages.

Arthropods, being poikilothermic are highly sensitive to changes in thermal environments (Chown & Terblanche, 2006; Chidawanyika *et al.*, 2020). Hence, fluctuations in ambient temperatures have a direct bearing on life history traits such as survival, reproduction and development (Jaworski & Hilszczanski, 2013, Ahn *et al.*, 2016; Chidawanyika *et al.*, 2017). Their sensitivity and assemblages thus make them good indicators of changes in microclimatic conditions (Strobl *et al.*, 2019). Their trophic association with plants as primary consumers also makes them suitable as model organisms for studying IAP impact (Mooney *et al.*, 2010). Species diversity has been found to be conditioned by vegetation structure, with dominant plant species playing a larger role (Farrell *et al.*, 2015) where a decline in arthropod populations could be used as indicators of natural ecosystem degradation (Lister & Garcia, 2018).

Robinia pseudoacacia, a deciduous tree originating from North America which is currently widely distributed across the globe is one of the alien trees invading Afriomontane grasslands in South Africa where it is regarded as an IAP (Henderson & Wilson, 2017; Martin, 2019). Among its functional traits is the ability to change soil chemical properties and high growth rates especially in forest gaps (Cierjacks *et al.*, 2013). The tree has been reported to alter micro-arthropod, soil microbial and plant communities in invaded areas (Lazzaro *et al.*, 2018). Furthermore, in Central Europe, Kadlec *et al.* (2018) linked *R. pseudoacacia* to reduced richness in canopy arthropods, particularly lepidopterans due to lack of adaptation to feed on the tree.

Given the rapid expansion of *R. pseudoacacia* in South Africa (Martin, 2019), this study aimed to determine the species phenology, particularly in highland grasslands, in order to ascertain its influence on the microclimates below the canopy and its impact on the grassland arthropods in this biome. Specifically, we asked. 1. What are the seasonal growth characteristics of *R. pseudoacacia* in the southern hemisphere and South Africa? 2. What is the impact of *R.*

pseudoacacia canopies on understory temperature and light in highland grasslands? and, 3.
How *R. pseudoacacia* mediated habitat changes influence invertebrate assemblages?

2.2 Materials and methods

2.2.1 Study area

The study was conducted between February 2019 and October 2020 (21 months) on the Northern Slopes of the Maluti- Drakensberg Mountains in South Africa (28° 32' 33.08" S; 28° 29' 30.91"E). Climate for the area according to the Köppen-Geiger classification is subtropical highland (Cwb) with annual temperatures and rainfall averaging 13.7 °C and 693 mm respectively (Beck *et al.*, 2018). Due to the high altitudes >1600m sub-zero temperatures, frequent frost and snow are also common in winter. The area falls within the high altitude or Drakensberg grassland biome where the native vegetation is dominantly grasses (Poaceae) although woody natives such as *Leucosidea sericea* Eckl. & Zeyh. (Rosaceae) and *Protea* species are also found (Mucina & Rutherford, 2006). Landuse in the area is a mixture of range based animal production, cereal and fruit production interspaced with conservation areas. Surveys were conducted on two farms, Clifton and Sunnyside where *R. pseudoacacia* invasions (hereafter 'invaded') and open grassland (hereafter 'uninvaded') were sampled (Fig. 2.1). Vegetation in invaded habitats on both farms is dominated by early successional ruderals such as *Tagetes minuta* L. (Asteraceae) as well as alien grasses such as *Bromus catharticus* Vahl. (Poaceae), *Brachypogon distachyon* (L.) P. Beauv. (Poaceae) and *Dactylis glomerata* L. (Poaceae). In contrast, uninvaded habitats are dominated by native grasses such as *Themeda triandra* Forssk. (Poaceae), *Tristachya leucothrix* Trin.(Poaceae) and *Eragrostis* species (Chikowore *et al.*, 2021). The linear distance between the two farms is approximately 11km and an altitudinal difference of 100m above sea level.

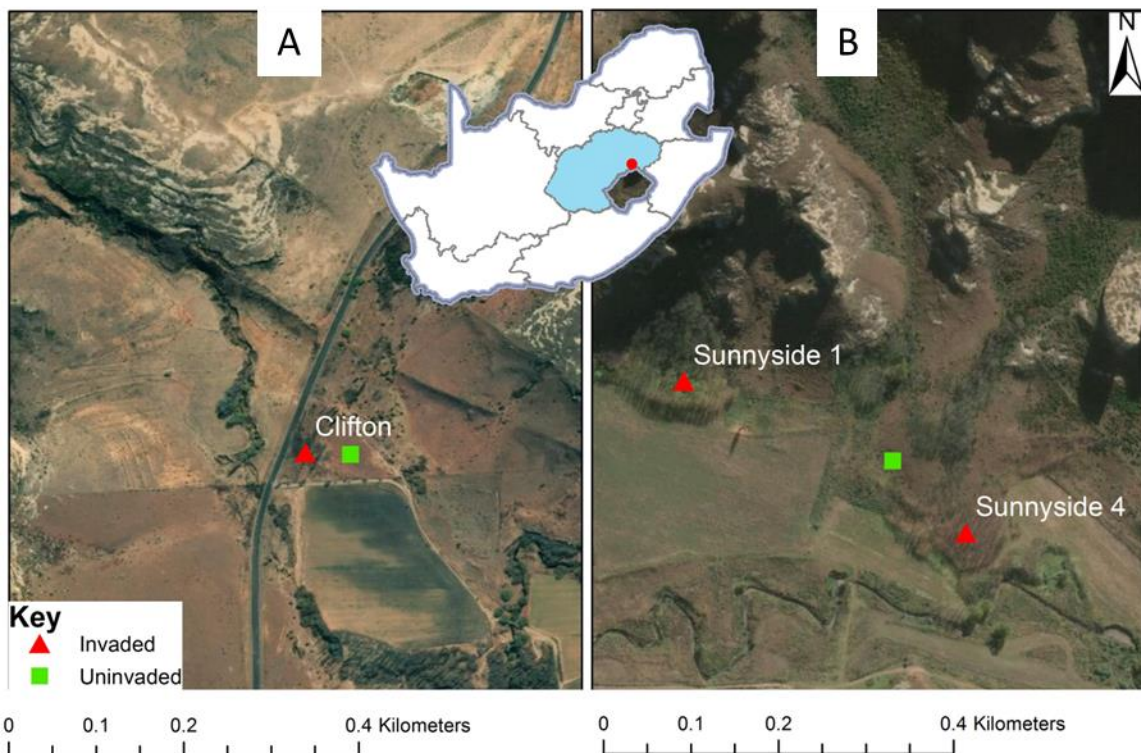


Figure 2.1: Study area showing *Robinia pseudoacacia* stands (Red triangles) and adjacent grasslands (Green squares) sampled at Clifton (A) and Sunnyside (B) farms in eastern Free State Province, South Africa. Background satellite imagery source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community.

2.2.2 *Robinia pseudoacacia* phenology

To determine the phenology of *R. pseudoacacia* in high altitude grasslands of South Africa, two Wingscapes PlantCam® with Red, Green and Blue (RGB) capability were mounted on positions overlooking stands at Clifton and Sunnyside between October 2019 and October 2020 (Fig 2.2). Cameras were set facing the south, to avoid direct sunlight on the lenses, at a setback of 5m from the target area. Still images were captured at one hour intervals between 7am and 4pm, at a resolution of 2560×1920 pixels giving a total of 10 images per day. At the end of the recording season, images were downloaded for processing and analysis as explained later.



Figure 2.2: Plant camera (left) and images (right) showing *Robinia pseudoacacia* canopy development at one of the sites in eastern Free State, South Africa.

2.2.3 Temperature and light measurements

The influence of *R. pseudoacacia* on grassland thermal and illumination environments were measured for 499 days between February 2019 and October 2020. Four HOBO MX2202 (Onset, www.onsetcom.com), temperature and light loggers were installed in three *R. pseudoacacia* stands and one in the adjacent open grassland. The loggers were programmed to record at 30-minute intervals giving 48 readings of each parameter per day. Loggers were serviced quarterly and data were downloaded using the Bluetooth linked HOBO mobile application.

2.2.4 Arthropod assemblages

Arthropod communities associated with uninvaded and habitats were sampled on two farms over two seasons (spring and summer) in October 2019 and February 2020. Sampling was done in *R. pseudoacacia* stands and adjacent open grassland with a distance of 50m between the treatments. At each site, 3 grids of pitfall traps were set in a 2×3 grid with an inter-trap distance of 2.5m and an inter-grid space of 10m. This configuration gave a total of 18 pitfall traps per treatment (invaded/uninvaded), 36 traps per site and 72 traps for the two sites. The traps consisted of 400ml plastic containers dug into the ground with the rim flush with the soil surface. Each trap was filled with 100ml of 100 % propylene glycol (propane-1,2-diol) and left open for five days. The traps were then emptied and specimens were preserved in 70% alcohol. All specimens were identified to order with the exception of Orthoptera which was further identified to family level. Taxonomic guides by Picker *et al.*, (2004) and Johnson & Triplehorn (2004) were used in the identification.

2.2.5 Statistical Analyses

To visualize temperature and light conditions in invaded habitats and open grassland, readings were plotted against sampling time in Excel (Microsoft Office 2016). Since the data conformed to normality tests, differences between habitats were compared using one-way analysis of variance (ANOVA) in STATISTICA version 7 (TIBCO Software). Tukey-Kramer *post hoc* tests were then used to separate statistically significant groups.

Plantcam images were analysed using the ‘phenopix’ package (Filippa *et al.*, 2016) in R software version 3.2.1 (R Development Core Team, 2020). Regions of interest were defined on a reference image using the ‘*DrawMultiroi*’ function while the ‘*extractVIs*’ function was used to extract indices from the Red, Blue and Green channels of the images. Maximum green chromatic coordinates were extracted using an ‘autofilter’ and plotted against the sampling

period to determine the growth season of the two stands. Vegetation greenness metrics were determined using the Green Chromatic Coordinates, computed using the following formula:

$$G_{CC} = \frac{G_{DN}}{R_{DN} + G_{DN} + B_{DN}}$$

Where: G_{DN} , R_{DN} , B_{DN} represent digital numbers from the green, red and blue image channels (Richardson *et al.*, 2009)

Since arthropod assemblage data did not conform to normality tests, they were subjected to multivariate generalised linear modelling using the ‘*mvabund*’ package in R (Wang *et al.*, 2012). The ‘*manyglm*’ function was used to model the effects of habitat (invaded/uninvaded), season (summer/winter) and farm (Clifton/Sunnyside) on arthropod abundances. A negative binomial distribution was applied to account for mild over-dispersion observed with a Poisson distribution. Univariate tests were further performed to assess the response of each insect order to the explanatory variables defined. Statistical significance of the effects was then assessed using Likelihood-Ratio Tests (LRT).

2.3 Results

2.3.1 *Robinia pseudoacacia* phenology

Robinia pseudoacacia canopy development started in October, peaking up between December and January (Fig. 2.3a). However, there was spatial variability in leaf-up periods with trees at Sunnyside farm developing leaves early (Fig. 2.3b) as indicated by high green chromatic coordinates (Gccs) of more than 0.4 at the time of camera set up when compared to Clifton farm which began from low Gccs of 0.32. A drop in canopy greenness was also evident at Clifton with the Gccs dropping from peak to values approximating senescence, indicating total defoliation (Fig. 2.3a). This defoliation was coincident with a sharp drop in temperatures (Fig. 2.4a). This only affected the stand at Clifton farm as the other stand showed a gradual decline in greenness consistent with normal canopy characteristics rather than stress. However, the stand at Clifton farm recovered and assumed a normal phenological curve. The phenological trajectory observed is typical of deciduous trees in the southern hemisphere where leaves are shed in winter and leaf development starts from spring into summer.

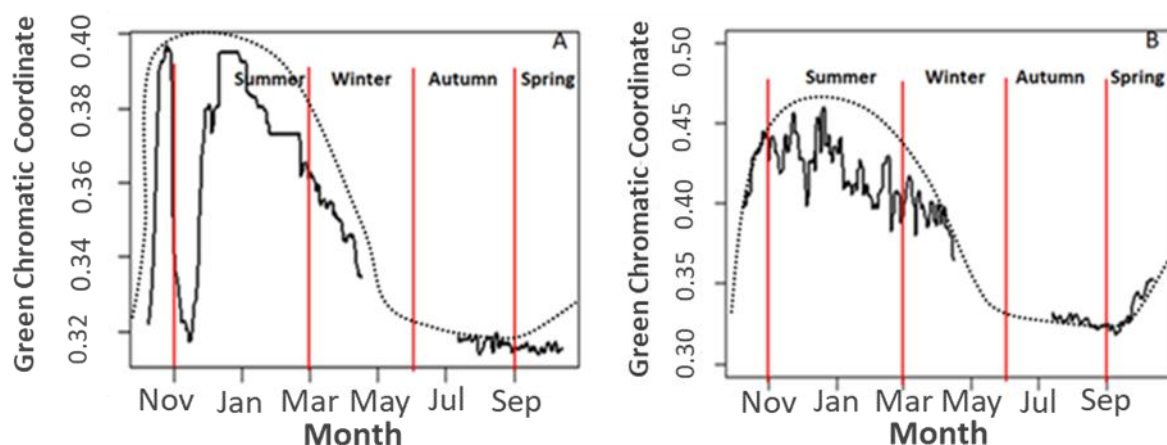


Figure 2.3: Changes in the Green Chromatic Coordinates (Gcc) on two *Robinia pseudoacacia* stands at Clifton farm (A) and Sunnyside farm (B). The broken line represents the expected phenological trajectory while the continuous line represents observed indices.

2.3.2 Thermal and illumination environments

Average temperatures were higher in open grassland (15.6°C) as compared to *R. pseudoacacia* understory (12.76 -13.77°C) (Fig. 2.4a). The site effect was significant in explaining variations in temperature ($p < 0.001$) (Table 2.1). *Post-hoc* tests showed that average temperatures were significantly higher in open grassland than all *R. pseudoacacia* stands (Fig. 2.5a). However, there were no significant differences in temperatures between stands. Similarly, light intensity was higher in open grassland (1788.1 lumens/ft²) than under *R. pseudoacacia* canopies (378.5 - 316.3 lumens/ft²) (Fig. 2.4b). Thus, site (invaded or uninvaded) significantly explained this variation (Table 2.2). *Post hoc* tests also indicated that open grassland received significantly high illumination than *R. pseudoacacia* stands while there were no differences between the stands (Fig. 2.5b). However, there was intra-stand variability in light intensity at Sunnyside farm 4 as indicated by the wide confidence interval of the mean (Fig. 2.5b). However, open grassland experienced extreme temperatures both minimum and maximum in comparison to understory habitats (Table 2.1).

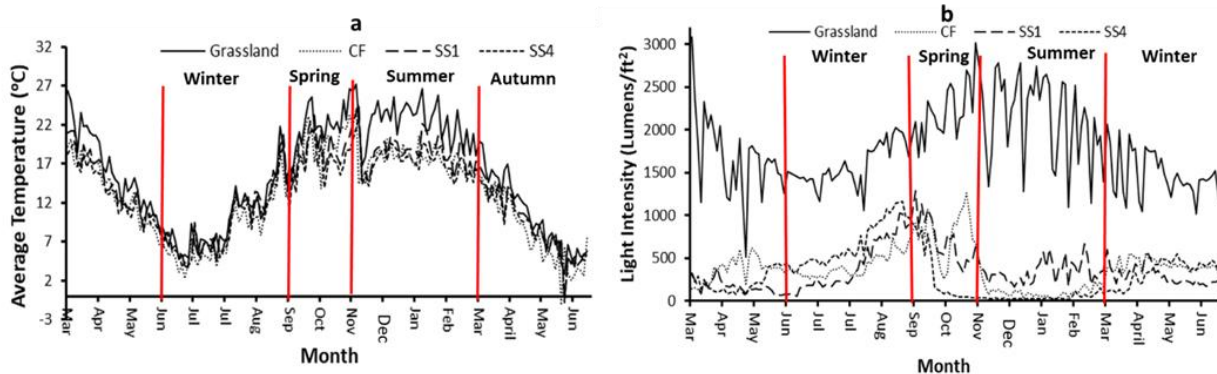


Figure 2.4: Three-day moving average and seasonal temperature (a) and light intensity (b) in open grassland and *Robinia pseudoacacia* stands in eastern Free State, South Africa (Sites are abbreviated as follows: CF = Clifton, SS1= Sunnyside 1 and SS4 = Sunnyside 4).

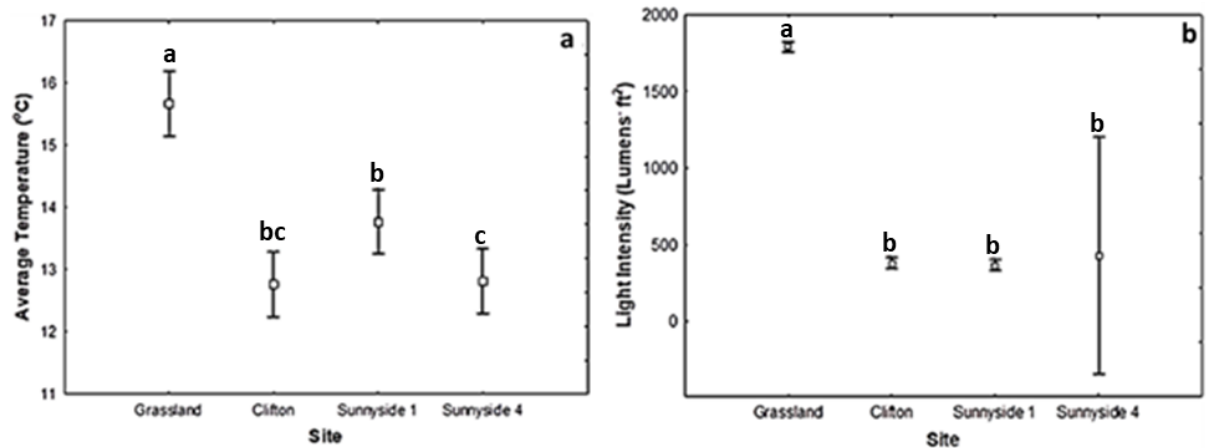


Figure 2.5: Differences in temperature (a) and light (b) (mean $\pm$ 95% confidence interval) between grassland and three *Robinia pseudoacacia* stands. Means with different letters are significantly different from each other.

Table 2.1: Minimum and maximum temperatures and maximum light intensity recorded at microhabitats in eastern Free State. Data represents the extreme recording for each site.

Site	Elevation (m)	Minimum Temperature (°C)	Maximum Temperature (°C)	Maximum light intensity (Lumens/ft ²)
Grassland	1731	-16.99	56.54	13003
Clifton	1712	-14.72	43.58	7460
Sunnyside 1	1833	-7.81	50.53	9578
Sunnyside 4	1832	-12.01	47.83	7247

Table 2.2: Summary of ANOVA on temperature and light intensity of sampled *Robinia pseudoacacia* stands

Parameter	Effect	Sum of Squares	Df	Ms	F	p-value
Temperature	Intercept	375989.5	1	375989.5	10676.27	<0.0001
	Site	2737.7	3	912.6	25.91	<0.0001
	Error	69871.1	1984	35.2		
Light Intensity	Intercept	8712079	1	8712079	56.04	<0.0001
	Site	667037681	3	222345894	1430.11	<0.0001
	Error	232279151	1494	155475		

2.3.3 Invertebrate assemblages

The most abundant arthropods in both invaded and uninvaded habitats were Hymenopterans, particularly from the Formicidae family (Fig. 2.6). These were followed by Coleopterans and Araneae, respectively. However, unlike Hymenopterans, which were more abundant in open grassland, the latter orders were more abundant under *R. pseudoacacia* trees. The family Acrididae (Orthoptera) was only recorded in open grassland while the family Gryllidae was common to both habitats although more abundant in the understory of *R. pseudoacacia*. From the multivariate analysis of variance, habitat (LRT = 55.85, $p = 0.001$), season (LRT = 25.34, $p < 0.05$), as well as interaction of habitat and season (LRT = 21.2, $p < 0.05$) had significant effects on arthropod abundances (Table 2.3). Univariate analysis showed that habitat had a significant effect on Acridids, Coleopterans, Lepidoptera and Hymenopterans while season significantly affected Gryllids (Fig. 2.6).

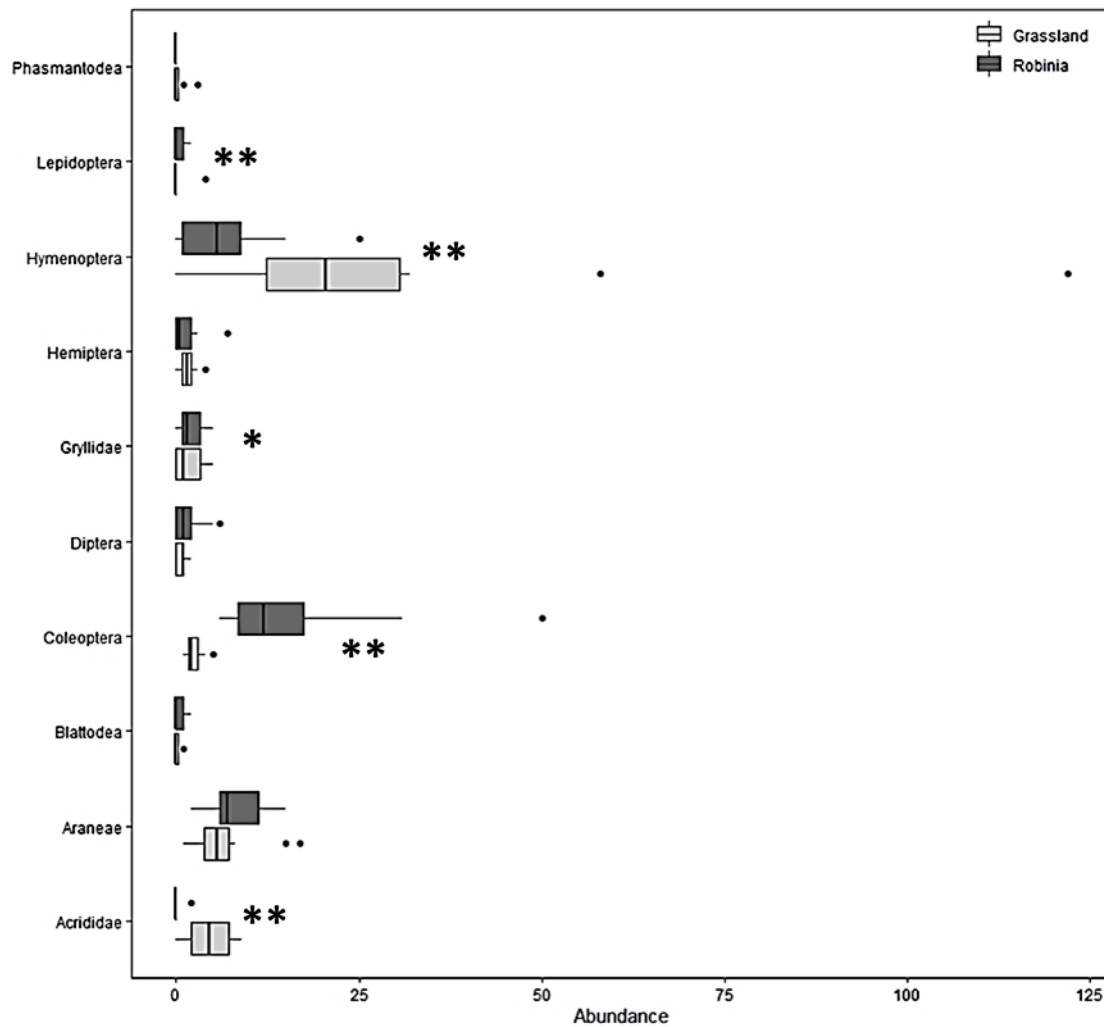


Figure 2.6: Abundance of arthropods (median, minimum, and maximum) in habitats invaded by *Robinia pseudoacacia* and uninvaded grasslands. All arthropods were grouped into orders except Orthopterans (which were grouped into 2 families, Acrididae and Gryllidae). Groups that significantly differed between habitats are indicated by ** while groups differing between seasons are indicated by *.

Table 2.3: Multivariate analysis for arthropod abundance in habitats invaded by *Robinia pseudoacacia* and adjacent uninvaded grassland at Clifton and Sunnyside Farms, South Africa

Effect	Residual Df	Df Diff	Deviance	Pr(>Dev)
Intercept	23			
Habitat	22	1	55.85	0.001
Farm	21	1	15.14	0.055
Season	20	1	25.34	0.002
Habitat * Farm	19	1	9.76	0.160
Habitat*Season	18	1	24.40	0.002
Farm * Season	17	1	13.96	0.054
Habitat*Farm*Season	16	1	4.49	0.296

2.4 Discussion

This study suggests that *R. pseudoacacia* is transforming grassland arthropod ecosystems through alteration of microclimatic conditions in the eastern Free State of South Africa. This is mainly due to its canopy characteristics which reduce temperature and light availability for understory communities, creating habitats with novel microclimates in the process. The results indicate that Orthopterans, particularly from the family Acrididae, declined significantly in invaded habitats while conditions created by the IAP seemed to favour the proliferation of Coleopterans. This therefore suggests that habitat transformation by IAPs differentially affects endemic arthropods.

The phenology of *R. pseudoacacia* in the southern hemisphere showed leaf emergence and expansion in spring (September), peaking between December and January. In the northern hemisphere, leaf expansion occurs in the spring months of April and May (Cierjacks *et al.*, 2013). Thus, environmental cues are responsible for phenological events in trees. However, its phenology varies with local climatic conditions as our study showed spatial variability in leaf development as well as the length of growth season between sites. Moreover, its ability to recover rapidly following frost damage gives an insight into the competitive advantage in invaded ecosystems. Our results further showed that in sync with other deciduous trees in South Africa, *R. pseudoacacia* develops leaves early before the emergence of annual grasses. However, unlike native trees that are often sparsely populated in South African grasslands (Mucina & Rutherford 2006), the impact of *R. pseudocacia* is exacerbated by monospecific

continuous canopies covering relatively larger areas. This limits seedling recruitment of native vegetation including grasses due to lower temperatures and low illumination under the canopies (Chikowore *et al.*, 2021). These realised microclimates influence community assembly cascading to other trophic levels. Indeed, *R. pseudoacacia* has been associated with a decline in both above and below-ground biodiversity. For example, Lazzaro *et al.* (2018) reported a decline in micro-arthropod, microbial and plant communities underlying the potent effects of *R. pseudoacacia* on ecosystem integrity.

In this study, average temperatures were consistently higher in the open grasslands as opposed to the invaded understory. However, when temperature readings were decoupled into minimum and maximum, grasslands recorded lower minimum temperatures and higher maximum temperatures. These findings are consistent with several studies that attribute these differences to the buffering effect of trees which ensures higher minimum and lower maximum temperatures in the understory (von Arx *et al.*, 2013; Gaudio *et al.*, 2017; Davis *et al.*, 2019). In all these studies, temperature was influenced by the sheltering effect of trees, canopy cover and soil moisture. Although we did not measure hydrological parameters such as relative humidity and soil moisture in our study, their influence on temperatures observed is highly probable. For example, Breshears *et al.* (1998) asserted that trees can lower temperatures through evaporative cooling, underscoring the importance of moisture in temperature regulation. In addition, interception of incident solar radiation by tree canopies could also be the reason for lower temperatures.

750 The absence of Acridids (Orthoptera) from invaded stands is an indication of the impacts of *R.*
751 *pseudoacacia* on microhabitats. While their exclusion might be due to invasion mediated
752 vegetation changes which places limitations on food resources (Andersen *et al.*, 2019), there is
753 also a likelihood that environmental conditions influenced their absence, especially considering
754 the generalist nature of their feeding habit. For example, Highland *et al.* (2013) found that
755 habitat structure rather than plant diversity affected arthropod diversity in woodlands while
756 Pawson *et al.* (2010) tied variation in arthropod herbivores to physical habitat characteristics
757 such as light availability and stand structure. Thus it is plausible that changes in arthropod
758 communities observed in this study were influenced in part by changes in the structure of the
759 grassland habitat as well as a shift in microclimatic conditions as a result of *R. pseudoacacia*
760 invasion. Furthermore, Samways (1990) observed that Orthopterans are more susceptible to
761 cold conditions hence they seek refuge on mountain slopes receiving more sunlight and ruled
762 out vegetation type and cover as determinants of the distribution of this taxa. Similarly, Rada
763 *et al.* (2015) found a strong correlation between temperature and Acrididae life history traits in
764 central Europe, emphasizing the importance of microclimates on the taxa. While grazing by
765 ungulates has been proven to alter community composition, particularly trophic cascades of
766 grassland arthropods (Farrell *et al.*, 2015), in our study both habitats were equally open to
767 grazing by cattle. Therefore, differences in taxa across habitats could be a result of invasion
768 mediated habitat modifications.

769 Our results also showed that there were more Coleopterans in invaded habitats than open
770 grassland. This taxon is comprised of a wide range of detritivores, hence, their abundance might
771 have been influenced by the quantity and quality of litter from *R. pseudoacacia*. In
772 corroboration with these findings, Alerding and Hunter (2013) found that *Alliaria petiolata* M.
773 Bieb. (Brassicaceae) invasion was associated with an increase in the depth of litter which
774 increased the abundance of detritivores. Although social arthropods such as ants can regulate
775 their internal and surrounding environments through elaborate nest designs and physical
776 mechanisms (Kadochova & Frouz 2014), our study showed that habitat had a significant effect
777 on their abundance. Although there was a possibility of some trapping positions located close
778 to ant nests and foraging grounds, the role of microclimates cannot be ruled out. In support of
779 these findings, Silva *et al.* (2011) reported that the choice of ant nesting sites is also influenced
780 by microclimatic conditions. The authors further stated that the quality and quantity of leaf
781 litter also influences the distribution of ants. However, since we did not measure these
782 parameters in our study, there is a possibility that *R. pseudoacacia* leaf litter makes understory
783 habitats unfavourable for ants.

784 In conclusion, invasion by *R. pseudoacacia* alters thermal and illumination environments of
785 grassland ecosystems. Through canopy characteristics, microclimatic conditions within tree
786 stands vary significantly from immediate local environments.

2.4.1 Conservation implications

The main goal of tackling biological invasions ‘should be the conservation or restoration of ecosystems to preserve or re-establish native biodiversity and functions’ (Hulme, 2006). Countries are under increasing pressure to mitigate the negative effects of invasive species on ecosystem dynamics. However, in the absence of baseline data on how invasive species are directly or indirectly changing the ecosystems, it is difficult to measure a return to a functioning state following management (Richardson & van Wilgen 2004; Gallardo *et al.*, 2019). Due to the significant impacts of *R. pseudoacacia* in South African grasslands (Martin, 2019; Chikowore *et al.*, 2021), there is a need for its sustainable management. Motitsoe *et al.* (2020) showed recovery of an aquatic ecosystem following control of *Salvinia molesta* D.S. Mitch. (Salviniaceae) through monitoring algae and macroinvertebrate communities. Thus a similar approach might be taken for *R. pseudoacacia* using arthropod community assembly. While not all arthropod taxa observed in this study were affected by the physical and structural changes induced by the IAP, some showed enough sensitivity to enable their monitoring as indicators. For these taxa, before and after abundances can be used to assess the success of any control programme against *R. pseudoacacia*.

CHAPTER 3

Reduction of grazing capacity in high elevation rangelands following Black locust invasion in South Africa*

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3.1 Introduction

Despite social and economic benefits, globalisation has brought new challenges, notably the proliferation of transboundary movement of invasive species (Pysek & Richardson, 2010). Some of the invasive alien species are ecosystem transformers and drivers of environmental change (Rilov *et al.*, 2012), severely disrupting ecosystem integrity and provision of services necessary for human wellbeing (Pysek & Richardson, 2010). For instance, invasive alien plants (IAPs) can substantially impact native vegetation (Hejda *et al.*, 2009; Vitkova *et al.*, 2020), thereby threatening biodiversity (Gaertner *et al.*, 2009).

Upon establishment, IAPs disrupt complex ecosystem interactions leading to changes in the structure and function of ecosystems (Wardle & Peltzer, 2017). Hence, IAPs ultimately lead to shifts in the composition of native communities due to loss of habitats or environmental modifications (Rilov *et al.*, 2012). For example, invasion by nitrogen-fixing trees enriches soil nitrogen levels filtering out non-nitrophilous native plants in historically nutrient poor ecosystems (Benesperi *et al.*, 2012). This has been shown in South Africa where Australian acacias have been widely associated with a decline in native vegetation communities due to nitrogen enrichment in nutrient poor soils of the fynbos biome (Witkowski, 1991; Musil, 1993; Le Maitre *et al.*, 2011).

The high elevation rangelands of South Africa were defined by Mucina *et al.* (2006) as herbaceous, relatively short and simple structured vegetation dominated by Poaceae graminoids. This biome is regarded as a key biodiversity hotspot with high plant endemism (Clark *et al.*, 2014). However, biodiversity is increasingly affected by IAPs (Carbutt, 2012). Although native species diversity has been identified as a barrier to invasion (Kennedy *et al.*, 2002), rangelands present reduced resistance to invasion by tall woody species which often dominate and cover native species (Hejda *et al.*, 2009). Van Wilgen *et al.* (2008) identified the

rangelands as one of the terrestrial ecosystems that require alien plant control primarily to conserve biodiversity. The biome is also an important resource supporting livestock production where invasion driven changes in native flora has in some cases altered the grazing capacity of rangelands (Yapi *et al.*, 2018).

Despite an increasing body of knowledge on the impacts of IAPs and their management, (Richardson & van Wilgen, 2004; van Wilgen *et al.*, 2012; van Wilgen *et al.*, 2020), the spatial extent of invasion and number of invading plants continues to evolve. One of the emerging IAPs in the high elevation rangelands of South Africa is *Robinia pseudoacacia* L. (Fabaceae, Papilionoideae) (Henderson, 1991; Martin, 2019). The tree is native to North America and has now been declared invasive in 21 countries worldwide (CABI, 2020). It can proliferate under a variety of environmental conditions (Nicolescu *et al.*, 2018) and transforms the environment through shading (Haerdtle *et al.*, 2003; Cierjacks *et al.*, 2013) and soil nitrogen fixation (Dzwonko & Loster, 1997; Vitkova *et al.*, 2017; Campagnaro *et al.*, 2018). Its ability to fix nitrogen often leads to the proliferation of nitrophilous understory vegetation (Benesperi *et al.*, 2012) and as the soil nitrogen increases over time, transient colonization, followed by competitive displacement occurs (Dzwonko & Loster, 1997; Lazzaro *et al.*, 2018). In South Africa, *R. pseudoacacia* is listed as a category 1b invasive alien plant under the National Environmental Management: Biodiversity Act (NEM: BA) (DEA, 2016). Category 1b invasive alien plants are those whose unregulated importation and propagation is prohibited.

Increasing invasion of rangelands by *R. pseudoacacia* in South Africa has the potential to reduce the availability of grazing. Potential economic losses from invasion of rangelands by this IAP in South Africa alone were estimated to be approximately US\$39 million (Humphrey *et al.*, 2019). Woody IAPs create novel conditions in rangelands that are unfavourable for native species (O'Connor & van Wilgen, 2020). This can lead to species displacement and a

reduction in the grazing capacity of rangelands, especially when low palatability forage species take over (Bankovich *et al.*, 2016; O'Connor & van Wilgen, 2020).

The impacts of *R. pseudoacacia* on native biodiversity in South Africa remain poorly understood as evidence of its impacts are mainly anecdotal. A paucity of data on the tree was also noted in the southern hemisphere as compared to the northern hemisphere (Martin, 2019). However, extrapolation of findings from studies of *R. pseudoacacia* in the northern hemisphere is difficult as there are often variations in the extent of invasion, the environment, and taxonomic groups affected (Clusella-Trullas & Garcia, 2017).

To understand and adequately respond to the challenges of IAPs, there is need to assess impacts on a continuum linking plant traits, ecosystem processes, and services (Walker & Smith, 1997; Parker *et al.*, 1999). This is critical in defining management goals that are specific and benchmarking future evaluation of interventions. The present study therefore investigated the impacts of *R. pseudoacacia* on native plant communities and cascading effects on the grazing capacity of montane rangelands in eastern Free State Province, South Africa. The aims were to determine 1) if *R. pseudoacacia* is driving changes in the richness and composition of grass and forb communities, 2) if there are any relationships between vegetation community dynamics and *R. pseudoacacia* stand characteristics, and 3) whether there are any changes in the grazing capacity of rangelands as a result of invasion.

3.2 Materials and Methods

3.2.1 Study site

The study was conducted at Clifton (28° 32' 48.08" S; 28° 25' 08.91" E) and Sunnyside (28° 32' 12.87" S; 28° 31' 59.60" E) properties in the Eastern Free State Province, South Africa, between February 2019 and February 2020. This area falls under the subtropical highland climate (Cwb) according to the Köppen-Geiger climate classification with temperatures averaging 13.7 °C

and an average annual rainfall of 693 mm (Beck *et al.*, 2018). Vegetation in this area is mainly composed of grasses with climax indigenous species such as *Themeda triandra* Forssk. (Poaceae) and *Cymbopogon dieterlenii* Stapf ex Schweick. (Poaceae) dominating (Du Preez & Venter, 1992). However, there is an increasing incidence of woody species, most of which are non-native (Carbutt, 2012). Agriculture is the primary land-use within this region with livestock, cereal and fruit production as the major enterprises (Hensley *et al.*, 2006).

3.2.2 *Robinia pseudoacacia* stand characteristics

Stand characteristics based on one population parameter, density (trees/hectare), and one plant level measurement, Diameter at Breast Height (DBH) were assessed for four tree stands, two at Clifton and two at Sunnyside properties. Sampling was done within three 10m × 10m quadrats established 10m apart along 50m transects randomly selected within each stand. The number of trees in each quadrat was counted and DBH of each tree was measured at approximately 1.3m from the soil surface using a steel diameter tape (West, 2009). For forked trees, measurements were taken just below the fork whilst for multi-stemmed trees, the base was measured. To measure variation in light penetration through *R. pseudoacacia* canopy, hemispherical images were captured from 10 randomly selected locations in each of the four stands. The images were captured using a 170°, Voyager Explorer® 4K Ultra HD activity camera in February 2020 when *R. pseudoacacia* trees were in full leaf and on a cloudy day to avoid the effects of the sun. The images were then transferred to a Samsung Galaxy J2 Core smartphone for determination of canopy cover indices using the mobile application, Gap Light Analysis Mobile Application (GLAMA) (Tichy, 2016). The application distinguishes between dark and light pixels from the canopy hemispherical images, classifying them as either vegetation or sky in the process. The Canopy Cover index (CaCo), a projection of canopy cover onto the surface, is then calculated and expressed as a percentage (Fig. 3.1) (Tichy, 2016).

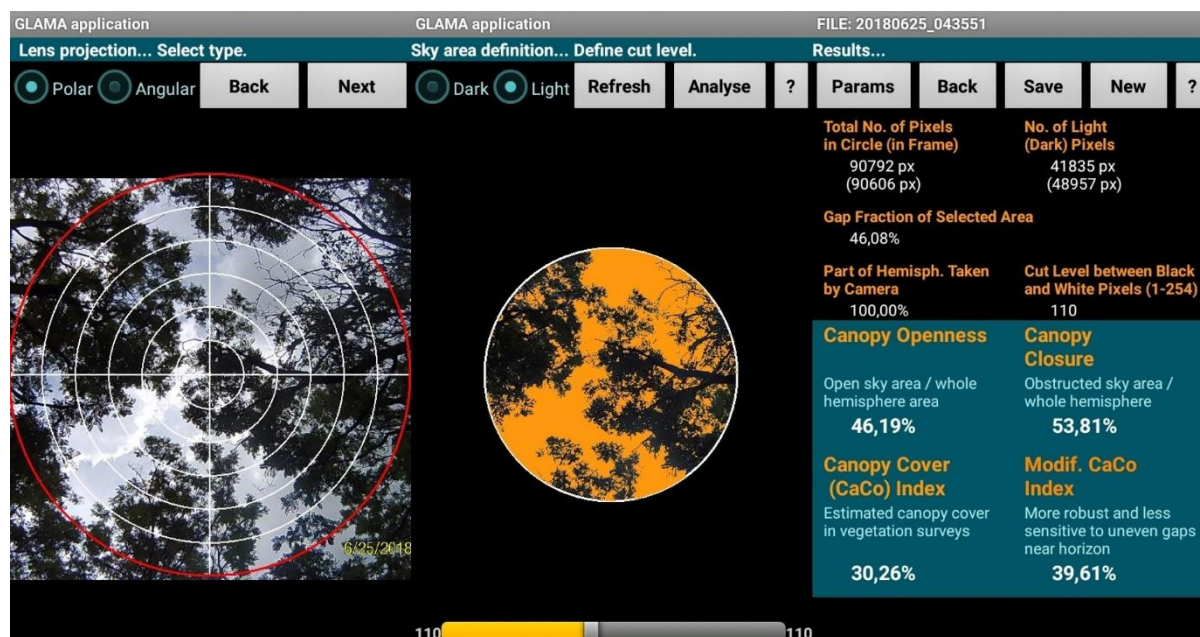


Figure 3.1: Determination of *Robinia pseudoacacia* Canopy Cover (CaCo) index using Gap Light Analysis Mobile Application (GLAMA).

3.2.3 Understory vegetation composition

These surveys were conducted between December 2019 to February 2020 when most of the grasses were flowering for ease of identification. Sampling was done in two habitats, patches of *R. pseudoacacia* (n=3) (hereafter ‘invaded’), and in adjacent open rangeland (n=3) (hereafter ‘uninvaded’). The fourth site, Sunnyside 4 had no vegetation hence it was excluded from these surveys. Invaded and uninvaded sites 50m apart were surveyed, while sampling within invaded sites was done at least 5m from the edge to minimize edge effects. Pairing of sites was done to ensure similar environmental conditions. Although uninvaded sites chosen were used as pasture, care was taken to select areas where there was no history of soil disturbance based on property owners’ accounts. Invaded and uninvaded sites were exposed to the same grazing pressure by livestock. The line-point intercept method was used to estimate plant species cover and composition. The method uses a length of non-stretch rope marked at 1m intervals and a handheld rod. Starting at zero, and working from left to right and on the same side of the line

each time, one moved to the first point on the line and dropped a rod from a height of 50cm without guiding it to the ground. Once the rod reached the ground, all plant species it intercepted in descending order were recorded from top to ground cover. (Herrick *et al.*, 2005). Sampling was done at each site along 4 transects, 50m long and 5m apart, giving a total of 200 points per site, and a total of 1200 points in both invaded and uninvaded habitats. Plants were grouped into two functional types i.e. grasses and forbs (non-graminoid herbaceous plants) and these were identified to species level by taxonomists with the aid of guides by Moffett (1997) and Van Oudtshoorn (1999).

3.2.4 Invaded and uninvaded rangeland condition similarities

The total number of top canopy strikes for each species was calculated as a percentage of total observations for the species. Grazing index values (GIV), which range from 0 (low quality) to 10 (high quality) for grasses sampled in this survey were obtained from lists compiled by Esler *et al.* (2006). Grazing index values are determined using volumes, amount of graze-able dry matter and chemical composition of the specific species (Du Toit, 1995). For species without listed GIVs, the average for two phylogenetically close species was used. Range condition indices were then calculated for both habitats by summing the product of the percentage cover of species and their GIVs (Du Toit, 1995).

3.2.5 Statistical analyses

Data analyses were carried out using STATISTICA, version 7 (TIBCO Software), R software version 3.2.1 (R Development Core Team, 2020), and Paleontological Statistics Software Package (PAST) (Hammer *et al.*, 2001). *Robinia pseudoacacia* canopy cover, density and DBH were analysed using one-way analysis of variance (ANOVA) in STATISTICA and Tukey-Kramer *post hoc* tests were used to separate statistically significant groups. Vegetation data from invaded and uninvaded transects were grouped into two functional groups, grasses and

forbs, and tested for sampling saturation using the Mao tau sample-based rarefaction method at 95% confidence interval in PAST. Since the curve for forb sampling did not reach an asymptote and preliminary analyses resulted in a high stress value (>0.2), the data was not subjected to distance based analyses (Fig. 3.2). To visualize the separation of grass communities between invaded and uninvaded plots, non-metric multidimensional scaling (nMDS) was performed and the Bray-Curtis distance was applied using the “vegan” package (Oksanen *et al.*, 2019) in R. Permutational Multivariate Analysis of Variance (PERMANOVA) was then used to test for differences in grass community composition between the invaded and uninvaded sites. To explore the contribution of each species to dissimilarity between sites, similarity percentages (SIMPER) were computed also using the Bray-Curtis distance. To test variation in species composition between the two habitats, the data was tested for the assumption of homogeneity of multivariate dispersion (Anderson, 2006).

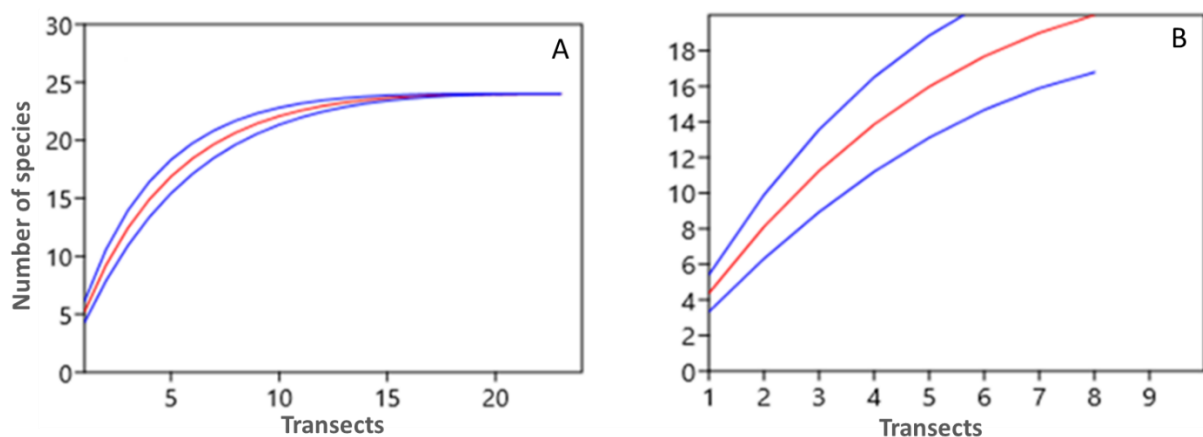


Figure 3.2: Sampling saturations curves based on Mao tau sample-based rarefaction method (number of species $\pm$ 95% confidence limit) for grasses (A) and forbs (B) at Clifton and Sunnyside properties in eastern Free State, South Africa.

Differences in Range Condition Index were analysed using a Linear Mixed Model (LMM) specified with gaussian errors and a log lik function (Bolker *et al.*, 2009), with site (Clifton/Sunnyside) and habitat (invaded/uninvaded) as fixed effects. Quadrat and transect

were included as random effects to account for potential non-independence between data points (Bolker *et al.*, 2009). A global (maximal) model was specified including an interaction term between site and habitat, and both random effects. The ‘*dredge*’ function from ‘*MuMIn*’ R package (Barton, 2019), which specifies all possible combinations of fixed effects as possible models, was then used in a model selection framework to assess the importance of predictor variables. Model selection was performed by ranking all candidate models using Akaike’s information criterion corrected for small sample sizes (AICc). Moreover, Akaike weights (w_i) were calculated to determine the degree of support for each candidate model being the best model. The sign and magnitude of the effect of each model term on range condition index scores was assessed by semi-parametric bootstrapping of parameter estimates, using bootstrap replicates, implemented with the ‘*bootMer*’ function from the ‘*lme4*’ R package (Bates *et al.*, 2015).

Preliminary analyses showed that inclusion of transect as a random effect did not improve model fit when analysing Shannon-Weiner (H') diversity and percentage grass and forb cover variables. As such, Shannon-Weiner diversity was analysed using a GLM with gaussian errors and a log link function, and vegetation cover analysed using a logistic GLM with binomial errors and a logit link function. For both models, site (Clifton/Sunnyside) and habitat (invaded/uninvaded) were specified as fixed effects.

3.3 Results

3.3.1 *Robinia pseudoacacia* population demographics and canopy characteristics

Robinia pseudoacacia canopy cover varied significantly between stands (Table 3.1). However, results of *post-hoc* tests showed that there was no significant difference in canopy cover between three stands (Clifton 1, Clifton 2 and Sunnyside 1) whilst Sunnyside 4 had significantly higher canopy cover (> 60%) (Fig. 3.3). Similarly, tree densities varied significantly across stands (Table 3.1). Sunnyside 4 had the highest density of trees whilst the lowest densities were recorded at Sunnyside 1 (Fig. 3.3). Diameter at breast height (DBH) varied significantly across sites (Table 3.1) and the widest trees stems were found at Sunnyside 1 whilst stem diameter did not differ significantly at the other three sites (Fig. 3.3).

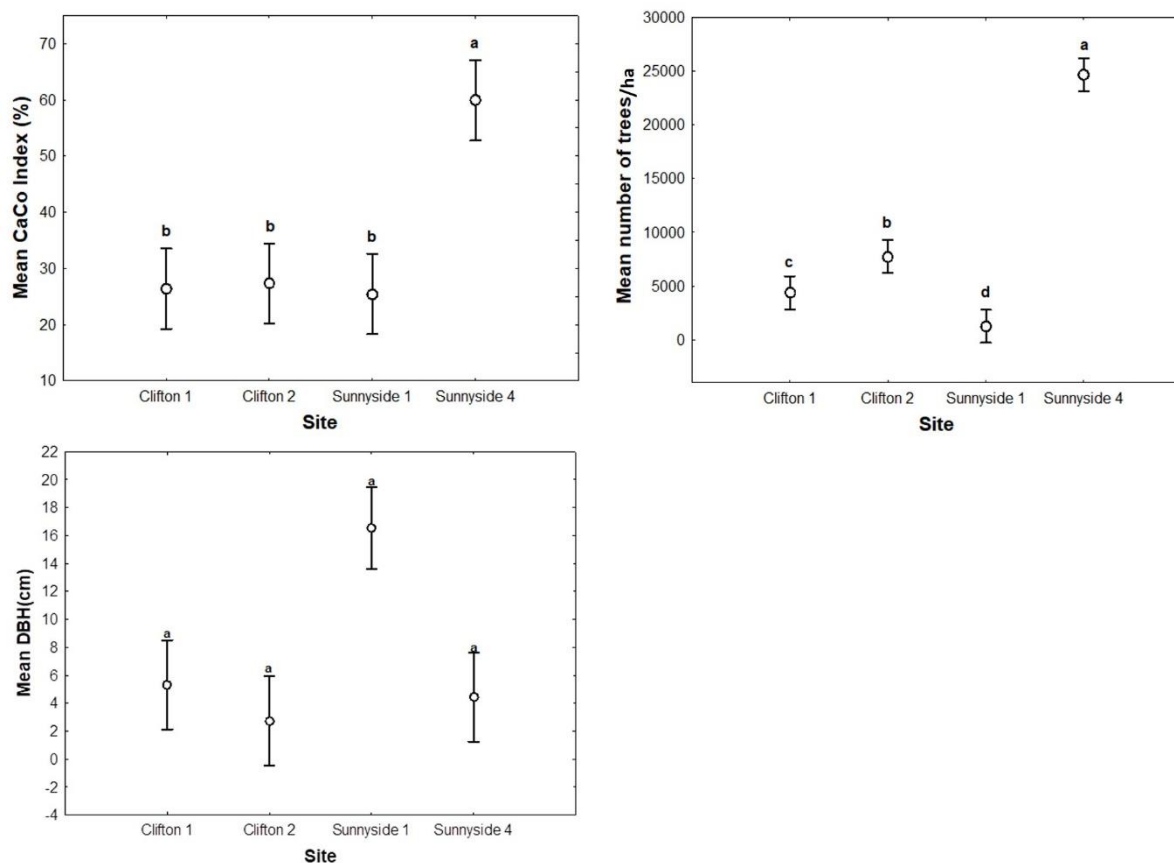


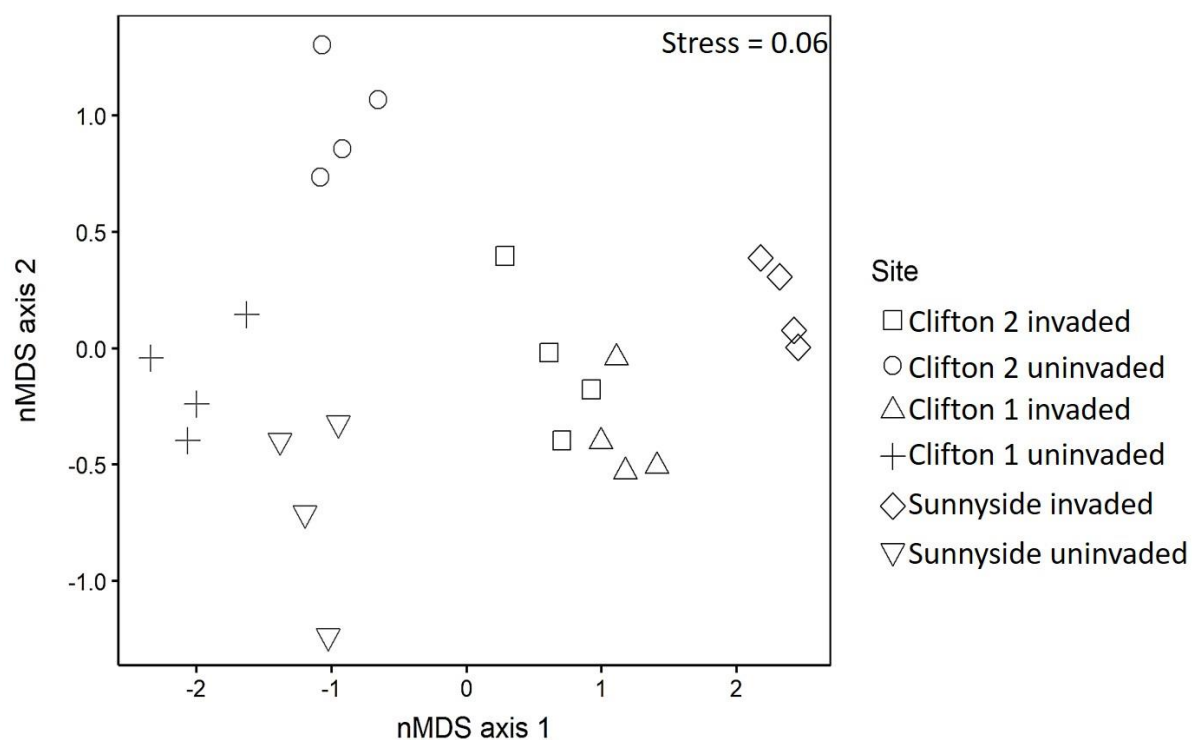
Figure 3.3: Differences in canopy cover (CaCo) (A), densities (B) and diameter at breast height (DBH) (C) of *Robinia pseudoacacia* across sites (mean $\pm$ 95% confidence limits). Means with the same letter are not significantly different from each other.

Table 3.1: Summary of ANOVA on canopy cover, tree densities and Diameter at Breast Height (DBH) of sampled *Robinia pseudoacacia* stands

Parameter	Effect	Ss	Df	Ms	F	p-value
Canopy cover	Intercept	48353.94	1	48353.94	388.01	< 0.001
	Site	8457.51	3	2819.17	22.62	< 0.001
	Error	4486.33	36	124.62		
Tree density	Intercept	2.17E+09	1	2.17E+09	665.06	< 0.001
	Site	1.96E+09	3	6.54E+08	200.35	< 0.001
	Error	6.53E+07	20	3.27E+06		
DBH	Intercept	1309.3	1	1309.3	93.00	< 0.001
	Site	791.00	3	263.90	18.74	< 0.001
	Error	295.67	21	14.08		

3.3.2 Effects of invasion on grass and forb species community composition

The nMDS plot showed that grass community composition differed between invaded and uninvaded rangeland with no overlaps between the two habitats (Fig. 3.4). The grass community in uninvaded rangeland was mainly composed of native species (e.g. *Tristachya leucothrix* and *Cymbopogon dieterlienii*) while exotic species such as *Dactylis glomerata* L. (Poaceae) and *Bromus catharticus* Vahl. (Poaceae) were dominant in habitats invaded by *R. pseudoacacia* (Fig. 3.5). There was a significant site (49%) and habitat (36%) effect ($p < 0.001$) in explaining the variation in grass community composition between the two vegetation communities (Table 3.2). Although the grass community uninvaded habitats seemed to be more dispersed than in invaded habitats, the homogeneity multivariate dispersion assumption was met ($F = 1.16, p = 0.29$). The SIMPER analysis further showed that dissimilarity between invaded and uninvaded rangeland was 96% of which more than 50% was due to *E. biflora* (14.3%), *B. catharticus* (13.4%), *Tristachya leucothrix* (12.3%), *Cymbopogon dieterlenii* (10.2%) (Table 3.3). The two native grasses common to both habitats, *C. dieterlienii* and *T. triandra*, contributed significantly to differences in community composition between invaded and uninvaded habitats ($p < 0.05$).



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1039 **Figure 3.4:** Two-dimensional non-metric multidimensional scaling (nMDS) plot showing
 1040 grass species composition in plots invaded by *Robinia pseudoacacia* and adjacent uninvaded
 1041 rangeland based on Bray-Curtis distance.

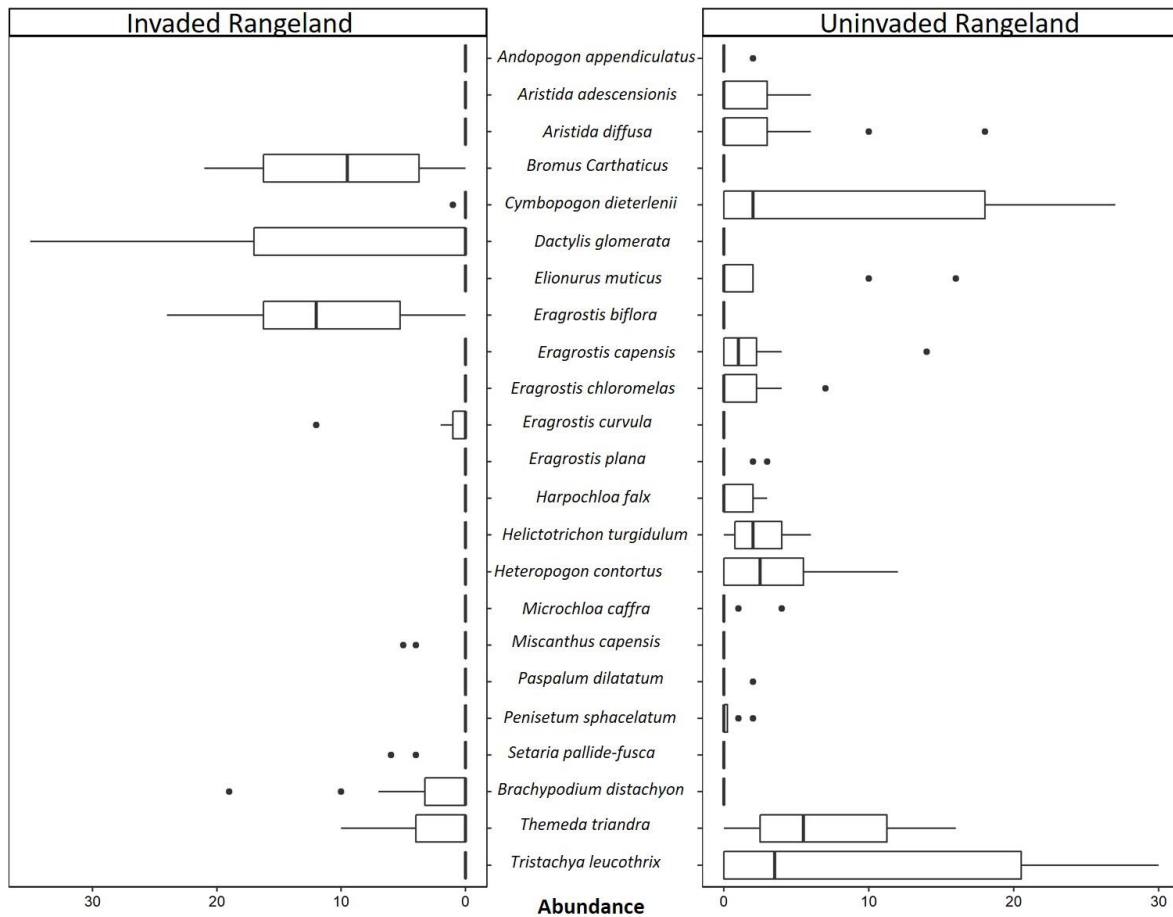


Figure 3.5: Grass species abundance in uninvaded rangeland and habitats invaded by *Robinia pseudoacacia* in eastern Free State, South Africa.

Table 3.2: Permutational multivariate analysis of variance (PERMANOVA) on grass communities under *Robinia pseudoacacia* stands and uninvaded veld

Effect	Df	SS	MS	F	R ²	p-value
Habitat	1	2.97	2.97	41.23	0.36	< 0.001
Site	5	3.10	0.80	11.08	0.49	< 0.001
Residuals	17	1.23	0.07		0.15	
Total	23	8.19			1	

Table 3.3: Similarity percentages for grasses between open rangeland and under *Robinia pseudoacacia* stands based on Bray-curtis distance

Species	Average	SD	Ratio	Mean Veld	Mean Invaded	Cumulative sum	p - Value
<i>Eragrostis biflora</i> Hack. ex Schinz (Poaceae)	0.14	0.10	1.34	0	10.67	0.14	0.001
<i>Bromus catharticus</i> Vahl. (Poaceae)*	0.13	0.10	1.32	0	10.33	0.28	0.001
<i>Tristachya leucothrix</i> Trin.(Poaceae)	0.12	0.14	0.86	10.08	0	0.40	0.005
<i>Cymbopogon dieterlenii</i>	0.1	0.12	0.80	8.00	0.08	0.50	0.003
<i>Dactylis glomerata</i> L. (Poaceae)*	0.09	0.14	0.66	0	8.25	0.60	0.083
<i>Themeda triandra</i>	0.07	0.06	1.33	6.67	2.17	0.68	0.044
<i>Heteropogon contortus</i> L. (Poaceae)	0.05	0.05	0.90	4.00	0	0.73	0.003
<i>Aristida diffusa</i> Trin. (Poaceae)	0.04	0.07	0.57	3.08	0	0.76	0.018
<i>Brachypogon distachyon</i> (L.) P. Beauv. (Poaceae)*	0.04	0.07	0.54	0	3.17	0.80	0.158
<i>Elionurus muticus</i> (Spreng.) Kuntze. (Poaceae)	0.03	0.06	0.54	2.67	0	0.83	0.012
<i>Helictotrichon turgidulum</i> (Stapf) Schweick.(Poaceae)	0.03	0.02	1.24	2.25	0	0.86	0.001
<i>Eragrostis capensis</i> (Thunb.) Trin. (Poaceae)	0.03	0.04	0.58	2.17	0	0.89	0.005
<i>Aristida adescensionis</i> L.(Poaceae)	0.02	0.04	0.62	1.50	0	0.91	0.001
<i>Eragrostis curvula</i> (Schräd.) Nees. (Poaceae)	0.02	0.05	0.42	0	1.42	0.93	0.140
<i>Eragrostis chloromelas</i> Steud. (Poaceae)	0.02	0.03	0.61	1.33	0	0.95	0.031
<i>Harpochloa falx</i> (L.f.) Kuntze. (Poaceae)	0.01	0.02	0.80	0.92	0	0.96	0.001
<i>Setaria pallide-fusca</i> (Schumach.) Stapf and C.E. Hubb. (Poaceae)	0.01	0.03	0.43	0	0.83	0.97	0.364
<i>Miscanthus capensis</i> (Nees) Andersson. (Poaceae)	0.01	0.02	0.44	0	0.75	0.98	0.420
<i>Microchloa caffra</i> Nees. (Poaceae)	0.01	0.01	0.37	0.42	0	0.99	0.074
<i>Eragrostis plana</i> Nees. (Poaceae)	0.01	0.01	0.44	0.42	0	0.99	0.088
<i>Penisetum sphacelatum</i> (Nees) T.Durand and Schinz (Poaceae)	0.01	0.01	0.55	0.33	0	1.00	0.001
<i>Paspalum dilatatum</i> Poir. (Poaceae) *	0.00	0.01	0.30	0.17	0	1.00	0.130
<i>Andropogon appendiculatus</i> Nees. (Poaceae)	0.00	0.01	0.30	0.17	0	1.00	0.138

*Non-native grasses

Grass species diversity varied significantly between uninvaded and habitats invaded by *R. pseudoacacia* ($F = 29.41$, $p < 0.001$), but not sites ($F = 2.10$, $p = 0.152$). There was no significant interaction effect between habitat and site ($F = 1.06$, $p = 0.366$). Results of *post-hoc* tests showed that grass species diversity was significantly higher in uninvaded rangeland at Clifton (Fig. 3.6). While grass species diversity at Sunnyside was marginally higher in uninvaded than in invaded sites, the differences were not statistically significant. On the

contrary, forb species were more diverse in sites invaded by *R. pseudoacacia* at Clifton than uninvaded and invaded sites at Sunnyside (Fig. 3.6). The effect of site contributed significantly to variation in forb diversity ($F = 11.71, p = 0.001$). All forb species in invaded sites were early successional invasive alien species as compared to natives in uninvaded habitats.

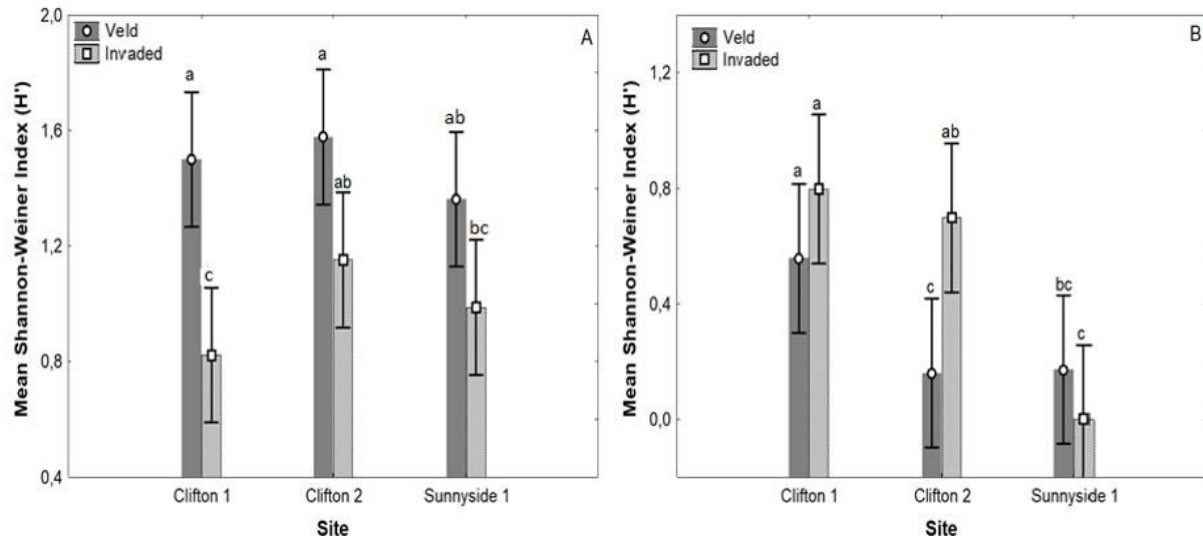


Figure 3.6: Differences in grass (A) and forb (B) diversity (Shannon-Weiner index) (mean $\pm$ 95% confidence limits) between uninvaded rangeland and habitats invaded by *Robinia pseudoacacia* in eastern Free State, South Africa. Means with the same letter are not significantly different from each other.

3.3.3 Effects of invasion on rangeland grazing index values

Site, habitat and the interaction between site and habitat were all statistically significant in explaining range condition index values (Table 3.4). Range condition was significantly lower in invaded habitats (180 ± 24.3) than uninvaded rangeland (401 ± 24.3) at Clifton. However, these differences were less noticeable at Sunnyside where range condition, in both invaded (432 ± 34.4) and uninvaded habitats (450 ± 34.4), was better than both invaded and uninvaded habitats at Clifton. *Dactylis glomerata* which had the highest grazing index value of 7.59

occurred under *R. pseudoacacia* at Sunnyside while in all uninvaded sites *T. triandra* which was most abundant had a grazing value of 6.81.

Vegetation cover also followed a similar trend as it was significantly higher in uninvaded than invaded habitat at Clifton while at Sunnyside rangeland had slightly higher but insignificant cover than invaded habitat (Fig. 3.7). Habitat had a significant influence on grass cover ($F = 24.03, p < 0.001$).

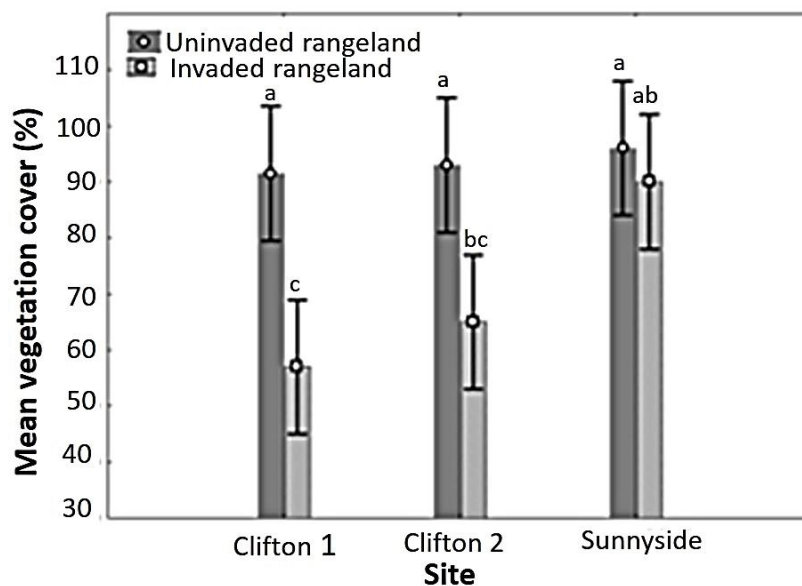


Figure 3.7: Differences in vegetation cover (mean $\pm$ 95% confidence limits) between uninvaded rangeland and habitats invaded by *Robinia pseudoacacia* in eastern Free State, South Africa. Means with the same letter are not significantly different from each other.

Table 3.4: Linear Mixed Model selection matrix based on semi-parametric bootstrapping of parameter estimates for Range Condition Index values in open rangeland and habitats invaded by *Robinia pseudoacacia* at Clifton and Sunnyside properties in eastern Free State, South Africa.

Model structure	K	AICc	ΔAICc	LogLik	w _i
Site * Habitat	7	244.7	0.00	-111.85	1.00
Site + Habitat	6	264.1	18.40	-123.58	0.00
Habitat	5	275.0	30.30	-130.83	0.00
Site	5	286.6	41.90	-136.65	0.00
Random effects only	4	297.8	53.10	-143.82	0.00
Null model ^a	2	304.58	59.88	-150.00	0.00

^a Null model = ~1 (random intercept model)

3.4 Discussion

This study highlights the impact of *R. pseudoacacia* on plant community dynamics in rangelands. There was a reduction in abundance and diversity of native grasses followed by succession by alien grasses and forbs thereby reducing the grazing capacity of the rangeland. Emergent communities following invasion were dominated by nitrophilous and shade-tolerant species, demonstrating the impact of *R. pseudoacacia* on microclimates. To our knowledge, this is the first study assessing the impacts of this species on native plant community composition and associated ecosystem services in South Africa and the southern hemisphere.

3.4.1 *Robinia pseudoacacia* population demographics and canopy characteristics

Overstory vegetation characteristics influence understory vegetation communities (Ali *et al.*, 2019). In this study, we observed an influence of canopy cover on light availability and subsequently understory vegetation communities. Other studies noted the influence of the density of invasive alien species such as Australian *Acacias*, *Prosopis* species and *R. pseudoacacia* on understory vegetation locally (Ndhlovu *et al.*, 2011; Yapi *et al.*, 2018) and in

Europe (Benesperi *et al.*, 2012). To further affirm this, we did not find any significant undergrowth at Sunnyside 4 which had the highest canopy cover and density, demonstrating the importance of light availability on understory vegetation dynamics. Tree diameter can be used as a proxy for tree age (McElhinny *et al.*, 2005). Hence, we suggest that stand age influences successional dynamics in understory vegetation. Campagnaro *et al.* (2018), also found an influence of stand successional dynamics and other stand characteristics such as stand basal area, tree diameter and stand vertical structure, on understory vegetation communities. It is therefore plausible that vegetation dynamics in our study were as a result of invasion.

3.4.2 Effects of *R. pseudoacacia* invasion on grass species composition

In the present study, *R. pseudoacacia* invasion led to a marked reduction in native grass and forb diversity, consistent with studies on the same species in Europe (Benesperi *et al.*, 2012; Sitzia *et al.*, 2012) as well as other invasive alien species such as *Pereskia aculeata* (Hejda *et al.*, 2009; Paterson *et al.*, 2011). These changes could be a result of its active nitrogen fixation activity and deposition of nitrogen-rich leaf litter (Cierjacks *et al.*, 2013), which leads to the recruitment of nitrophilous grasses and forbs. In nutrient poor soils, such nitrogen enrichment gives *R. pseudoacacia* a competitive advantage over natives adapted to nutrient poor soils (Rawlik *et al.*, 2018). Indeed, grass species found underneath *R. pseudoacacia* stands in our study, *D. glomerata*, *B. catharticus* and *E. biflora* reportedly have a high affinity for nitrogen (Van Oudtshoorn, 1999) underlying its role in understory composition. By contrast, other studies, despite linking *R. pseudoacacia* to nitrophilous understory vegetation, suggested that the tree contributes to biodiversity after association with equally species rich vegetation communities (Campagnaro *et al.*, 2018; Slabejova *et al.*, 2019). However, in our study although understory vegetation at Sunnyside appeared to be as diverse as in uninvaded habitat, it was composed of alien instead of native species.

Our study showed that invasion of rangelands by *R. pseudoacacia* leads to the succession of native grass communities by mainly alien taxa. This indicates that invasive alien species can act as drivers for further invasion by other alien species. Even though the sources of the alien understory vegetation in this biome are not clear, we posit that some of the grasses could have escaped from planted pastures whilst the rest of forb species are generally weedy species transported inadvertently through various vectors, primarily birds. Tecco *et al.* (2006), also recorded higher richness of exotic species under an invasive alien tree, *P. angustifolia* than *Condalia montana* A. Cast. (Rhamnaceae), a native plant. These results also indicate approximately 100% species turnover due to invasion and in keeping with this observation, Vitkova *et al.* (2020) recorded a 20% decline in the frequency of native species under *R. pseudoacacia* stands in xeric rangelands of Southern Europe. Whilst there may be variations in the composition of understory vegetation, our results are consistent with findings by Sitzia *et al.* (2018) in Mediterranean lowlands of Europe that generally *R. pseudoacacia* stands are associated with aliens, ruderals and habitat specialists. Invasion by *R. pseudoacacia* therefore creates conditions necessary for the recruitment of these alien species from incoming invaders due to creation of enabling niches. Moreover, there was a shift in composition of grass species which follow the C₄ carbon fixation pathway, which are common in this biome as the case with uninvaded plots, to those following the C₃ pathway in this study. C₄ grasses are adapted and highly competitive under high light and temperature intensities and also thrive best in low nitrogen soils (Taylor *et al.*, 2010). All the 16 native grasses recorded in this study are adapted to the C₄ pathway whilst exotic species, found under *R. pseudoacacia* all follow the C₃ pathway (Milton, 2004), suggesting an influence of the tree in the recruitment of species with special adaptations different from natives.

Robinia pseudoacacia is a light demanding and fast-growing species that creates light deficient conditions suitable for the shade tolerant species in the understory (Cierjacks *et al.*, 2013). It is therefore possible that shade tolerance could have played a role in the grass assemblages underneath *R. pseudoacacia* in our study. For example, *D. glomerata* that we recorded was previously associated with shade tolerance in a silvi-pastoral agroecosystem, where its nutritional value increased with shading (Kyriazopoulos *et al.*, 2012) indicating its adaptation to low illumination conditions. Shading was also found to shape grass communities underneath another invasive alien plant, *Chromolaena odorata* R.M.King & H.Rob. (Asteraceae) (Te Beest *et al.*, 2015). Our results therefore suggest that, in all likelihood, the interplay between shading and nitrogen fixation influences the emerging communities underneath *R. pseudoacacia* stands and the reduction of native grass and forb diversity.

3.4.3 Effects of invasion on grazing quality

Invasion of rangelands by alien taxa has been associated with a corresponding reduction in grazing capacities, affecting livestock enterprises, which are largely dependent on rangelands (Palmer & Ainslie, 2005; Suttie *et al.*, 2005). In this study, there was a marked reduction in range condition at one of the properties. Consistent with these findings, several studies have linked the reduction in grazing capacity to invasive alien species locally and globally (Duncan *et al.*, 2004; Ndhlovu *et al.*, 2011; Yapi *et al.*, 2018) and potential reduction in gross margins (Humphrey *et al.*, 2019). Through quantification of the increased losses in grazing capacities of the invaded rangelands our study further demonstrates how invasive species can compromise livestock production. While overstocking can also drive rangeland degradation and alter grass community composition (Van der Merwe *et al.*, 2018), our habitats were subjected to the same grazing intensities. Hence, we suggest that differences in community composition and grazing capacity observed in this study were a result of invasion by *R. pseudoacacia*. High range

condition indices under *R. pseudoacacia* at Sunnyside were likely influenced by the dominance of *D. glomerata*, a species with high grazing value, high understory vegetation cover and low tree densities. However, this good condition might be offset if considered together with Sunnyside 4, one of the invaded sites on the property without understory vegetation possibly due to high tree density and low light penetration. Not only is *R. pseudoacacia* capable of reducing grazing capacity through alteration of grass communities, but it also takes up grazing space and is also poisonous to livestock (Humphrey *et al.*, 2019).

3.4.1 Implications

The current study highlights the negative impacts of *R. pseudoacacia* in rangelands. Invasion mediated replacement of native grass communities by alien grasses and forbs reduces the grazing capacity of the rangelands. Given these adverse effects, urgent control measures together with ecological restoration are required. With property owner reports indicating failure of chemical and mechanical control due to the vigorous coppicing capabilities of *R. pseudoacacia*, biological control offers better prospects for successful management of this invasive species. Furthermore, to allow for predictable successional trajectories as suggested by Sheley & Krueger-Mangold (2003), long term management approaches should be adopted. While integrated approaches which promote desirable species while suppressing the proliferation of undesirable ones are recommended (DiTommaso *et al.*, 2017), there are no prescribed methods which are effective against *R. pseudoacacia*. Moreover, control of the IAP through utilization as a browse species is curtailed by its toxicity to animals. Elimination of *R. pseudoacacia* might be possible through aggressive mechanical techniques which involve the complete destruction of the rooting system. However, this form of management besides its practical limitations at larger scales, introduce further disturbance leading to invasion by secondary weeds. Overall, there is need to develop a sustainable management regime for *R.*

1207 *pseudoacacia* and other invasive species with similar functional traits. In the meantime,
1208 rangeland managers should focus on early detection and prevention while those with existing
1209 infestations should minimize mechanical disturbance to limit rapid spread.

1210

CHAPTER 4

**Integration of invasive tree, Black locust, into agro-ecological flower visitor
networks induces competition for pollination services***

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4.1 Introduction

Pollination ensures reproductive success in most flowering plants and thus contributes to terrestrial ecosystem functioning (Daniels & Arceo-Gomez, 2019; Keherberger & Holzschuch, 2019). Many flowering plants are insect-pollinated hence they require pollinator visits for successful seed set (Sheffield, 2014). In agricultural crop production, entomophilous pollination is a key ecosystem service that improves yields (Klein *et al.*, 2007; FAO, 2018). However, a number of factors including climate variability, land-use change, and invasive alien species may disrupt insect-mediated pollination through modification of bottom-up and top-down factors (Chidawanyika *et al.*, 2019). For example, invasive alien plants (IAPs) can displace the resources available for native insects, including pollinators, leading to a decline in the population and available services provided by these insects (Litt *et al.*, 2014). Furthermore, the differential abundance and quality of floral resources may be modified such that interactions between available pollinators and native plants are disrupted through alteration of flower visitation rates (Gibson *et al.*, 2013; Daniels & Arceo-Gomez, 2019; Ojija *et al.*, 2019), inducing heterospecific pollen transfer (Albrecht *et al.*, 2014, Johnson & Ashman, 2019) and threatening pollinator health (Vanbergen *et al.*, 2018). Indeed, the literature is replete with studies showing changes in visitation rates and how IAPs impact pollinator visitation to native plants (Gibson *et al.*, 2013, Ojija *et al.*, 2019). It remains unclear whether such a shift is due to superior floral quality in both attractive odour and/ or subsequent nectar rewards in IAPs compared to native plants or just a matter of the limited pollinators being stretched over abundant plant resources.

Among ecosystems, the functional diversity and biotic interactions therein are key elements that determine ecosystem integrity and subsequent ecosystem service provision (Pysek & Richardson, 2010). Hence, understanding networks among functional groups such as plants-pollinators remains a key priority for conservation of beneficial taxa and their ecosystem

services (Bluthgen *et al.*, 2008; Bartomeus, 2013; Chidawanyika *et al.*, 2019). It is therefore not surprising that network metrics such as connectance and number of shared partners within trophic levels provide an indication of network stability (Rabeling *et al.*, 2019) and possibly conservation value (Heleno *et al.*, 2012).

Pollinators and flowering plants form intricate ecological interaction networks (Hansen *et al.*, 2018). As such, a number of plants have coevolved with their pollinators, with flower phenology, morphology and volatilome specifically adapted to attract certain pollinators (Steenhuisen *et al.*, 2010; Lazaro *et al.*, 2013; Daniels & Arceo-Gomez, 2019). However, there are also generalist flower visitors that can be lured by larger floral blooms and copious pollen rewards (Bartomeus *et al.*, 2008; Traveset & Richardson, 2014). Hence, co-flowering plants can share or compete for generalist pollinators. In consequence, co-flowering plants that are poor competitors for pollinators may receive reduced visitation rates and this can drive evolutionary shifts in flowering phenology (Mosquin, 1971). Furthermore, there is increasing evidence that for plant species which share pollinators, flowering earlier in the season than other plants is advantageous (Herbertsson *et al.*, 2017; Keherberger & Holzschuch, 2019).

Invasive alien plants owe their establishment success partly to high seed production rates emanating from large floral displays (Dawson *et al.*, 2009). In addition, some species form large monospecific patches with a huge abundance of flowers making them superior competitors for pollinators (Traveset & Richardson, 2014). For example, Hansen *et al.* (2018) reported a larger decrease of flower visitation rate among native plants following American bramble (*Rubus cuneifolius* Pursh) invasion compared to fragmentation in South African grasslands.

1283 Agroecosystems are characterised by low plant species diversity due to the general nature of
1284 land use, which involves frequent landscape disturbances that prioritise specific plants, leading
1285 to monocultures (Altieri, 1999). For pollinators, monocultures and fragmentation present a
1286 challenge of limited floral resources at various spatial and temporal scales leading to limited
1287 nutrition and ultimately population decline (Heller *et al.*, 2019). Moreover, indiscriminate
1288 application of agrochemicals in agroecosystems further induces stress on biodiversity (Goulson
1289 *et al.*, 2015) and a net decline in pollinators owing to their high sensitivity (Connolly, 2013;
1290 Aoun, 2020).

1291 Agroecosystems are mainly dominated by introduced plants which now constitute more than
1292 90% of world food crop production (Pimentel, 2005). Lack of diversity in these managed
1293 ecosystems and frequent disturbances often leads to susceptibility to IAP invasion and
1294 competition for resources (Lozon & MacIsaac, 1997; Harker *et al.*, 2005). Thus, invasion of
1295 pollinator-dependent agroecosystems has the potential to reduce food production due to
1296 competition and disruption of plant-pollinator networks. While additional floral resources may
1297 be necessary in provision of food and nesting for pollinators in agroecosystems, these need to
1298 be managed to avoid an overlap in blooms in order to reduce competition (Heller *et al.*, 2019).

1299 The black locust (*Robinia pseudoacacia* L.: Fabaceae), native to North America, is a growing
1300 threat to both natural and agroecosystems invading the Afromontane region of South Africa
1301 (Martin, 2019). Besides its known impacts on native plant diversity through alteration of the
1302 biophysical components of the ecosystem such as nitrogen levels and soil microbiota (Lazzaro
1303 *et al.*, 2018), its role in pollinator interaction networks remains underexplored. Locally, the
1304 tree has already shown potential to reduce livestock margins by up to US\$ 39 million per annum
1305 through invasion of rangelands (Humphrey *et al.*, 2019). Although *R. pseudoacacia* is
1306 widespread in South Africa, a lot is still unknown regarding its ecology under local conditions
1307 (Martin, 2019). Its large floral displays together with high quantities of nectar potentially attract

pollinators thereby affecting co-flowering plants (Ciejacks *et al.*, 2013). In some countries, such as Hungary, *R. pseudoacacia* is the major contributor to honey production, suggesting its role in attracting bees of the *Apis* genus (Sitzia *et al.*, 2016). Furthermore, various studies investigating the impacts of IAPs on pollinator visits are largely focused on native non-crop plants. Yet, disruption of crop pollination can have dire consequences on crop yield and profitability of farming together with food and nutritional security. Therefore, this study sought to determine diversity, abundance and interactions between flower visitors, as a proxy for pollination, on two co-flowering plants: the orchard plant *Malus domestica* L. Borkh. (apple), and an IAP, *R. pseudoacacia*. Specifically, we sought to answer the following questions (i) Which insect groups visit *M. domestica* and *R. pseudoacacia*? (ii) Is there competition between *R. pseudoacacia* and neighbouring co-flowering orchard plants for flower visitors? We hypothesise that there will greater diversity, abundance and interaction of flower visitors to *R. pseudoacacia* than *M. domestica*.

4.2 Materials and methods

4.2.1 Study Area

The study was conducted in October 2019 at Ionia Farm in the eastern Free State (20°46'49.93" S; 28°02'18.03" E), South Africa, during an overlapping flowering period for *R. pseudoacacia* and *M. domestica*. The area is located in the grassland biome (Mucina & Rutherford, 2006) and according to the Köppen-Geiger classification, the climate is subtropical highland (Cwb) with annual temperatures and rainfall averaging 13.7 °C and 693 mm respectively (Beck *et al.*, 2018). The Eastern Free State is an expanding fruit growing region which, according to a local bulletin (The Farmers Weekly), has ~400 ha under apple production. In order to supplement pollination, it is common for growers to hire bee colonies during the flowering period.

4.2.2 Study plant species

4.2.2.1 *Robinia pseudoacacia*

Robinia pseudoacacia is a leguminous tree, commonly known as Black locust, from the Fabaceae family, originating from the Appalachian Mountains in North America. The tree is now widely distributed across the globe with extensive populations in Europe and Asia (Brundu & Richardson, 2016). Its status as an invasive species varies with country although according to CABI (2020), 21 countries regard it as invasive. In South Africa, it is a category 1b invasive alien plant according to the National Environmental Management: Biodiversity Act (NEM: BA) 10 of 2004 . The tree produces white to cream fragrant flowers, in pendulous, many-flowered, axillary racemes (Cierjacks *et al.*, 2013). Flowering in the southern hemisphere occurs during spring, between September and October. Trees normally start flowering at 6 years and continue until approximately 40 years. However, biennial flowering is also common. *Robinia pseudoacacia* exhibits compensatory growth following disturbance through aggressive coppicing, which makes it difficult to manage through mechanical and chemical means.

4.2.2.2 *Malus domestica*

Malus domestica L. Borkh (Rosaceae), commonly known as apple is a plant originating from Tian Shan Mountains in Kazakhstan (Spengler, 2019). Flower initiation in the Eastern Free State of South Africa usually occurs for approximately three weeks, between September and October. However, flowering may be inhibited by nearby developing fruitlets resulting in biennial bearing (Eccher *et al.*, 2014). Flowers in *M. domestica* form clusters called corymbs, which are characterised by proportionally longer lower stalks so that the flowers form a flat or slightly convex head. Apples are obligate cross-pollinated and under commercial production systems, pollen donors (pollenizers) are interspaced with the main fruit trees. Inadequate

pollination can result in misshapen apples which take long to ripen, or immature fruits which drop early (Sheffield, 2014).

4.2.3 Plant-pollinator interaction and flower visitation

To determine plant-pollinator interactions, six 25 m² quadrats were randomly established, three over a patch of *R. pseudoacacia* and the other three in an orchard of *M. domestica* 100m away (Fig. 4.1). Both plant species were observed twice, once in the morning (08:00–12:00) and once in the afternoon (14:00–18:00), for 15 minutes over three clear and calm days when there was maximum arthropod activity. Two observers noted arriving flower visitor groups and their numbers in each quadrat. Only those visitors that contacted floral and reproductive parts of a flower during the 15-minute period were considered as potential pollinators (Gibson *et al.*, 2013). The first insect encountered for each visitor group was captured using either a sweep net or an aspirator, and preserved in labelled vials with 70% alcohol for later identification. Some flower visitors were also photographed for identification purposes. To determine flower visitation rates, the number of floral units for the two focal plant species was established. Visitation rates were then calculated as the average number of visits/number of open flowers/hour for every visitor group. This was done to avoid the bias of unequal flower numbers between the two plant species.

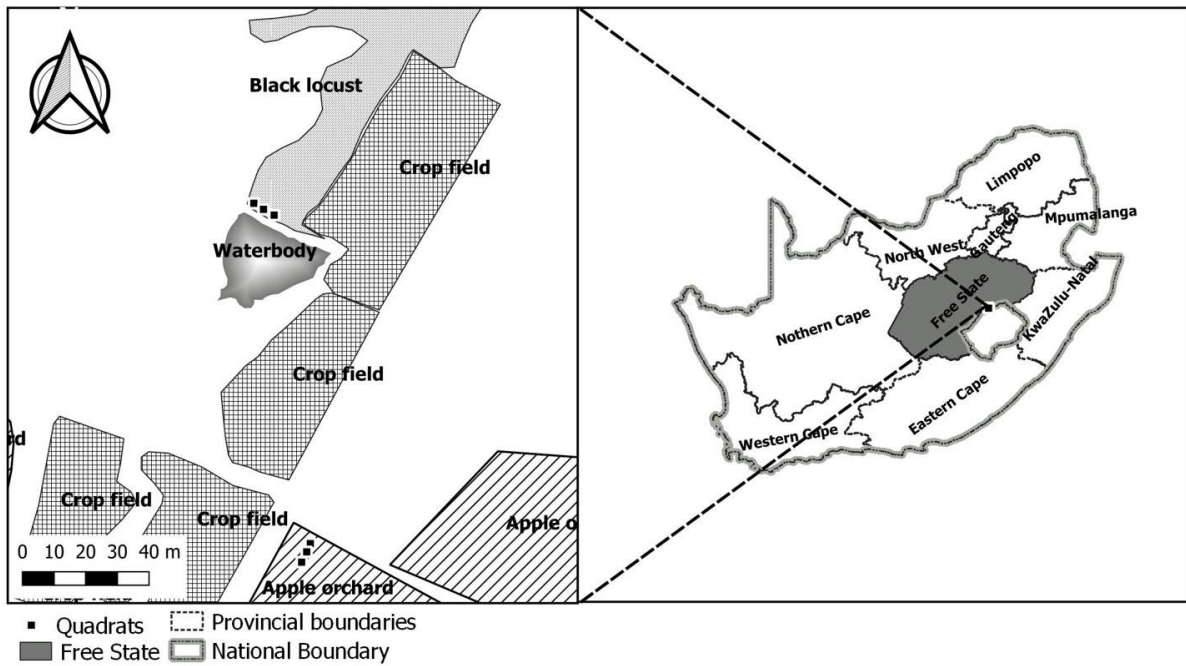


Figure 4.1: Map of the study area showing location of the apple orchards (*Malus domestica*) in relation to the Black locust (*Robinia pseudoacacia*) patches at Ionia in the eastern Free State, South Africa.

4.2.4 Statistical Analyses

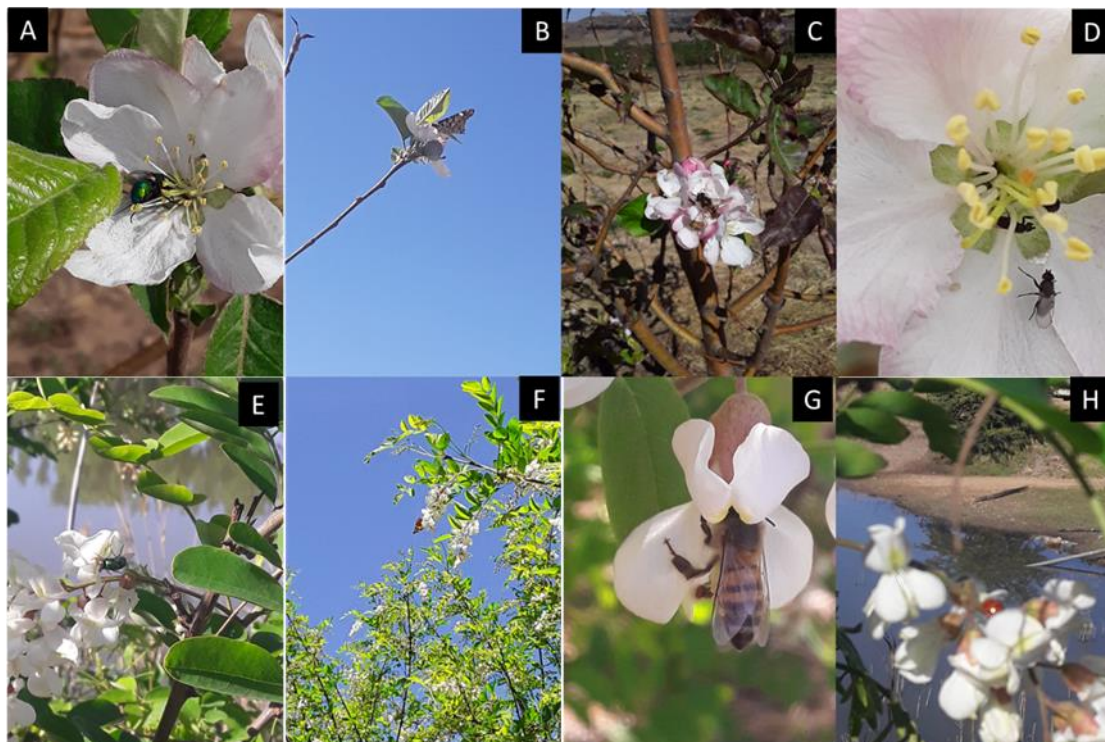
All data analyses were carried out using R software version 3.2.1 (R Development Core Team, 2020). Variation in flower visitation rates by different arthropod orders on the same plant species and across plant species were analysed using the non-parametric Kruskal –Wallis test as the data did not conform to normality. To further affirm the suitability of the test, the data were first subjected to Bartlett’s test with results showing unequal variances. Where the Kruskal-Wallis test indicated significant differences, post hoc tests were further performed to separate significantly different pairs using the Kruskal-Wallis multiple comparison p-values adjusted with the Bonferroni method or Dunn test using the R package, “FSA” (Dunn, 1964). Due to low abundance of some flower visitors, analyses were conducted at species level for the most prominent visitors, while some were grouped into families (e.g. Formicidae, Syrphidae and Bibionidae) and the rest were aggregated into orders.

To explore the interaction of flowers and visitors, a quantitative network was constructed for combined visitors observed on both sites using the Bipartite package in the R software (Dormann *et al.*, 2008). The two co-flowering plants, *M. domestica* and *R. pseudoacacia*, constituted the lower trophic level while flower visitors categorized mainly into orders, (Coleoptera, Diptera, Hemiptera, Hymenoptera and Lepidoptera), families (Formicidae, Bibionidae and Syrphidae) and species (*Apis mellifera* L. and *Xylocopa caffra* L.), formed the higher trophic level. To create a visual representation of the interaction network, the ‘*plotweb*’ function was used to create a web of interactions between the two trophic levels. Then using the ‘*networklevel*’ function of the package, network level metrics were calculated (Bluthgen *et al.*, 2008).

4.3 Results

4.3.1 Abundance of flower visitors

Diptera were the most abundant flower visitors recorded in the *M. domestica* orchards with the march fly (Bibionidae) family being the most common visitor within the order (Fig. 4.2D, Fig. 4.3). These were followed by the African honey bee (*Apis mellifera*). Calyptrate dipterans mainly from the Calliphoridae family (Fig. 4.2A) as well as hoverflies (Syrphidae) also constituted a significant proportion of apple flower visitors. However, in the *R. pseudoacacia* stands, honey bees were the dominant flower visitors while the rest of the arthropod families and orders occurred as occasional visitors (Fig. 4.3). When compared across sites, *A. mellifera* was more abundant in *R. pseudoacacia* than *M. domestica* (Fig. 4.3). However, there were more dipterans in *M. domestica* orchards (n = 908) in comparison to *R. pseudoacacia* (n = 74) (Table 4.1). Whilst all the other flower visitors were common to both trees, ants (Formicidae) and the solitary carpenter bee (*Xylocopa caffra*) were not observed on *M. domestica* flowers (Table 4.1).



1411

1412 **Figure 4.2:** Some of the flower visitors observed on *Malus domestica* (top row) and *Robinia*
 1413 *pseudoacacia* (bottom row) belonging to Diptera (A, D & E), Lepidoptera (B & F),
 1414 Hymenoptera (C & G) and Coleoptera (H). Photos: Chikowore G., 2019.

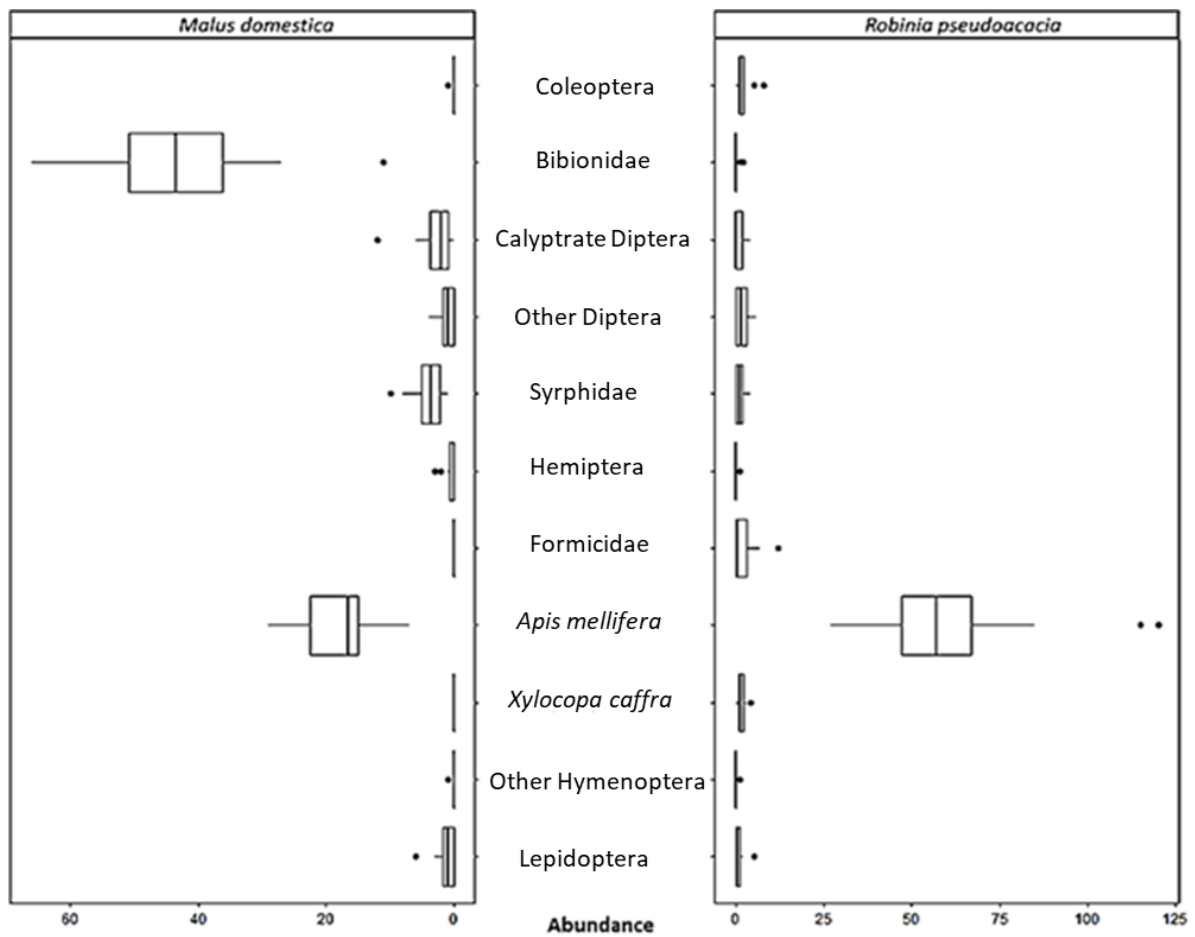


Figure 4.3: Abundance of flower visitors in *Malus domestica* orchard and adjacent *Robinia pseudoacacia* patches at Ionia Farm in Eastern Free State, South Africa. Note difference in scale when comparing visitor abundance to each plant species.

Table 4.1: Total number of visiting insects observed (n), and percentage (%) of total for each flower visitor on *Malus domestica* (Apple) and *Robinia pseudoacacia* (Black locust) flowers, observed twice a day for three days.

Order	Flower visitor	<i>M. domestica</i>		<i>R. pseudoacacia</i>	
		n	%	n	%
Coleoptera	Beetles	2	0.18	29	1.71
Diptera	Flies	49	4.33	15	0.89
Diptera	Bibionidae	768	67.84	3	0.18
Diptera	Syrphidae	71	6.27	21	1.24
Diptera	Other Diptera	20	1.77	35	2.07
Hymenoptera	Formicidae	0	0.00	38	2.24
Hymenoptera	<i>Apis mellifera</i>	192	16.96	1505	88.9
Hymenoptera	<i>Xylocopa caffra</i>	0	0.00	28	1.65
Hymenoptera	Other Hymenoptera	2	0.18	1	0.06
Lepidoptera	Butterflies	20	1.77	16	0.95
Hemiptera	Hemiptera	8	0.71	2	0.12
Total		1132	100	1693	100

4.3.2 Pollinator visitation rate

Flower visitation rates by Diptera were significantly different between families and sites ($\chi^2 = 89.868$, $df = 7$, $p < 0.001$). The post-hoc Dunn test further showed Bibionids recording the highest flower visitation rate in apple orchards relative to other dipteran species (Fig. 4.4A). Visitation rates by Dipteran insects did not vary significantly for *R. pseudoacacia* ($p > 0.05$) (Fig. 4.4A). When compared across sites, visitation rates by Bibionidae, Calliphoridae and Syrphidae differed significantly between *M. domestica* and *R. pseudoacacia* (adjusted $p < 0.001$) (Table 4.2). For the order Hymenoptera, flower visitation also varied significantly within and across sites ($\chi^2 = 120.23$, $df = 7$, $p < 0.001$). Post hoc tests showed that the visitation rate for *A. mellifera* was significantly higher than other species for both *M. domestica* and *R. pseudoacacia* (Fig. 4.4B). Although flower visitation rates by *A. mellifera* were marginally higher in *R. pseudoacacia* than *M. domestica*, the difference was not statistically significant

(adjusted $p > 0.05$). Visitation to *R. pseudoacacia* flowers by Coleopterans was significantly higher than Apple flowers (Fig. 4.4C). In contrast, the visitation rate by Hemipterans was significantly higher on *M. domestica* than *R. pseudoacacia* flowers. There was no significant difference in visitation rates to both plant species by Lepidopterans (Fig. 4.4C).

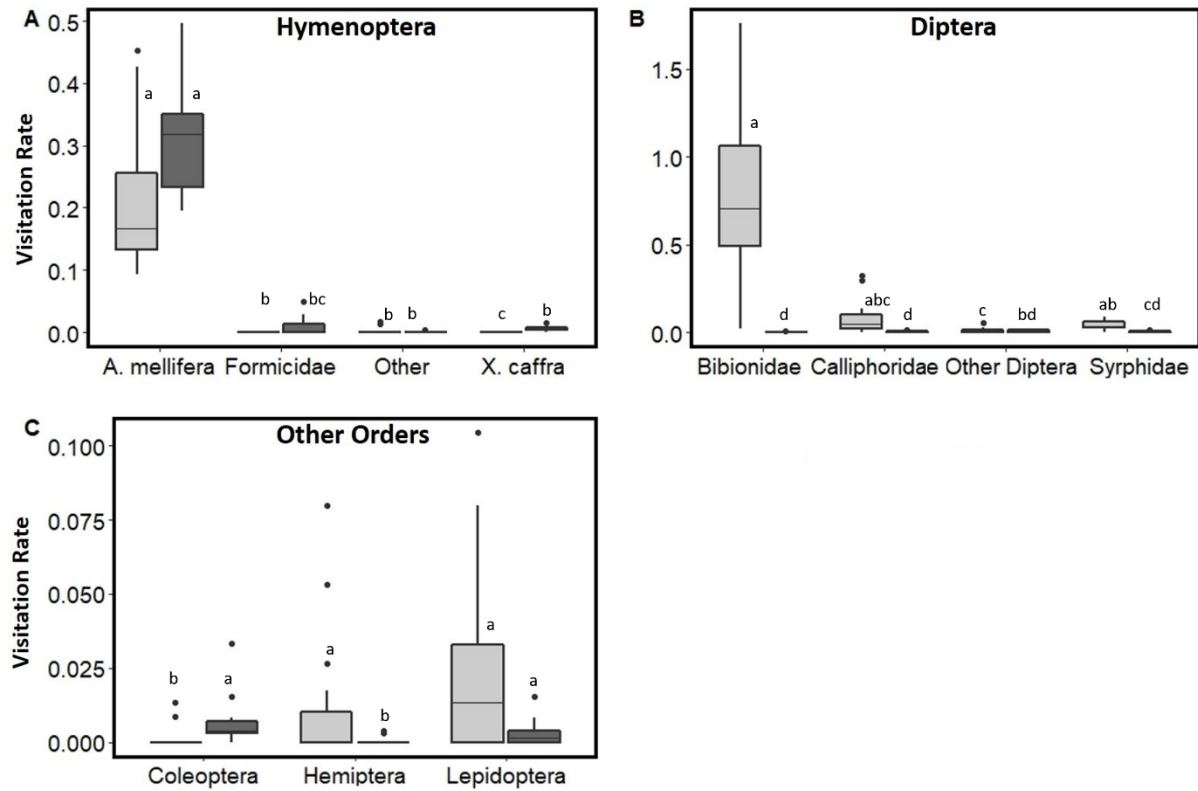


Figure 4.4: Flower visitation rates (visitors flower⁻¹ hour⁻¹) (median) on *Malus domestica* (Apple) in light grey and *Robinia pseudoacacia* (Black locust) in dark grey. The visitors were grouped into (A) Hymenoptera, (B) Diptera and (C) other orders. Boxplots with different letters indicate significant differences in visitation rates between plant species.

Table 4.2: Rank sum pair-wise Kruskal –Wallis comparison test with p-values adjusted with the Bonferroni method results for variation in visitation rates (visitors flower⁻¹ hour⁻¹) between *Robinia pseudoacacia* and *Malus domestica* flowers. Significant effects are denoted by a bold font.

Order	Group	Z	p-value (unadjusted)	p-value (adjusted)
Hymenoptera	<i>Apis mellifera</i>	-0.83	0.409	1
	Formicidae	-2.3	0.021	0.604
	<i>Xylocopa caffra</i>	-3.6	0.002	0.008
	Other Hymenoptera	0.36	0.751	1
Diptera	Bibionidae	7.48	<0.0001	<0.0001
	Calyptrate Diptera	3.74	0.0002	0.0052
	Syrphidae	3.35	0.0008	0.027
	Other Diptera	-0.71	0.479	1
Coleoptera		-3.35	0.0008	0.012
Hemiptera		1.39	0.163	1
Lepidoptera		1.51	0.131	1

4.3.3 Plant-pollinator visitation network

The bipartite interaction network for the two sites showed that *A. mellifera* was the most common visitor, mostly frequenting *R. pseudoacacia*, while march flies (Bibionidae) were the major flower visitors on *M. domestica* flowers (Fig. 4.5). Shared interactions were 1.54 for the higher trophic level which translated to a niche overlap of approximately 67%, while the two sites shared 9 of the 11 flower visitor groups (Fig. 4.5). Of all flower visitors in the network, only those from the family Formicidae as well as carpenter bees (*X. caffra*) were not shared between the two sites. However, at species level, a number of individuals were also not shared although it is not reflected in the interaction due to taxonomic resolution. The network metrics indicated high nestedness and connectance indicating high redundancy hence there were no indications of one plant totally excluding the other (Table 4.3).

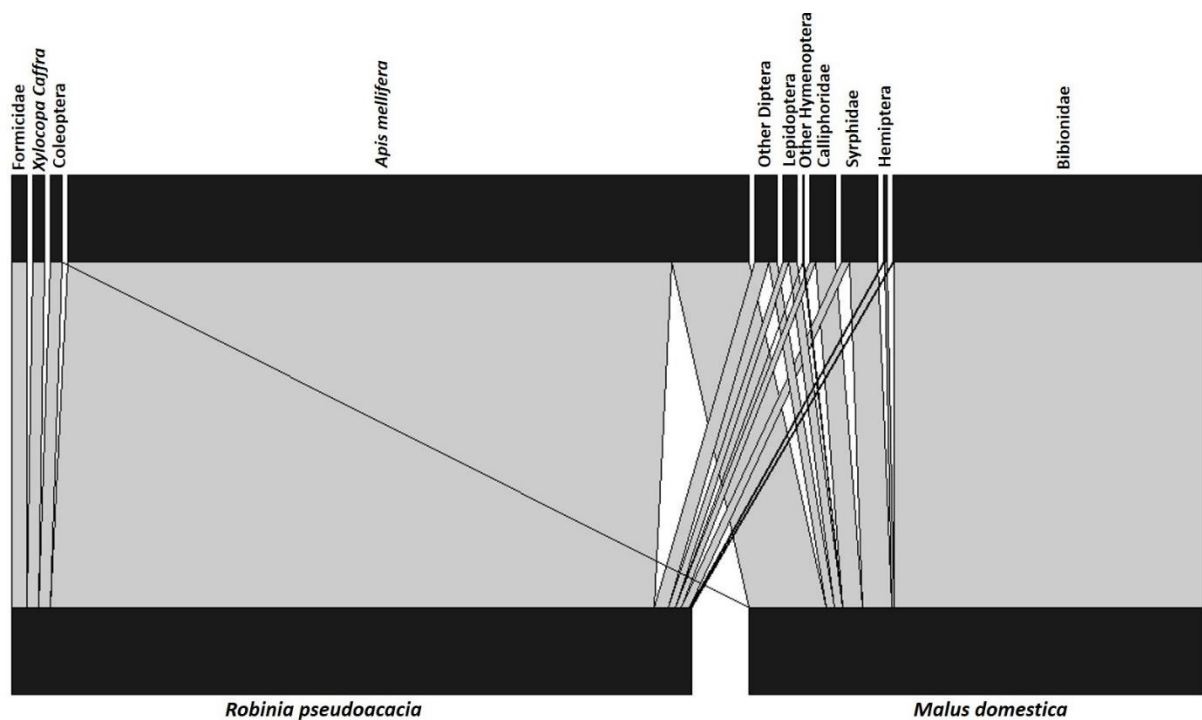


Figure 4.5: Bipartite interaction network for *Malus domestica* (Apple) and *Robinia pseudoacacia* (Black locust) visitors. Black boxes represent flower-visiting insects in the upper level, and plants in the lower level, their widths indicate the number of visits recorded. Grey links indicate plant–flower visitor interactions, and the breadth of the links represent visitation frequency.

Table 4.3: Network level metrics for plant - flower visitor interactions in *Malus domestica* and *Robinia pseudoacacia*

Parameter	Level
Connectance	0.91
Nestedness	0.02
H ₂ ' index	0.63
Niche overlap Lower Trophic Level	0.38
Niche overlap Higher Trophic Level	0.67
Linkage per species	1.54

4.4 Discussion

This study showed that invasion of agro-ecological ecosystems by *R. pseudoacacia* results in the sharing of flower visitors, potentially reducing pollination and ultimately yields. These findings are consistent with a number of studies showing the impact of invasive alien plants such as *Acacia saligna* (Labill.) H.L.Wendl. (Fabaceae), *Rubus cuneifolius* Pursh (Rosaceae) and *Parthenium hysterophorus* L. (Asteraceae) on native plant flower visitation (e.g. Gibson *et al.*, 2012, 2013; Hansen *et al.*, 2018; Ojija *et al.*, 2019). However, in contrast to these studies our study focused on the impact of an invasive alien plant, *R. pseudoacacia* on another exotic commercially cultivated orchard plant *M. domestica*. As the two plants are both exotic, it is most likely that they depend entirely on generalist pollinators, putting them in direct competition. Hence, visitors such as the social *A. mellifera* (Bartomeus, 2013), were the most commonly shared pollinators between the two indicating a competitive rather than mutual relationship.

Similarities in flower morphology have also been discovered to be a determinant of co-option of IAPs into native pollination networks (Munoz & Cavieres, 2019). Our results showed a marked preference for apple flowers by dipteran flies. It is possible that this preference was driven by differential appeal mediated by their odour emission and /or flower morphology as apple flowers are more open with easily accessible reproductive parts compared to *R. pseudoacacia*. This preference in the presence of strong competition for native bee pollination may contribute to successful pollination of apples by dipterans. However, this depends on the efficiency of this taxa as pollinators as Garrat *et al.* (2016) states, flies are less efficient as apple pollinators than bees. Although bibionids were the most abundant visitors on apple flowers, their movement between flowers and plants seemed to be limited suggesting that they may play a role in autogenous pollination. Although Chirango *et al.* (2019) observed high abundances of

bibionids on *Eustegia minuta* R.Br. (Apocynaceae) in South Africa, there is still need to examine if indeed they contribute to apple pollination. There is also a possibility that they might be acting as nectar thieves rather than pollinators. Since apples are obligatory cross-pollinated, this might be compromised as pollen has to be transferred from pollen donors. However, this aspect requires further investigation.

Although we found more honey bees in *R. pseudoacacia* than in apple orchards in this study, the visitation rate by the bees did not vary significantly in both sites. This similarity seems to be driven by the abundance of flowers in *R. pseudoacacia* when compared to apple trees. In an aerial study to quantify *R. pseudoacacia* flowers, Carl *et al.* (2017) found an average of ~255 inflorescences bearing ~5000 flowers on trees. Dietzsch *et al.* (2011) observed a decline in flower visitation rates to native *Digitalis purpurea* L. (Plantaginaceae) with an increase in the abundance of an invasive alien plant, *Rhododendron ponticum* L. (Ericaceae) in Ireland. This further confirms that the IAPs can attract more pollinators from the community when compared with co-flowering plants. Similarities in visitation rates in this study show that *R. pseudoacacia* is a strong competitor especially for native honey bees, *A. mellifera*, with potential negative effects for apple production. This genus has also been found to be attracted to IAPs with potential impacts on pollination for co-flowering plants (see Gibson *et al.*, 2013). It is also common practice for farmers to supplement pollinators by hiring bee colonies. However, in light of this competition posed by *R. pseudoacacia*, larger colonies might be required as some are bound to be lured away from the orchards. Moreover, only a fraction of bees successfully transfer pollen from donors thus any competition further reduces chances of successful pollen transfer (Adler & Irwin, 2006).

Our results showed that there were no crawling insects visiting apple flowers, such as ants. In all likelihood, this might be a consequence of management, particularly pesticide application. However, the effect on flying arthropods needs to be investigated especially when contact

pesticides are applied. Some studies have highlighted the negative impacts of pesticides on pollinator abundances especially in managed ecosystems (Goulson *et al.*, 2018). In the presence of alternative floral resources provided by invasive trees, this might further serve to reduce production in pollinator dependent agro-ecosystems.

The interaction network constructed in this study was highly nested, suggesting that pollinators visiting both *R. pseudoacacia* and apple trees belong to the same community and one is a subset of the other. While this metric is highly robust as it is not influenced by sampling, it might be influenced by network size (Bascompte *et al.*, 2003; Bartomeus, 2013). Nevertheless, our results clearly show an integrated network of flower visitors for *R. pseudoacacia* and apples. This has implications on pollination of economically important trees such as apple as the sharing of pollinators potentially reduces fruit set. Although fruit production was not measured in the current study, this warrants further investigation to fully elucidate the competitive effects of *R. pseudoacacia* on *M. domestica* fruit set and yield. While some studies have recommended the provision of additional flower resources for spring pollinators (e.g. Heller *et al.*, 2019), care must be taken to avoid overlapping of flowering periods to reduce competition. Our results also showed that honey bees frequented *R. pseudoacacia* more than apple trees suggesting that the former might be more attractive. This has been the case in all flower visitor networks involving invasive alien plants (see Ojija *et al.*, 2019; Hansen *et al.*, 2018) and flower abundance has been identified as the primary reason for attraction.

In conclusion, invasion of pollinator dependent agro-ecological systems by *R. pseudoacacia* can potentially compromise productivity due to competition for pollinators. Therefore, effective pollinator conservation should be adopted and control of IAPs should be integrated into those strategies. In this study, we did not quantify the floral attributes mediating the differential visitation rates nor the subsequent impact on yield. Future studies should therefore

1553 consider investigating the actual drivers of the visitation rates together with yield in order to
1554 prioritise management decisions.

CHAPTER 5

Contributions of Black locust (*Robinia pseudoacacia* L.) to livelihoods of peri-urban dwellers in the Free State Province of South Africa*

*Submitted to: Geojournal

5.1 Introduction

Globalisation mediated by human movement and trade are among the leading drivers of biological invasions (Mack, 2003; IPBES, 2019; Essl *et al.*, 2020). While the introduction of biological material might be accidental, for several species, it is driven by societal needs. For instance, invasive alien plants (IAPs) have been introduced for food, fuel, land reclamation, construction resources and amelioration, among other needs (Richardson & Rejmanek, 2011; Shackleton *et al.*, 2019). However, despite some of these plants providing services, many have become invasive and are threatening human wellbeing through their impact on various ecosystem services (Shackleton *et al.*, 2015). Several studies have documented the adverse impacts of invasive alien species on ecosystem structure and functioning (Ehrenfeld, 2003; Gorgens & van Wilgen, 2004; Livingstone *et al.*, 2020). However, the extent to which IAPs have integrated into and their importance to local livelihoods remains underexplored. This is particularly important in developing countries where ecosystem services chiefly influence livelihoods.

Although many alien trees were introduced globally for defined purposes, communities at times develop additional uses for some of the species over time. For example, *Acacia cyclops* A.Cunn. ex G.Don (Fabaceae) was introduced into South Africa for sand dune stabilisation but is now used as fuelwood for domestic and commercial purposes (Adair, 2004). Even for accidentally introduced species, communities also explore their beneficial properties. For instance, one of the widespread agricultural weeds globally, *Bidens pilosa* L. (Asteraceae) is reportedly utilised as food and medicine across Africa, Asia, America and Oceania (Bartolome *et al.*, 2013). Some authors have described utilisation of alien species as opportunistic (Howard, 2019; Martinez & Manzano-Garcia, 2019). However, there is clear integration of such species into local livelihoods (de Neergaard *et al.*, 2005). Residence time plays a role in the acceptance and utilisation of some species as locals become adventitious and diversify their livelihoods

(Rai *et al.*, 2012). Furthermore, ubiquity and declining native species can also result in local communities migrating to alien species (Geesing *et al.*, 2004). In such cases, a direct proportion of the alien species environmental presentation and not their superior traits dictate community usage (Shackleton *et al.*, 2007). In addition, the need to conserve natural diversity has also been proffered as the reason behind utilisation of alien species. Kull *et al.* (2011) reported that uses of invasive alien trees are influenced by a number of factors among them, land use traditions, historical and current economic opportunities as well as subsistence needs. While there is evidence on the adaptations of invasive alien species to local environments (Vila & Weiner, 2004; Oduor *et al.*, 2016), there is lack of information on the adaptation of invaded communities to alien species (Howard, 2019).

In some parts of North America, Africa and the Middle East, proposals have been put forward to control species that that communities can use through consumption (Geesing *et al.*, 2004; Varble & Secchi, 2013). This view stems partly from the realisation that it might be difficult to restore ecosystems as secondary invasions have been noted following clearing (Holmes *et al.*, 2020). However, the sustainability of control through utilisation has been questioned due to its potential to create a dependence on the resource. Nunez *et al.* (2012), postulated that utilisation of invasive alien species especially those that are edible potentially creates market actors which fuel further invasion. In South Africa, utilisation of *Acacia mearnsii* De Wild. (Fabaceae) and *Acacia dealbata* Link (Fabaceae) has influenced the perception of locals to view these invasive alien trees as indigenous natural resources (de Neergaard *et al.*, 2005).

Context is therefore important in determining the impacts and benefits of invasive alien species to avoid conflict scenarios between environmental impacts and community needs (Shackleton *et al.*, 2019). In many instances, benefits and negative impacts variably affect different stakeholders. For example, while plantation trees have quantifiable benefits to private entities and national economies, their spread into public spaces may affect surrounding communities.

Where invasive alien species are utilised, there are often trade-offs between the negative impacts and benefits (Ewel *et al.*, 1999). For example, de Wit *et al.* (2001) reported that environmental damage caused by the invasive plantation tree, *A. mearnsii* outweighs its economic contributions in South Africa. Similarly, *Eucalyptus camaldulensis* Dehnh. (Myrtaceae) is reportedly the most widespread conflict generating species in South Africa as perceptions on its benefits and costs vary across regions and social sectors (Hirsch *et al.*, 2020). While it is easier to do cost-benefit analyses for commercially introduced species, the same is difficult for those without clearer purposes. This is due to the complexity in apportioning value to biological forest products exploited by poor rural communities in Africa (Shackleton *et al.*, 2007). While trading in forest products generally offers a social safety net, incomes generated are insignificant for poverty alleviation (Shackleton *et al.*, 2008) unless these resources are exploited formally (Shackleton *et al.*, 2007)

In South Africa, considerable evaluations on the impacts of invasive alien trees on rural livelihoods have been done with both positive and negative impacts reported (Shackleton *et al.*, 2007; Semanya *et al.*, 2012; Shackleton *et al.*, 2015). For instance, Shackleton *et al.* (2015) reported that while *Prosopis* contributed to livelihoods through non-timber products, some stakeholders cited many negative impacts including obstruction to land access, destruction of houses, and reduction in water resources. However, very little is known about the interactions between peri-urban communities and invasive alien plants. Peri-urban communities are defined as landscapes of mixed land use and livelihoods encompassing both rural and urban traits (Diaz-Caravantes & Sanchez-Flores, 2011). In addition, they have higher population densities than rural areas and are often informal. Like most rural communities, they rely on forest products primarily for fuel. Even for those with access to power infrastructure, the increasing strain on conventional gridded power, as well as the escalating cost of alternative power

sources, has seen a number turning to firewood for heating and other domestic purposes (Shackleton *et al.*, 2007).

Robinia pseudoacacia is a tree of North American origin that is now widely distributed across the globe (Nicolescu *et al.*, 2020). The tree was described by Vitkova *et al.* (2017) as ‘beloved and despised’ due to its highly conflicted nature. As a result, there are contradictions on invasive status across Europe depending on jurisdiction (Vitkova *et al.*, 2017). Among its notable benefits are honey production, erosion control, rot-resistant timber (Cierjacks *et al.*, 2013). However, these are weighed down by its rapid spread, management difficulty, and impacts on native diversity (Benesperi *et al.*, 2012; Lazzaro *et al.*, 2018). In South Africa *R. pseudoacacia* is present in all provinces of the country although the largest stands have been recorded in the grassland biome (Henderson & Wilson, 2017; Martin, 2019). A survey in the eastern Free State of South Africa showed that the plant reduces the diversity of native vegetation and can potentially reduce gross margins in livestock enterprises (Humphrey *et al.*, 2019; Chikowore *et al.*, 2021). While negative effects of the tree are emerging, its current utilisation and potential contribution to local livelihoods in South Africa is undocumented. Native grasslands provide essential ecosystem services such as grazing for domestic and wild animals, thatching grass, medicines, and sweeping brooms (Moffett, 1997). However, habitants of grassland-dominated landscapes also require other services provided by trees which are scarce and where available do not suit desired purposes. Thus, some alien woody species are utilised whenever available through deliberate or accidental introductions. The montane grasslands of South Africa are a reflection of the transformation of local landscapes to satisfy societal needs. Native woody species in this biome are composed of short brushy vegetation dominated by the genus *Leucosidea* and some *Protecea* species (Mucina & Rutherford, 2006). Given this structure, native vegetation fails to meet some needs such as construction, fencing, and fuel. In this biome, perceived suitable species of exotic origin are

often introduced and utilised. In the Maluti–Drakensburg grasslands, besides commercial plantation trees, many invasive species have also been introduced (Carbutt, 2012). It has been argued that invasive alien species have a competitive advantage over local species due to lack of natural enemies (Vila & Weiner, 2004). In this regard, they may produce unblemished products when compared to native species leading to their increased utilisation.

Given the expanding populations of *R. pseudoacacia* in the grassland biome of South Africa (Martin, 2019), there is need to understand its utilisation patterns by various stakeholders. Peri-urban settlements are particularly a vulnerable section of society which is overlooked. Shackleton *et al.* (2015) showed that they are equally affected by invasive alien plants. Furthermore, Reynolds *et al.* (2020) highlighted that poverty increases vulnerability of communities to invasive alien plants. This study sought to determine the level of utilisation for *R. pseudoacacia* by peri-urban communities in eastern Free State Province of South Africa. Specifically, we asked 1. What is the level of knowledge regarding trees in grasslands and their origins? 2. What are the utilisation patterns of invasive alien trees and *R. pseudoacacia* in particular? 3 What is the relative contribution of *R. pseudoacacia* to the livelihoods of peri-urban dwellers in eastern Free State Province of South Africa?

5.2 Materials and methods

5.2.1 Study area

The survey was undertaken between March and July 2020 in the eastern Free State of South Africa. Three peri-urban communities near Clarens, Fouriesburg and Phuthadijhaba were surveyed. Targeted settlements were mainly informal on the peripheries of these towns. Clarens and Fouriesburg fall under the Dihlabeng Municipality of the Thabo Mofutsanyane District. The two are small towns typical of the eastern Free State which serve primarily as service centres for surrounding agricultural communities. However, there is significant tourist activity especially in Clarens due to its proximity to Golden Gate National Park and scenic grassland mountains (Halseth & Meiklejohn, 2009; Campbell, 2016). According to the Municipalities of South Africa (www.municipalities.co.za), a local governance body that tracks service delivery across South Africa, the unemployment rate in the district was approximately 28.7% in 2011 while informal settlements constituted around 23.3%. Phuthadijhaba falls under the Maluti-A-Phofung municipality and the major economic sectors are social services and agriculture. The area also experiences severe winter temperatures which go below -10°C. This drives the demand for fuelwood for heating purposes. Native vegetation in the area is dominated by grasses and forbs with scattered woody trees, most of which are of alien origin (Fig. 5.1).

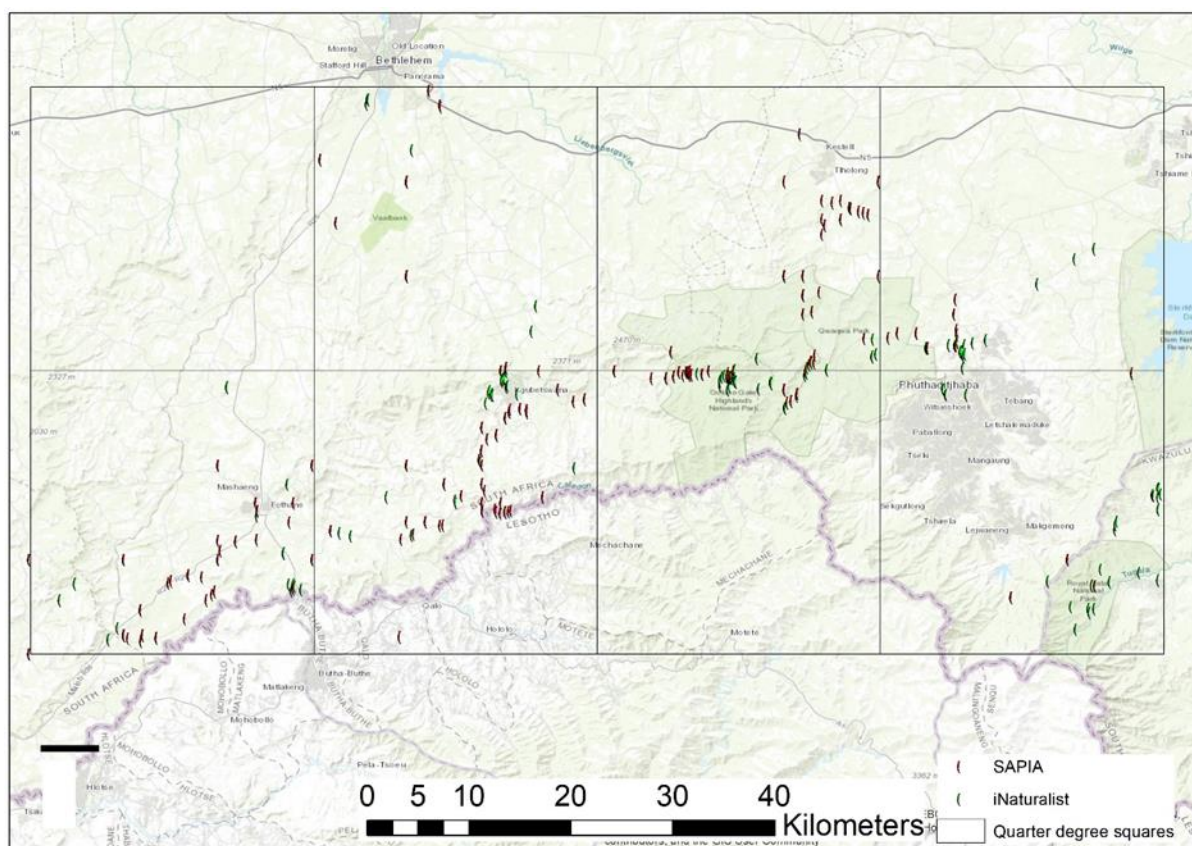


Figure 5.1: Distribution of invasive alien plants in the eastern Free State, South Africa. Data sources: The South African Plant Invaders Atlas (SAPIA) and iNaturalist.

5.2.2 Sampling approach

Semi-structured and structured interviews with a total of 36 interviewees from settlements surrounding Clarens, Fouriesburg, and Phuthadichaba towns, chosen intentionally by the snowballing method. Prior to administration of the questionnaire, a survey was done to identify settlements close to established large *R. pseudoacacia* populations as well as physical signs of harvesting on the stands. Furthermore, common invasive alien trees were also recorded to enable comparison with *R. pseudoacacia*, the target species. The study proposal was submitted to the General/Human Research Ethics Committee (GHREC) of the University of the Free State and granted ethical approval (UFS-HSD2020/1448/1111). Initial respondents were drawn from individuals who were found harvesting *R. pseudoacacia* trees and these snowballed to

other respondents. The data saturation criterion, where the next interviewee did not provide new information, was used to determine the sample size. Informed consent was obtained and participation was voluntary while confidentiality was maintained. Interviews were conducted in the local language- Sotho, targeting adults from different households. These focussed on the knowledge of available trees, their significance to livelihoods, and potential trade-offs. The focus of respondents was particularly drawn to incomes generated from *R. pseudoacacia* and its products as well as management efforts directed towards the species.

5.2.3 Data analysis

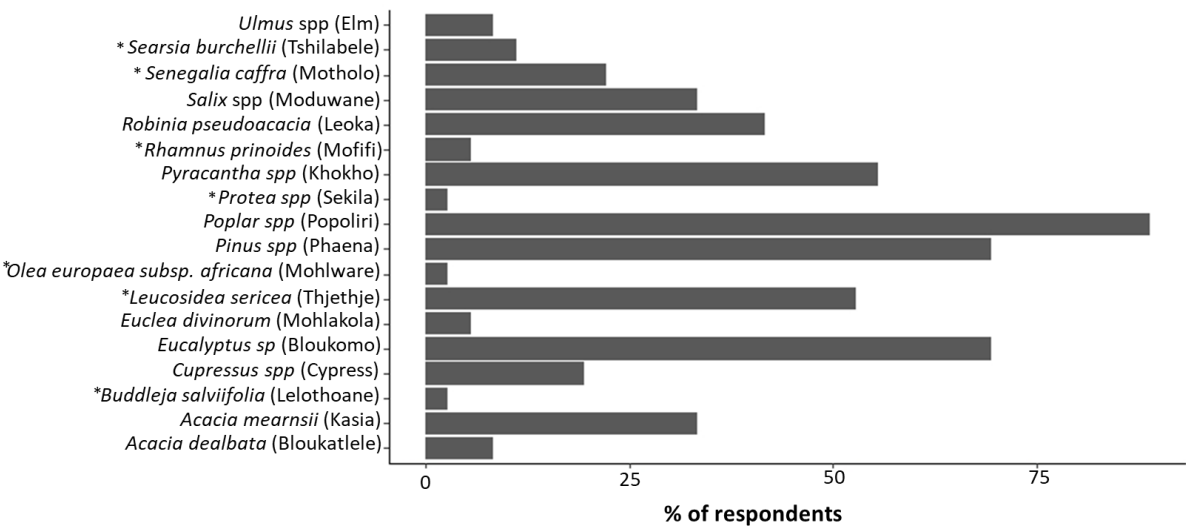
Since deductive coding was used in the design of the questionnaire, data was captured and organised into its respective codes. These codes were botanical knowledge, utilisation of forest products, and management of *R. pseudoacacia*. Microsoft Excel was then used to compare the frequency of responses to particular thematic areas. To compare the utility of *R. pseudoacacia* with other trees within the landscape, the pairwise ranking approach was applied based on the number of uses for each tree species.

5.3 Results

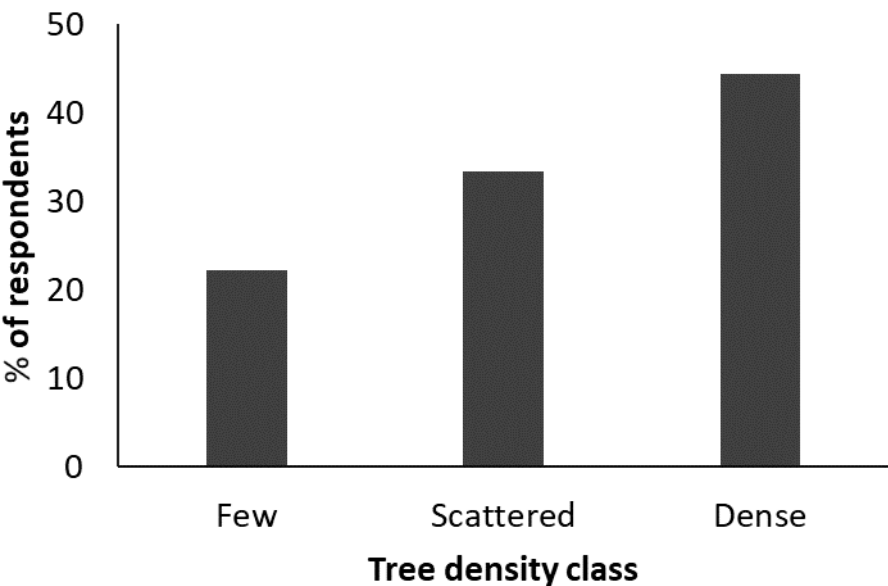
5.3.1 Botanical knowledge

The majority of respondents (61%) exhibited knowledge of local flora as they easily mentioned common trees in their environs. However, only 42% of these could distinguish between indigenous and alien trees. When further asked to name common trees in the area, commonly mentioned species were of alien origin with *Poplar* species getting the most mentions (89%) (Fig. 5.2). A total of 18 tree species were listed and 33% of these are native to South Africa. The most common native species was *Leucosidea sericea* Eckl. & Zeyh. (Rosaceae) which was mentioned by 53% of the respondents. *Robinia pseudoacacia* appeared to be fairly common as it was mentioned by 42% of respondents. When questioned further, those respondents who did

1751 not list *R. pseudoacacia* could identify the tree. All respondents had *R. pseudoacacia* trees
 1752 close to their homesteads and densities were described and the majority of respondents (44%)
 1753 perceived the stands to be very dense (Fig. 5.3).



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 1755 **Figure 5.2:** Tree species mentioned by respondents in eastern Free State, South Africa. Species
 1756 names are given with native Sotho names in brackets. Native species are marked by an asterisk.



1757
 1758 **Figure 5.3:** Perceptions of respondents to densities of *Robinia pseudoacacia* in eastern Free
 1759 State, South Africa.

5.3.2 Utilisation of forest products

All respondents used both timber and non-timber forest products collected from surrounding areas. However, the frequency of utilisation varied from daily to annual usage (Fig. 5.4). Most of the respondents (47%) said they used trees and tree products daily. The most frequent use for selected trees of alien origin was fuelwood followed by timber and fencing poles (Fig. 5.5). From the overall pairwise ranking, *Eucalyptus* species ranked higher than all species while *Pyracantha* species were the least valuable. However, there were variations when comparisons were based on single uses (Table 5.1). Respondents mainly used *R. pseudoacacia* for fuel, timber (house construction materials), and fencing poles. However, some respondents stated that firewood from *R. pseudoacacia* produces bad smoke and did not last long thus making it inferior to other species. Minor uses for the species were related to carving and furniture. The species was never ranked highly for any of the uses. A few respondents perceived the tree to be useful and need to be propagated together with *Pinus* and *Poplar* species.

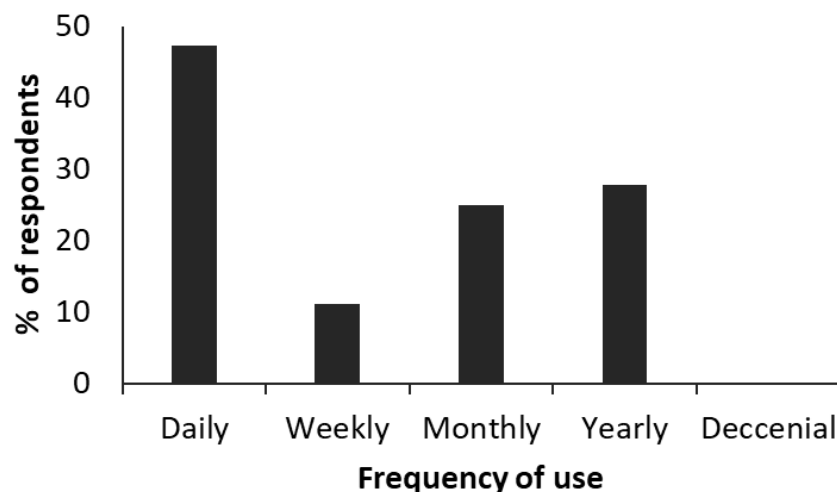


Figure 5.4: Frequency of *Robinia pseudoacacia* and its products use by peri-urban inhabitants in eastern Free State, South Africa.

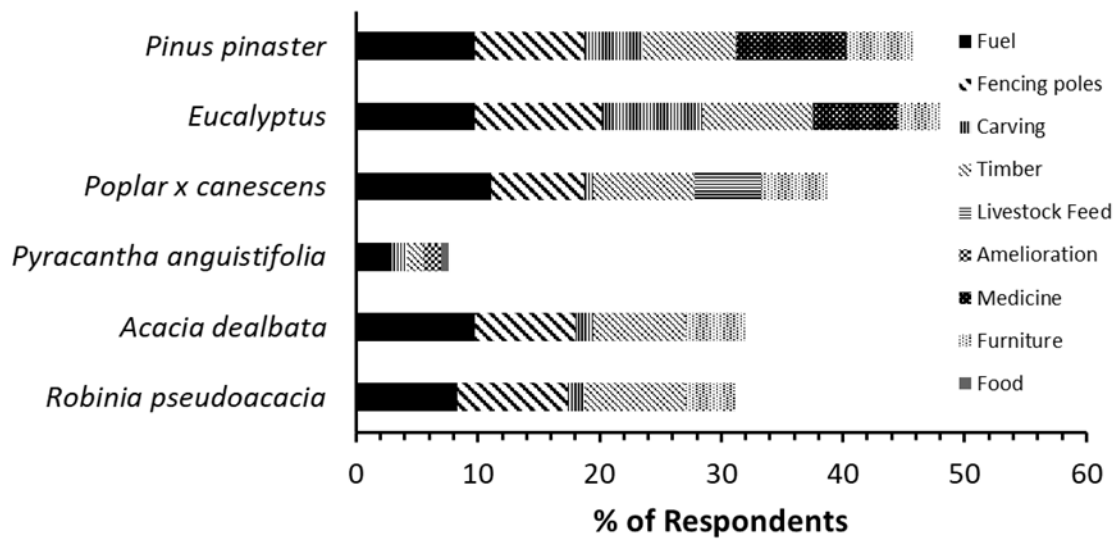


Figure 5.5: Uses of common alien invasive trees by peri-urban dwellers in eastern Free State, South Africa.

Table 5.1: Uses frequently cited by respondents for common invasive alien plants in eastern Free State grasslands

Use	Species	Frequency (%)	Rank
Firewood	<i>Robinia pseudoacacia</i>	75	3
	<i>Acacia dealbata</i>	87.5	2
	<i>Pyracantha angustifolia</i>	25	4
	<i>Poplar x canescens</i>	100	1
	<i>Eucalyptus</i>	87.5	2
	<i>Pinus pinaster</i>	87.5	2
Fencing poles	<i>Robinia pseudoacacia</i>	81.25	2
	<i>Acacia dealbata</i>	75	3
	<i>Pyracantha angustifolia</i>	0	5
	<i>Poplar x canescens</i>	68.75	4
	<i>Eucalyptus</i>	93.75	1
	<i>Pinus pinaster</i>	81.25	2
Construction	<i>Robinia pseudoacacia</i>	75	2
	<i>Acacia dealbata</i>	68.75	3
	<i>Pyracantha angustifolia</i>	12.5	4
	<i>Poplar x canescens</i>	75	3
	<i>Eucalyptus</i>	81.25	1
	<i>Pinus pinaster</i>	68.75	3
Livestock feed	<i>Robinia pseudoacacia</i>	0	2
	<i>Acacia dealbata</i>	0	2
	<i>Pyracantha angustifolia</i>	0	2

	<i>Poplar x canscens</i>	50	1
	<i>Ecalyptus</i>	0	2
	<i>Pinus pinaster</i>	0	2
Medicine	<i>Robinia pseudoacacia</i>	0	3
	<i>Acacia dealbata</i>	0	3
	<i>Pyracantha angustifolia</i>	0	3
	<i>Poplar x canescens</i>	0	3
	<i>Ecalyptus</i>	62.5	2
	<i>Pinus pinaster</i>	81.25	1
Amelioration	<i>Robinia pseudoacacia</i>	0	2
	<i>Acacia dealbata</i>	0	2
	<i>Pyracantha angustifolia</i>	12.5	1
	<i>Poplar x canescens</i>	0	2
	<i>Ecalyptus</i>	0	2
	<i>Pinus pinaster</i>	0	2
Carving	<i>Robinia pseudoacacia</i>	12.5	3
	<i>Acacia dealbata</i>	12.5	3
	<i>Pyracantha angustifolia</i>	12.5	3
	<i>Poplar x canescens</i>	6.25	4
	<i>Ecalyptus</i>	75	1
	<i>Pinus pinaster</i>	43.75	2
Food	<i>Robinia pseudoacacia</i>	0	2
	<i>Acacia dealbata</i>	0	2
	<i>Pyracantha angustifolia</i>	6.25	1
	<i>Poplar x canscens</i>	0	2
	<i>Ecalyptus</i>	0	2
	<i>Pinus pinaster</i>	0	2
Furniture	<i>Robinia pseudoacacia</i>	37.5	4
	<i>Acacia dealbata</i>	43.75	2
	<i>Pyracantha angustifolia</i>	0	5
	<i>Poplar x canescens</i>	50	1
	<i>Ecalyptus</i>	31.25	3
	<i>Pinus pinaster</i>	50	1

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1782 Only 5 respondents (14%) said they sold *R. pseudoacacia* products. The only commodity sold
1783 from this species was firewood and each individual sold a single bakkie load per year (~0.5
1784 ton), realising on average R367 (US\$1 ~ R16). This income was mainly spent on food, school
1785 uniforms, payment of debts, and other unspecified needs. A single respondent did also mention
1786 that *R. pseudoacacia* trees were also benefiting people from nearby larger towns such as
1787 Ficksburg who came and harvested at sites near Fouriesburg mainly during winter.

Despite its useful properties, *R. pseudoacacia* was viewed as a problematic species by all respondents. The majority of respondents (42%) stated that the tree forms thickets which makes movement difficult while 31% felt it was spreading uncontrollably (Fig. 5.6). Some individuals further mentioned that *R. pseudoacacia* was reducing the natural beauty of the landscape.

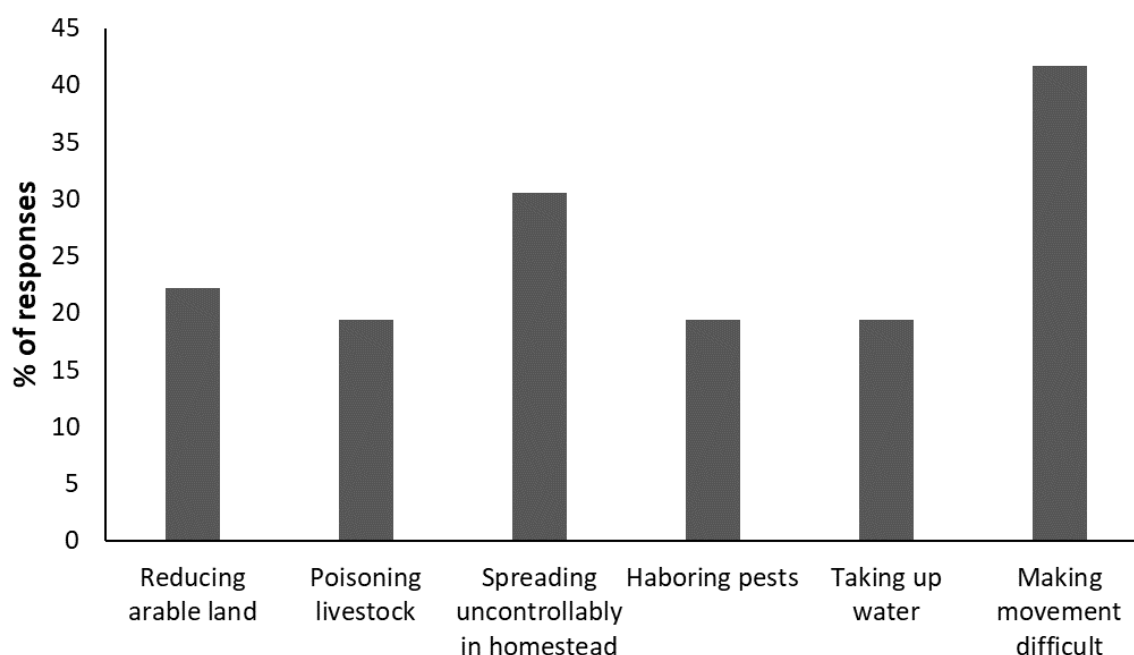


Figure 5.6: Perceived negative effects of *Robinia pseudoacacia* by peri-urban communities in eastern Free State, South Africa.

5.3.3 Management

There are attempts to clear the tree around Clarens town by both government and the local community. However, there are no similar efforts at Fouriesburg and in Puthadijthaba. In addition to negative effects noted by respondents (Fig. 5.6), other reasons for clearing were stated as space creation (25%), employment creation, and conservation (13%). All respondents were aware that both mechanical and chemical methods were being used. However, there were differences in opinion regarding effectiveness of the control measures with 44% saying the population is going down and 56% saying it is expanding.

5.4 Discussion

This study showed that peri-urban dwellers in eastern Free State Province make use of *R. pseudoacacia* to some extent. However, its utilisation appears to be opportunistic as it is not one of the species perceived to be of high importance by these communities. Moreover, its contribution to household incomes is very low. Its use is likely a function of its representation within the landscape as dense populations were reported and are evident.

This study further shows the importance of alien tree species in eastern Free State and the extent to which these have integrated into local communities. This is evident by the number of species of alien origin mentioned by respondents and their failure to distinguish between native and exotic trees. This is not surprising considering that native vegetation in this biome is predominantly grasses and forbs with a few scattered indigenous trees (Mucina & Rutherford, 2006). However, several authors have reported the rapid transformation of high altitude grasslands in eastern Free State and Lesotho by invasive alien trees (Carbutt, 2012; Kobisi *et al.*, 2019; Martin, 2019). Inevitably, these trees have become integrated into local communities with some now playing key socio-economic roles. For example, Kobisi *et al.* (2019) were able to show that of the 57 alien plants recorded in Lesotho, the majority were of ethnobotanical significance. The assignment of local vernacular names to alien trees also indicates the extent of integration of these species into local communities. This is because naming is often a descriptive process based on observed characteristics such as habitat, size, taste as well as functions of the plants (Moteetee & Van Wyk, 2006).

Multiple uses have been reported for *R. pseudoacacia* especially in European countries (Vitkova *et al.*, 2017; Kunes *et al.*, 2019). Documented uses are broader and inclined towards formal economic exploitation of the tree for example to support honey production, railway sleepers, mine supports, boat building among other large-scale uses (Dalby, 2004; Nicolescu

et al., 2020). Our results showed different utilisation patterns in South Africa with the tree mainly used for domestic purposes such as firewood, fencing posts, and house construction. However, these uses are also common for some alien trees in our study area with no evidence of a high preference for *R. pseudoacacia*. Similarly, Shackleton *et al.* (2015) reported a marked preference for products from native trees despite the widespread use of *Prosopis* products in South Africa. Results showed that respondents deemed *R. pseudoacacia* populations to be mainly dense. Therefore, the use of this tree in eastern Free State appears to be driven by its availability rather than superior traits. Although the tree reportedly produces durable, rot-resistant fencing poles (Cierjacks *et al.*, 2013), this characteristic did not make it preferable as *Eucalyptus* species which often grow straighter. However, this points towards lack of in-depth knowledge of the properties of the tree. In addition, flowers from *R. pseudoacacia* are reportedly edible (Martinez & Manzano-Garcia, 2019) which is also an unexplored use of the species locally.

Some studies have reported significant contributions of environmental products, especially tree resources to household incomes in Africa (Langat *et al.*, 2016; Shackleton *et al.*, 2007). This study shows that incomes generated by peri-urban dwellers from *R. pseudoacacia* exploitation are insignificant. This is despite severe winters experienced in eastern Free State which drive the demand for fuelwood. Kull *et al.* (2011) singled out poverty as the reason behind the widespread utilisation of Australian acacias outside commercial plantations as people take advantage of available resources. However, for *R. pseudoacacia* its fair representation in the study area does not translate into increased utilisation instance. Several reasons might be behind, firstly there are limited populations with trees with larger bole sizes (Chikowore *et al.*, 2021) which are attractive for many purposes such as firewood and poles. Secondly, smaller trees especially re-sprouts form thickets which are thorny and difficult to harvest thus limiting commercial exploitation. Thirdly, ownership of resources around urban centres limits

commercial exploitation. For instance, the area surrounding Clarens town is mainly a conservancy thus access to resources is limited. For some invasive alien trees, commercial exploitation has been practiced through value-addition into products such as charcoal (de Wit *et al.*, 2001). However, we found no evidence of processing of *R. pseudoacacia* or other invasive alien trees present in the area. This further shows that contributions of the species and others to household incomes in general is very limited.

For many invasive alien species, disparities in utilisation amongst stakeholders are often a source of conflict. van Wilgen *et al.* (2001) reported that invasive alien species may differentially impact stakeholders with benefits accruing to some while others bear the costs. However, common to all stakeholders in eastern Free State are the negative impacts of the tree. Humphrey *et al.* (2019) reported that farmers in eastern Free State perceived the species to be spreading uncontrollably and generally undesirable. This sentiment was shared by peri-urban dwellers even though their participation in the agricultural sector is limited. Moreover, there are no commercial plantations of the tree locally hence the tree invariably affects all stakeholders. Perceptions from respondents in this study indicated that efforts to control *R. pseudoacacia* using both mechanical and chemical means are largely ineffective. This is consistent with studies in Asia and Europe, controlling *R. pseudoacacia* through mechanical and chemical means individually or combined is ineffective (Nicolescu *et al.*, 2020).

In conclusion, although peri-urban dwellers make use of *R. pseudoacacia*, they have not integrated it fully into their livelihoods. However, there is great reliance on other alien species in addition to *R. pseudoacacia* for several services. In addition, there is need for awareness campaigns to sensitize communities on invasive alien species in order to avoid management conflicts. Attempts to control *R. pseudoacacia* are unlikely to result in socio-ecological shocks to peri-urban communities. Hence, efforts to find sustainable management options should continue.

CHAPTER 6

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General discussion

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This thesis highlights the importance of field studies in understanding the interactions between an invading species and the local environment. A geographical bias on the ecology of *Robinia pseudoacacia* in the southern hemisphere was reported by Martin (2019). Thus, this study is the first to provide quantitative information on the biotic and abiotic interactions of *R. pseudoacacia* in southern hemisphere montane grassland ecosystems. It provides empirical evidence on how it alters grassland climatic and habitat conditions to the detriment of native diversity. Several ecological parameters for the IAP, from individual plant to population level as well as responses of the invaded environment were considered in this study. In addition, the role of *R. pseudoacacia* in socio-ecological systems of invaded communities were explored.

6.1 Ecological impacts

This study showed how *R. pseudoacacia* alters grassland microhabitats through mechanisms such as shading and alteration of soil chemical composition. Differences in the thermal and illumination regimes between invaded and uninvaded grasslands were quantified. This research further showed how these shifts in microenvironments reduce native grassland plant and arthropod diversity especially in the understory. Exclusion of some key endemic grassland arthropod taxa (e.g Orthoptera: Acrididae) reported in this study (Chapter 2) provide evidence of the creation of novel microclimates following invasion. These findings are in agreement with several studies in Europe where *R. pseudoacacia* was found to be driving the homogenization of understory vegetation communities (Benesperi *et al.*, 2012) and altering micro-arthropod communities (Lazzaro *et al.*, 2018). However, unique to this study is the consideration of montane grasslands in the southern hemisphere which present unique environmental characteristics such as a wide temperature range. In addition, this study showed how *R. pseudoacacia* invasion creates a niche for further invasion by alien grasses. This was

shown in Chapter 3 of this study where understory vegetation was dominated by grasses of alien origin.

6.2 Ecosystem services and disservices

The loss of biological diversity mediated by *R. pseudoacacia* reported in this study has implications on key ecosystem services derived from montane grasslands. For instance, changes in the composition of grasses results in a reduction in grazing capacity of rangelands and subsequently livestock production. In South Africa, livestock production is mainly range-based hence the continued degradation of natural rangeland has serious implications on the viability and profitability of this enterprise. Humphrey *et al.* (2019), reported potential losses in livestock gross margins as a result of expanding *R. pseudoacacia* invasions. This study weighed in by quantifying losses in grazing capacity through alteration of grass communities. While other services such as provision of construction materials and medicinal plants by grasslands were not measured in this study, the loss in native diversity reported in this study implies that these are equally affected. The impact of IAPs on pollination has been widely reported (Gibson *et al.*, 2013; Ojija *et al.*, 2019; Hansen *et al.*, 2018), this study (Chapter 4) is among the few to consider these impacts in an agro-ecosystem. Based on arthropod visitation rates and abundance, competition for key pollinators such as the genus *Apis* is highly likely. Hence, there is likelihood of reduced pollination of fruit trees co-flowering with *R. pseudoacacia*.

One of the contentious issues in the control of IAPs is trying to strike a balance between benefits and impacts. This study addressed these concerns through a survey on the potential contributions of *R. pseudoacacia* to peri-urban communities in the eastern Free State Province (Chapter 5). This was motivated by reported multiple uses of *R. pseudoacacia* particularly in Europe. Conflicts of interest may pose a problem in the development and implementation of a

biological control program against *R. pseudoacacia* as the spread of agents, once initiated, cannot be spatially contained. However, this study revealed that in South Africa the tree is not grown commercially and does not pose any significant conflict of interest. In addition, the value of biological forest products is highly contextual. For instance, *R. pseudoacacia* is regarded highly in most European countries due to properties such as rot-resistant timber and support to the honey industry (Ciejacks *et al.*, 2013), poor rural and peri-urban communities in South Africa do not value the tree based on these. These conflicting values led Sadlo *et al.* (2017) to propose site specific management for the species especially in the Czech Republic, ranging from conservation of the species to ecological restoration. However, in South Africa this approach might not be necessary considering that *R. pseudoacacia* does not play a specific role particularly in the livelihoods of poor communities which depend more on biological forest products.

6.3 Conclusion and recommendations

The approach taken in this study of selecting ecological parameters at different organizational levels within the same site in order to effectively quantify the impacts of invasive alien plants as recommended by Kumschick *et al.* (2015). Moreover, the research showed among other factors, the importance of localised ecological studies to guide the design, implementation and evaluation of control programmes against species of alien origin. It is clear from this study that *R. pseudoacacia* is a problematic species especially in grasslands of South Africa. Management of this species and others invading high elevation grasslands will ensure the conservation of native biodiversity which underpins several ecosystem services. However, from this research a biologically based management approach appears to be the most feasible for *R. pseudoacacia* hence recommendations outlined below.

6.3.1 Management considerations

Currently, management of *R. pseudoacacia* is still problematic with no reports of successful clearance. Observations made on local management efforts using mechanical and chemical means show that these have driven further landscape spread. The major challenge is the clonal spread of the species which is fuelled by mechanical clearance of the trees (Radtke *et al.*, 2013). Several management options have been proposed based on combinations of mechanical and herbicide applications. In natural forests there are also proposals to let natural succession to take place (Sadlo *et al.*, 2017). However, for the South African context the “do nothing approach” proposed by Sadlo *et al.* (2017) may not work in grassland ecosystems as they are less resistant to invasion by woody species. Furthermore, occurrence of *R. pseudoacacia* in broken terrain makes it logistically challenging to apply mechanical and chemical control methods. In addition, chemical control may be expensive, environmentally damaging as well as lead to resistance development. In view of these management difficulties associated the plant, biological control offers better prospects of controlling the species. Since this work was conducted in the context of this management technique, it provides several success indicators following implementation.

In view of the difficulties of conventional management options in South Africa, *R. pseudoacacia* is currently being considered for biological control in South Africa (Martin 2021). Therefore, this work was considered in an effort to support Morin *et al.* (2009) and Schaffner *et al.* (2020) who highlighted the importance of pre-release ecological data especially in biological control programmes as it serves to measure a number of parameters such as potential agent success, devise performance targets and provision of a baseline for future evaluation work. Downey *et al.* (2021) as well as Paterson *et al.* (2021) further underscored the need for local ecological data in the prioritisation of target plants for biological control. While

1997 macrocosm studies provide an insight into large scale ecological interactions, field studies
1998 undertaken in this study capture a wide range of dynamics that occur at variable scales. This
1999 information can be applied to conceptualize, monitor and evaluate the management of *R.*
2000 *pseudoacacia* particularly in high altitude grasslands. This kind of pre-release data should be
2001 collected for any species that is being considered so that accurate quantitative post release
2002 evaluations can be made

2003 A system to prioritize future target species for biological control adapted to the South African
2004 context, the Biological Control Target Selection (BCTS) system was recently developed
2005 (Downey *et al.*, 2021; Paterson *et al.*, 2021). The system makes use of 13 attributes assigned
2006 to three sections, impacts, likelihood of success and investment to assign a score to target plants
2007 (Paterson *et al.*, 2021). Therefore, comprehensive ecological data is critical to enable accurate
2008 scoring for all attributes of the IAP. *Robinia pseudoacacia* is one of the species that was highly
2009 prioritized for control in South Africa based on the BCTS system (Canavan *et al.*, 2021).
2010 Similarly, the tree is amongst the top 20 candidates for classical biological control in Europe
2011 (Sheppard *et al.*, 2006). However, in South Africa, scoring for *R. pseudoacacia* was in some
2012 parts based on impacts recorded elsewhere due to lack of information. Thus, this study provides
2013 some of the key local data and helps confirm the species as a priority as already assigned. It
2014 will be interesting to monitor if *R. pseudoacacia* priority would be reduced should successful
2015 management options are found.

2016 Although there is no formal biological control programme against *R. pseudoacacia* in the
2017 world, prospects of developing a successful one are high. This is because of an increasing
2018 understanding of the ecology of the tree in varying environments to which this study adds. In
2019 addition, there are several natural enemies capable of inflicting extensive damage with some
2020 such as *Obolodiplosis robiniae* Haldeman (Diptera: Cecidomyiidae), *Odontota dorsalis*

2021 Thunberg (Coleoptera: Chrysomelidae) and *Agrilus difficilis* Gory (Coleoptera: Buprestidae)
2022 already being assessed (Martin, 2019; Nicolescu *et al.*, 2020). Data on the growth
2023 characteristics of the plant reported in this thesis (Chapter 2) also helps envisage the
2024 establishment and impacts of the agents in South Africa, considering the climatic and
2025 environmental conditions obtaining in high altitude grasslands locally. Current agents under
2026 consideration benefit from the findings of this study as they are aimed at altering the phenology
2027 of the species. For example, one of the agents *O. robiniae*, causes leaf galls and eventual die
2028 back of the trees in the long run (Duso *et al.*, 2005; Buhl & Duso, 2008) while *O. dorsalis* has
2029 been shown to result in early leaf drop of *R. pseudoacacia* in the USA. With the current
2030 knowledge of *R. pseudoacacia* phenology and other climatic parameters locally, issues of host-
2031 agent synchronisation under local conditions will be tackled.

2032 While the return to original ecosystem functioning is the desired state following the control of
2033 IAPs, it is prudent to note that this is difficult especially when dealing with plants which alter
2034 the biophysical components of the system. This study especially in Chapter 3, the proliferation
2035 of nitrophilous understory vegetation indicated alteration of soil chemical composition. Thus,
2036 there is a high likelihood of long term legacy effects which when coupled with depleted
2037 seedbanks of native species might compound the restoration process. Moreover, recovery to
2038 some extent is dependent on the rate and success of clearing the invasive alien tree, with
2039 evidence from other studies showing secondary invasion following mechanical clearance
2040 (Ruwanza & Tshililo, 2019). Nevertheless, this study provides a quantitative baseline to enable
2041 monitoring progress towards ecological restoration through parameters such as grazing
2042 capacity, arthropod diversity and flower visitation rates.

6.3.2 Further research

- Since *Robinia pseudoacacia* has invaded all provinces of South Africa, there is need to cascade studies to all biomes in order to obtain site specific interactions to guide local management efforts.
- This study inferred the impact of *R. pseudoacacia* on apple pollination from arthropod visitation rates and abundance. However, there is need to investigate potential edge effects in relation to apple quality and yields. In addition, emphasis should be placed on assessing the constancy of *Apis mellifera* given the two co-flowering plants. This will give a comprehensive picture on the impact of IAPs on agricultural productivity.
- Soil seedbanks play an important role in the restoration of natural vegetation post IAP control. Thus, it is necessary to look at seedbanks for both native and alien species in habitats invaded by *R. pseudoacacia* in order to predict the direction of plant community succession following removal of the species in grasslands.
- Much of this thesis has investigated the effects of *R. pseudoacacia* on mountain grassland ecosystems. Hence, a thorough investigation into drivers of *R. pseudoacacia* invasion in the mountain grasslands would provide valuable data to assist management.

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2894

Appendices

Appendix 1



Contributions of invasive alien tree, Black locust (*Robinia pseudoacacia* L.) to livelihoods of peri-urban dwellers in Clarens, South Africa

Gerald Chikowore¹

¹Department of Zoology and Entomology, University of the Free State, Bloemfontein

Rural utilization questionnaire

The University of the Free State in conjunction with the Centre for Biological Control at Rhodes University is conducting research into possible control of Black locust (*Robinia pseudoacacia*), an invasive alien tree using its natural enemies. The purpose of this questionnaire therefore is to assess utilization of *R. pseudoacacia* and its products by resource limited communities in South Africa.

2915

2916 The questionnaire consists of 20 questions in 4 sections

2917

2918



2919

2920

2921 **A. PARTICIPANT DETAILS**

2922

AGE	
SEX	
HOUSEHOLD SIZE	
DISTRICT	
YEARS IN THE LOCATION	

2923

2924 **B. BOTANICAL KNOWLEDGE**

2925

2926 1. Do you know a lot about trees?

2927

2928 ☐Yes ☐No

2929

2930 2. Do you know any trees growing here that are not from South Africa?

2931

2932 ☐Yes ☐No

2933 3. Can you name six trees (Sotho or English)?

2934

Tree number	Common name
1	
2	
3	
4	
5	
6	

2935

2936 4. Do you know Black locust?

2937

2938 ☐Yes ☐No

2939

2940 5. Is there Black locust near your homestead?

2941

2942 ☐Yes ☐No

2943

2944 6. Are there many Black locust trees in your area?

2945

2946 ☐Few (Individual trees) ☐A number (scattered) ☐Many (Clusters)

2947

2948 **C. UTILIZATION**

2949

2950 7. Do make use of trees and tree products at your homestead?

2951

2952 ☐Yes ☐No

2953

2954 8. Do you make use Black locust?

2955

2956 ☐Yes ☐No

2957

2958 9. How often do you make use of tree and tree products?

2959

2960 ☐Daily ☐Weekly ☐Monthly ☐Yearly ☐Every 10 years

2961

2962 10. How do you get the tree or tree products?

2963

2964 ☐Own harvesting ☐Clearing programmes ☐Both

2965

11. Please indicate whether you make use of the following trees in any of the following ways

Black locust / Sotho name



☒					

Silver wattle/Bloukatlele



☒					

Red firethorn/k'hok'ho/ponaponana



☒					

Grey poplar/Popoliri



☒					

Bluegum/Boloukomo



☒					

Maritime pine/Phaena



☒					

- 1 11.2. For each of the trees listed above, can you think of any other uses of the tree that are not
2 covered above? If so, please list them below.

Black locust:

Silver wattle:

Red firethorn:

Grey poplar:

Bluegum:

Maritime pine:

- 3 12. Do you sell any or part of Black locust trees to make a living?

4 ☐ Yes ☐ No

- 5 13. If yes, please list the products that you sell in the table below.

Product	Quantity sold/year	Price/unit (ZAR)
Firewood	--- Bakkie loads	
Charcoal	--- Bags	

- 6 14. Can you list the major uses of the money generated from these sales? (E.g. To buy food)

7 _____

8 _____

9 _____

10

11

12

13 15. Does Black locust affect you in any of the following ways?

EFFECT	YES	NO	NOT SURE
It is taking up arable area			
It is poisoning livestock			
It is spreading uncontrollably on the homestead			
It is harboring pests (rats, biting flies etc.)			
Hiding criminals			

14

15 Please list any other negative impacts of Black locust not listed above

16 _____

17 _____

18 _____

19 _____

20 **D. MANAGEMENT:**

21

22 16. Is Black locust being cleared removed from your area?

23

24 ☒Yes ☐No

25 17. If yes, who is removing the trees?

26

27 ☐Government ☐Community ☐Individuals

28

29

18. Why they are removing the trees?

19. How are the trees being removed?

☐ **Mechanical** (Physical removal of plants, chopping)

☐ **Chemical** (Use of herbicides, poisons)

☐ **Both**

20. Is the number of Black locust trees going down?

☐ Yes ☐ No ☐ N/A

E. CONCLUSION

Your assistance, time and input are greatly appreciated.

If there is anything further that you would like to make comment on, note, suggest, raise or otherwise divulge please feel free to include it in the “Additional Notes” section at the end of this booklet.

May I please remind you that none of this information will be shared publicly without your prior consent and that if you have any issues, queries or otherwise wish to get hold of me my email address is 2018446340@ufs4life.ac.za and I am available on 0843542646

Thank you for your time and all the best in all your endeavours.

51 **Additional Notes:**

52 _____

53 _____

54 _____

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Animal Research Ethics

19-Aug-2019

Dear Dr Frank Chidawanyika

Student Project Number: UFS-AED2019/0103

Project Title: Biological control prelease studies on the ecological impacts and biotic interactions of Robinia pseudoacacia L. (Black locust) with indigenous fauna and flora in South Africa

Department: Zoology and Entomology Department (Bloemfontein Campus)

You are hereby kindly informed that, at the meeting held on , the Interfaculty Animal Ethics Committee approved the above project.

Kindly take note of the following:

1.
A progress report with regard to the above study has to be submitted Annually and on completion of the project. Reports are submitted by logging in to RIMS and completing the report as described in SOP AEC007: Submission of Protocols, Modifications, Amendments, Reports and Reporting of Adverse Events which is available on the UFS intranet.
2.
Researchers that plan to make use of the Animal Experimentation Unit must ensure to request and receive a quotation from the Head, Mr. Seb Lamprecht.
3.
Fifty (50%) of the quoted amount is payable when you receive the letter of approval.

Yours Sincerely

Mr. Gerhard Johannes van Zyl

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GENERAL/HUMAN RESEARCH ETHICS COMMITTEE (GHREC)

11-Nov-2020

Dear Dr Frank Chidawanyika

Application Approved

Research Project Title:

Contributions of invasive alien tree, Black locust (*Robinia pseudoacacia* L.) to livelihoods of peri-urban dwellers in Clarens, South Africa

Ethical Clearance number:

UFS-HSD2020/1448/1111

We are pleased to inform you that your application for ethical clearance has been approved. Your ethical clearance is valid for twelve (12) months from the date of issue. We request that any changes that may take place during the course of your study/research project be submitted to the ethics office to ensure ethical transparency. Furthermore, you are requested to submit the final report of your study/research project to the ethics office. Should you require more time to complete this research, please apply for an extension. Thank you for submitting your proposal for ethical clearance; we wish you the best of luck and success with your research.

Yours sincerely

Dr Adri Du Plessis

Chairperson: General/Human Research Ethics Committee

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An assessment of the invasive alien tree, *Robinia pseudoacacia* canopy traits and its effect on grassland microclimates and subsequent arthropod assemblages

Gerald Chikowore^{1,2} · Grant D. Martin² · Frank Chidawanyika^{1,3} Received: 27 January 2021 / Accepted: 30 March 2021
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Abstract

Invasive alien trees transform landscapes and subsequent ecosystem function. For grassland ecosystems, fragmentation following invasion by alien woody species is of concern. In this study, we examined how an invasive alien tree, *Robinia pseudoacacia* L. (Fabaceae) impacts grassland microclimates and the subsequent assemblages of endemic arthropods. The phenological trajectory of *R. pseudoacacia*, temperature and light intensity, as well as arthropod abundances in both invaded and uninvaded habitats, were monitored. Results showed that *R. pseudoacacia* significantly lowers average understory temperatures and light penetration in grassland ecosystems. Average temperatures were at least 2 °C lower in understory habitats than open grasslands while light intensity was lower by more than 1200 lumens/ft². In addition, arthropod communities differed between invaded and uninvaded habitats. The most affected taxa were Acridids from the order Orthoptera as they were excluded from understory habitats while Coleopterans were more abundant under *R. pseudoacacia*. Apart from absence of main host plants, invasion mediated differences in microenvironments appeared to be the main driver of this shift in affected arthropod taxa.

Implications for insect conservation These results highlight the need to sustainably manage *R. pseudoacacia* invasions especially in grassland ecosystems. In addition to their conservation value due to their role in nutrient cycling, grassland arthropods also serve as important indicators of ecosystem health. Hence, they can be used to monitor ecosystem recovery post invasive alien tree management.

Keywords Afromontane · Biological invasions · Ecological interactions · Microhabitats

Introduction

Habitat transformation, climate change and invasive alien species are the major threats to ecosystem structure, integrity and function (Erviner et al. 2012; Walther et al. 2009). By interfering with coevolved interactions between biotic and abiotic components, invasive alien species modify

ecosystems, to the detriment of their integrity and human wellbeing (Shackleton et al. 2019; Walther 2010). Impacts of invasive alien species on ecological interactions can be direct or indirect. For example, dietary shifts have been observed especially in pollinators following invasion by plants with showy inflorescences (Gibson et al. 2013) or increased dominance of canopy dwelling arthropods when grasslands are invaded by woody trees (Andersen et al. 2019). Of interest, is how invasive plants can alter ecosystem structure, community assembly and subsequent trophic interactions such as predation and competition.

Plants have the highest number of taxa invading terrestrial and aquatic environments and as such, many studies have been conducted to quantify their impacts (van Wilgen et al. 2020). For example, there is ample evidence to show that invasive alien plants (IAPs) modify ecosystems through reduction of water availability and quality (Le Maitre et al. 2002), change soil chemical composition

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(Marchante et al. 2008) and reduce native plant and animal diversity (Hejda et al. 2009). These impacts result from complex interaction between the invaded environment and IAP functional traits (Yelenik et al. 2007). Thus, IAPs, through their functional traits, which include canopy characteristics, seed production rates, water use and biological nitrogen fixation can alter microclimates and microhabitats (van Kleunen et al. 2010; Milanovic et al. 2020). Furthermore, the release of root and leaf leachates may have inhibitory effect on the growth of competing plants (Thiébaud et al. 2019). Alteration of the physical environment by changing thermal regimes and illumination due to the shading effect of IAPs may also result in the creation of novel environments in which natives fail to adapt or compete (Lazzaro et al. 2018). This can lead to a gradual change in plant succession trends, an effect that cascades through trophic levels (Schirmel et al. 2016).

Grasslands are the largest biome covering over 40% of the global surface area, making them habitat to many endemic plant and animal species worldwide (Suttie et al. 2005). Moreover, grasslands provide natural grazing as a service to both wildlife and range-based livestock production systems (Palmer and Ainslie 2005; Humphrey et al. 2019; O'Connor and van Wilgen 2020). Unfortunately, this biome is rapidly being transformed due to natural and anthropogenic disturbances (van Wilgen et al. 2008). For example, highland grasslands in South Africa are becoming fragmented due to agricultural activity as well as invasion by a number of alien plants (Wigley et al. 2010; Carbutt 2012). Woody plants, in particular, have been successful invaders in grasslands as they have superior traits such as high canopy cover which alter habitat conditions for native grassland flora (Hejda et al. 2009; Chikowore et al. 2021).

To fully understand the influence of invasive alien plant traits on invaded communities, it is necessary to study the ecology of the species in their native and invaded ranges. This includes knowledge of the growth season and characteristics of the plants. The growth season of plants is defined by their phenology, which in turn influences certain ecological processes (Richardson et al. 2018). For instance, plant productivity, carbon storage, evapotranspiration and trophic interactions such as predation and pollination all depend on plant phenology (Brown et al. 2016; Richardson 2019). Spatio-temporal variability in phenology also offers an opportunity to monitor forest health as it is linked to climatic variation and other stresses such as pest damage (Richardson et al. 2009). For example, the impact of late spring frost on the deciduous tree, American buckeye, *Aesculus glabra* Wild. (Sapindaceae), induced stresses which persisted beyond the year of occurrence (Augspurger 2011). Therefore, monitoring the timing and intensity of canopy greenness enables the tracking of critical plant growth phases and helps to predict key ecological processes.

Temperatures also have a direct impact on plant and animal growth processes (Sendall et al. 2015). At the centre of the climate change debate temperature fluctuations are at the core. This is particularly important for endemic ectotherms, which rely on ambient temperature for their thermoregulation. Forest shelters modulate microclimates creating conditions that are unique and differentially suitable for other organisms. For instance, in a study to quantify the effect of canopy openness and seasonality on microclimates in France, Gaudio et al. (2017) found minimum temperatures to be higher and maximum temperatures to be lower under tree canopies than in open lands especially in summer. Microclimates also tend to vary significantly at very fine spatio-temporal scales (Pincebourde et al. 2016). In grassland ecosystems, the introduction of trees creates wetter and cooler conditions, which are novel to resident biota (von Arx et al. 2013). The growth of understory vegetation under these conditions will therefore depend on the incident solar radiation which influences temperatures. Moreover, invasive alien trees are associated with the homogenization of understory habitats, reducing food resources and habitats for specialist organisms (Andersen et al. 2019; Kadlec et al. 2018; Chikowore et al. 2021).

For ectotherms, body temperature is dependent on the environment (Chown and Nicolson 2004; Chidawanyika et al. 2017, 2020). Thus, invasion-mediated modification of vegetative structure can affect behavioural thermoregulation among ectotherms and ultimately their assemblages (Clusella-Trullas and Garcia 2017). For example, Watling et al. (2011) reported that alteration of microclimates by the invasive alien shrub, *Lonicera maackii* (Rupr.) Maxim. (Caprifoliaceae) the Amur honeysuckle negatively impacted amphibian species richness and composition in Missouri, USA. This was despite the absence of a direct trophic linkage between the two taxa. Similarly, heterogeneity on alpine plant assembly was recently shown as a mediating factor among flower visitation rates (Ohler et al. 2020), underlying the importance of vegetative structure on arthropod assemblages.

Arthropods, being poikilothermic are highly sensitive to changes in thermal environments (Chown and Terblanche 2006; Chidawanyika et al. 2020). Hence, fluctuations in ambient temperatures have a direct bearing on life history traits such as survival, reproduction and development (Jaworski and Hilszczanski 2013; Ahn et al. 2016; Chidawanyika et al. 2017). Their sensitivity and assemblages thus make them good indicators of changes in microclimatic conditions (Gerlach et al. 2013; Strobl et al. 2019). Their trophic association with plants as primary consumers also makes them suitable as model organisms for studying IAP impact (Mooney et al. 2010). Species diversity has been found to be conditioned by vegetation structure, with dominant plant species playing a larger role (Farrell et al. 2015)

where a decline in arthropod populations could be used as indicators of natural ecosystem degradation (Lister and Garcia 2018).

Robinia pseudoacacia, a deciduous tree originating from North America which is currently widely distributed across the globe is one of the alien trees invading Afro-montane grasslands in South Africa where it is regarded as an IAP (Henderson and Wilson 2017; Martin 2019). Among its functional traits is the ability to change soil chemical properties and high growth rates especially in forest gaps (Cierjacks et al. 2013; Vitkova et al. 2015). The tree has been reported to alter micro-arthropod, soil microbial and plant communities in invaded areas (Lazzaro et al. 2018). Furthermore, in Central Europe, Kadlec et al. (2018) linked *R. pseudoacacia* to reduced richness in canopy arthropods, particularly lepidopterans due to lack of adaptation to feed on the tree.

Given the rapid expansion of *R. pseudoacacia* in South Africa (Martin 2019), this study aimed to determine the species phenology, particularly in highland grasslands, in order to ascertain its influence on the microclimates below the canopy and its impact on the grassland arthropods in this biome. Specifically, we asked: 1. What are the seasonal growth characteristics of *R. pseudoacacia* in the southern hemisphere and South Africa? 2. What is the impact of *R. pseudoacacia* canopies on understory temperature and light in highland grasslands? and, 3. How *R. pseudoacacia* mediated habitat changes influence invertebrate assemblages?

Materials and methods

Study area

The study was conducted between February 2019 and October 2020 (21 months) on the Northern Slopes of the Maluti-Drakensberg Mountains in South Africa (28° 32' 33.08" S; 28° 29' 30.91"E). Climate for the area according to the Köppen-Geiger classification is subtropical highland (Cwb) with annual temperatures and rainfall averaging 13.7 °C and 693 mm respectively (Beck et al. 2018). Due to the high altitudes > 1600 m sub-zero temperatures, frequent frost and snow are also common in winter. The area falls within the high altitude or Drakensberg grassland biome where the native vegetation is dominantly grasses of the Poaceae family although woody natives such as *Leucosidea sericea* and *Protea* species are also found (Mucina and Rutherford 2006). Landuse in the area is a mixture of range based animal production, cereal and fruit production interspaced with conservation areas. Surveys were conducted on two farms, Clifton and Sunnyside where *R. pseudoacacia* invasions (hereafter 'invaded') and open grassland (hereafter 'uninvaded') were sampled (Fig. 1). Vegetation in invaded

habitats on both farms is dominated by early successional ruderals such as *Tagetes minuta* L. (Asteraceae) as well as alien grasses such as *Bromus catharticus* Vahl. (Poaceae), *Brachypogon distachyon* (L.) P. Beauv. (Poaceae) and *Dactylis glomerata* L. (Poaceae). In contrast, uninvaded habitats are dominated by native grasses such as *Themeda triandra* Forssk. (Poaceae), *Tristachya leucothrix* Trin. (Poaceae) and *Eragrostis* species (Chikowore et al. 2021). The linear distance between the two farms is ~ 11 km with an altitudinal difference of ~ 100 m above sea level.

Robinia pseudoacacia phenology

To determine the phenology of *R. pseudoacacia* in high altitude grasslands of South Africa, two Wingscapes Plant-Cam® with Red, Green and Blue (RGB) capability were mounted on positions overlooking stands at Clifton and Sunnyside between October 2019 and October 2020. Cameras were set facing the south, to avoid direct sunlight on the lenses, at a setback of 5 m from the target area. Still images were captured at 1 h intervals between 7 am and 4 pm, at a resolution of 2560 × 1920 pixels giving a total of 10 images per day. At the end of the recording season, images were downloaded for processing and analysis as explained later.

Temperature and light measurements

The influence of *R. pseudoacacia* on grassland thermal and illumination environments were measured for 499 days between February 2019 and October 2020. Four HOBO MX2202 (Onset, www.onsetcom.com), temperature and light loggers were installed in three *R. pseudoacacia* stands and one in the adjacent open grassland. The loggers were programmed to record at 30-min intervals giving 48 readings of each parameter per day. Loggers were serviced quarterly and data were downloaded using the Bluetooth linked HOBO mobile application.

Arthropod assemblages

Arthropod communities associated with uninvaded and habitats were sampled on two farms over two seasons (spring and summer) in October 2019 and February 2020. Sampling was done in *R. pseudoacacia* stands and adjacent open grassland with a distance of 50 m between the treatments. At each site, 3 grids of pitfall traps were set in a 2 × 3 grid with an inter-trap distance of 2.5 m and an inter-grid space of 10 m. This configuration gave a total of 18 pitfall traps per treatment (invaded/uninvaded), 36 traps per site and 72 traps for the two sites. The traps consisted of 400 ml plastic containers dug into the ground with the rim flush with the soil surface. Each trap was filled with 100 ml of 100% propylene glycol (propane-1,2-diol) and left open for 5 days. The traps were

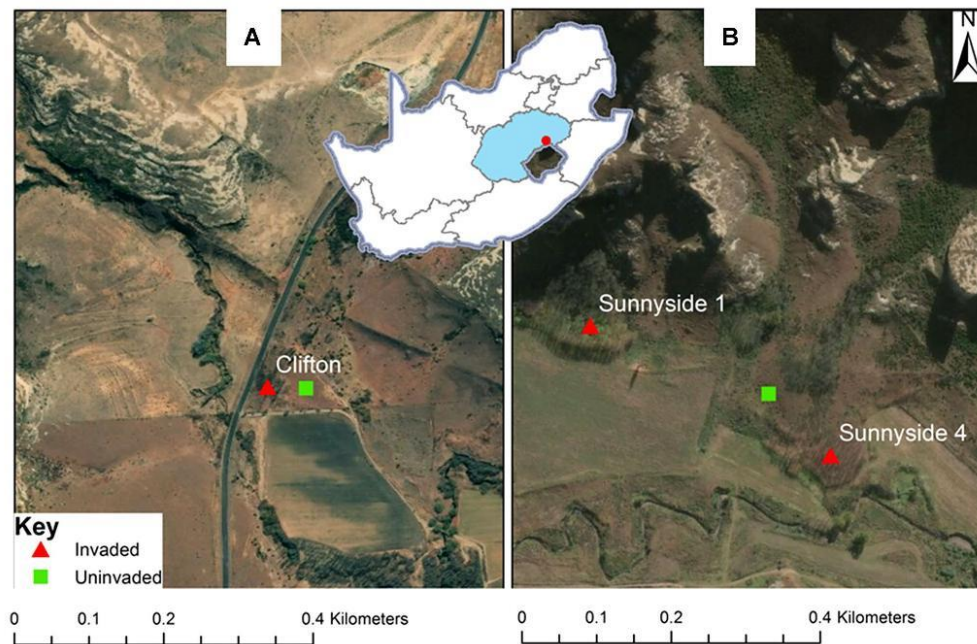


Fig. 1 Study area showing *R. pseudoacacia* stands (red triangles) and adjacent grasslands (green squares) sampled at Clifton (a) and Sunnyside (b) farms in eastern Free State Province, South Africa. Back-

ground satellite imagery source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community. (Color figure online)

then emptied and specimens were preserved in 70% alcohol. All specimens were identified to order with the exception of Orthoptera which was further identified to family level. Taxonomic guides by Picker et al. (2004) and Johnson and Triplehorn (2004) were used in the identification.

Statistical analyses

To visualize temperature and light conditions in invaded habitats and open grassland, readings were plotted against sampling time in Excel (Microsoft Office 2016).

Key assumptions of analysis of variances (ANOVA) were met for homogeneity of variance and normality of data distributions. Hence, differences among temperature and light conditions were compared using one-way ANOVA in STATISTICA version 7 (TIBCO Software). Tukey–Kramer post hoc tests were then used to separate statistically significant groups.

Plantcam images were analysed using the ‘phenopix’ package (Filippa et al. 2016) in R software version 3.2.1 (R Core Team 2020). Regions of interest were defined on a reference image using the ‘DrawMultiroi’ function while

the ‘extractVIs’ function was used to extract indices from the Red, Blue and Green channels of the images. Maximum green chromatic coordinates were extracted using an ‘auto-filter’ and plotted against the sampling period to determine the growth season of the two stands. Vegetation greenness metrics were determined using the Green Chromatic Coordinates, computed using the following formula:

$$G_{CC} = \frac{G_{DN}}{R_{DN} + G_{DN} + B_{DN}}$$

where G_{DN} , R_{DN} , B_{DN} represent digital numbers from the green, red and blue image channels (Richardson et al. 2009).

Since arthropod assemblage data did not conform to normality tests, they were subjected to multivariate generalised linear modelling using the ‘mvabund’ package in R (Wang et al. 2012). The ‘manyglm’ function was used to model the effects of habitat (invaded/uninvaded), season (summer/winter) and farm (Clifton/Sunnyside) on arthropod abundances. A negative binomial distribution was applied to account for mild over-dispersion observed with a Poisson distribution. Univariate tests were further performed to

assess the response of each insect order to the explanatory variables defined. Statistical significance of the effects was then assessed using Likelihood-Ratio Tests (LRT).

Results

Robinia pseudoacacia phenology

Robinia pseudoacacia canopy development started in October, peaking up between December and January (Fig. 2a). However, there was spatial variability in leaf-up periods with trees at Sunnyside farm developing leaves early (Fig. 2b) as indicated by high green chromatic coordinates (Gccs) of more than 0.4 at the time of camera set up when compared to Clifton farm which began from low Gccs of 0.32. A drop in canopy greenness was also evident at Clifton with the Gccs

dropping from peak to values approximating senescence, indicating total defoliation (Fig. 2a). This defoliation was coincident with a sharp drop in temperatures (Fig. 3a). This only affected the stand at Clifton farm as the other stand showed a gradual decline in greenness consistent with normal canopy characteristics rather than stress. However, the stand at Clifton farm recovered and assumed a normal phenological curve. The phenological trajectory observed is typical of deciduous trees in the southern hemisphere where leaves are shed in winter and leaf development starts from spring into summer.

Thermal and illumination environments

Average temperatures were higher in open grassland (15.6 °C) as compared to *R. pseudoacacia* understorey (12.76–13.77 °C) (Fig. 3a). The site effect was significant

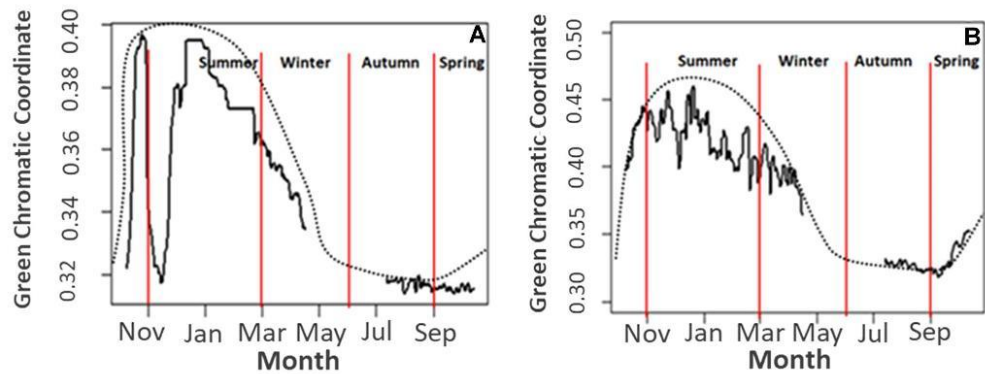


Fig. 2 Changes in the green chromatic coordinates (Gcc) on three *R. pseudoacacia* stands at Clifton farm (a) and Sunnyside farm (b). The broken line represents the expected phenological trajectory while the continuous line represents observed indices

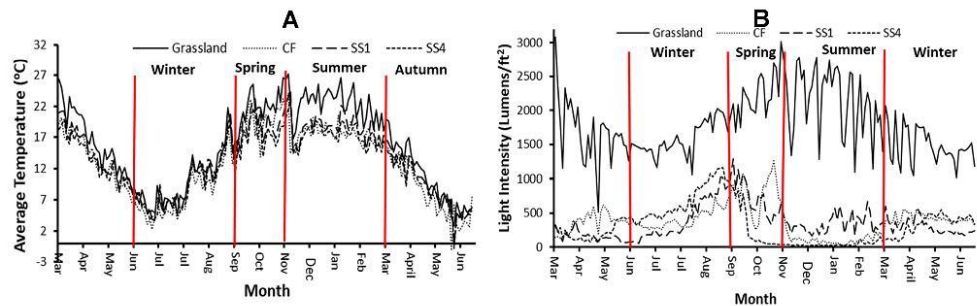


Fig. 3 Three-day moving average and seasonal temperature (a) and light intensity (b) in open grassland and *R. pseudoacacia* stands in eastern Free State, South Africa (Sites are abbreviated as follows: CF Clifton, SS1 Sunnyside 1 and SS4 Sunnyside 4)

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in explaining variations in temperature ($p < 0.001$) (Table 1). *Post-hoc* tests showed that average temperatures were significantly higher in open grassland than all *R. pseudoacacia* stands (Fig. 4a). However, there were no significant differences in temperatures between stands. Similarly, light intensity was higher in open grassland (1788.1 lumens/ft²) than under *R. pseudoacacia* canopies (378.5–316.3 lumens/ft²) (Fig. 3b). Thus, site (invaded or uninvaded) significantly explained this variation (Table 1). *Post hoc* tests also indicated that open grassland received significantly high illumination than *R. pseudoacacia* stands while there were no differences between the stands (Fig. 4b). However, there was intra-stand variability in light intensity at Sunnyside farm

4 as indicated by the wide confidence interval of the mean (Fig. 4b). However, open grassland experienced extreme temperatures both minimum and maximum in comparison to understory habitats (Table 2).

Invertebrate assemblages

The most abundant arthropods in both invaded and uninvaded habitats were Hymenopterans, particularly from the Formicidae family (Fig. 5). These were followed by Coleopterans and Araneae, respectively. However, unlike Hymenopterans, which were more abundant in open grassland, the latter orders were more abundant under *R.*

Table 1 Summary ANOVA output of prevalent temperature and light intensity in sampled *R. pseudoacacia* stands in South Africa

Parameter	Effect	Sum of squares	Df	Ms	F	p-value
Temperature	Intercept	375,989.5	1	375,989.5	10,676.27	< 0.0001
	Site	2737.7	3	912.6	25.91	< 0.0001
	Error	69,871.1	1984	35.2		
Light intensity	Intercept	8,712,079	1	8,712,079	56.04	< 0.0001
	Site	667,037,681	3	222,345,894	1430.11	< 0.0001
	Error	232,279,151	1494	155,475		

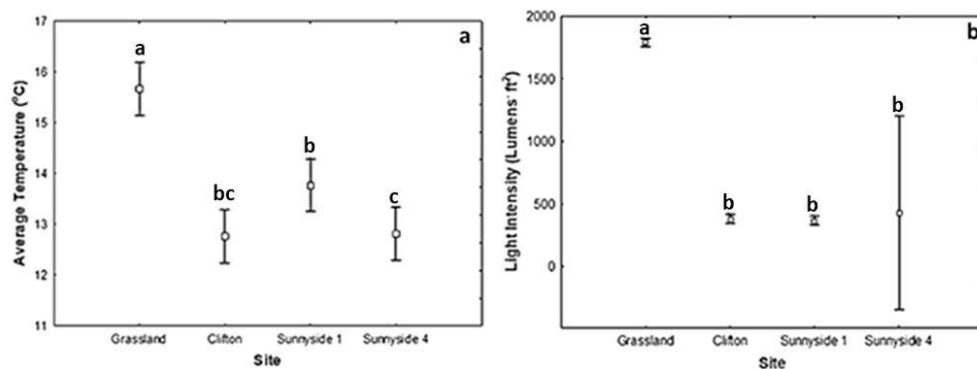


Fig. 4 Differences in temperature (a) and light (b) (mean $\pm$ 95% confidence interval) between grassland and three *R. pseudoacacia* stands. Means with different letters are significantly different from each other

Table 2 Minimum and maximum temperatures and maximum light intensity recorded at microhabitats in eastern Free State, South Africa

Site	Elevation	Minimum temperature (°C)	Maximum temperature (°C)	Maximum light intensity (Lumens/ft ²)
Grassland	1731	− 16.99	56.54	13,003
Clifton	1712	− 14.72	43.58	7460
Sunnyside 1	1833	− 7.81	50.53	9578
Sunnyside 4	1832	− 12.01	47.83	7247

Data represents the extreme recording for each site

Fig. 5 Abundance of arthropods (median, minimum, and maximum) in habitats invaded by *R. pseudoacacia* and uninvaded grasslands. All arthropods were grouped into orders except Orthopterans (which were grouped into 2 families, Acrididae and Gryllidae). Groups that significantly differed between habitats are indicated by ** while groups differing between seasons are indicated by *

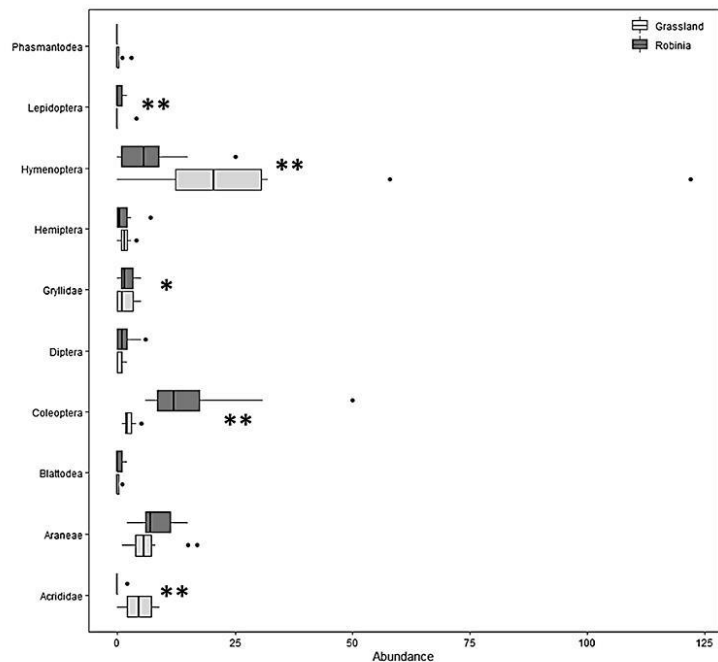


Table 3 Multivariate analysis for arthropod abundance in habitats invaded by *R. pseudoacacia* and adjacent uninvaded grassland at Clifton and Sunnyside farms, South Africa

Effect	Residual Df	Df diff	Deviance	Pr (> dev)
Intercept	23			
Habitat	22	1	55.85	0.001
Farm	21	1	15.14	0.055
Season	20	1	25.34	0.002
Habitat * Farm	19	1	9.76	0.160
Habitat*Season	18	1	24.40	0.002
Farm * Season	17	1	13.96	0.054
Habitat*Farm*Season	16	1	4.49	0.296

Bold denotes statistically significant groups

pseudoacacia trees. The family Acrididae (Orthoptera) was only recorded in open grassland while the family Gryllidae was common to both habitats although more abundant in the understory of *R. pseudoacacia*. From the multivariate analysis of variance, habitat (LRT = 55.85, $p = 0.001$), season (LRT = 25.34, $p < 0.05$), as well as interaction of habitat and season (LRT = 21.2, $p < 0.05$) had significant effects on arthropod abundances (Table 3). Univariate analysis showed that habitat had a significant

effect on Acridids, Coleopterans, Lepidoptera and Hymenopterans while season significantly affected Gryllids (Fig. 5).

Discussion

This study suggests that *R. pseudoacacia* is transforming grassland arthropod ecosystems through alteration of microclimatic conditions in the eastern Free State of South Africa. This is mainly due to its canopy characteristics which reduce temperature and light availability for understory communities, creating habitats with novel microclimates in the process. The results indicate that Orthopterans, particularly from the family Acrididae, declined significantly in invaded habitats while conditions created by the IAP seemed to favour the proliferation of Coleopterans. This therefore suggests that habitat transformation by IAPs differentially affects endemic arthropods.

The phenology of *R. pseudoacacia* in the southern hemisphere showed leaf emergence and expansion in spring (September), peaking between January and December. In contrast, leaf expansion occurs between April and May in the northern hemisphere (Cierjacks et al. 2013). While various environmental cues are responsible for phenological events

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in trees, this adaptation of *R. pseudoacacia* to climates in the southern hemisphere buttresses assertions of its high tolerance to a wide range of conditions. However, its phenology varies with local climatic conditions as our study showed spatial variability in leaf development as well as the length of growth season between sites. Moreover, its ability to recover rapidly following frost damage gives an insight into the competitive advantage in invaded ecosystems. Our results further showed that in sync with other deciduous trees in South Africa, *R. pseudoacacia* develops leaves early before the emergence of annual grasses. However, unlike native trees that are often sparsely populated in South African grasslands (Mucina and Rutherford 2006), the impact of *R. pseudoacacia* is exacerbated by monospecific continuous canopies covering relatively larger areas. This limits seedling recruitment of native vegetation including grasses due to lower temperatures and low illumination under the canopies (Chikowore et al. 2021). These realised microclimates influence community assembly cascading to other trophic levels. Indeed, *R. pseudoacacia* has been associated with a decline in both above and below-ground biodiversity. For example, Lazzaro et al. (2018) reported a decline in microarthropod, microbial and plant communities underlying the potent effects of *R. pseudoacacia* on ecosystem integrity.

In this study, average temperatures were consistently higher in the open grasslands as opposed to the invaded understory. However, when temperature readings were decoupled into minimum and maximum, grasslands recorded lower minimum temperatures and higher maximum temperatures. These findings are consistent with several studies that attribute these differences to the buffering effect of trees which ensures higher minimum and lower maximum temperatures in the understory (von Arx et al. 2013; Gaudio et al. 2017; Davis et al. 2019). In all these studies, temperature was influenced by the sheltering effect of trees, canopy cover and soil moisture. Although we did not measure hydrological parameters such as relative humidity and soil moisture in our study, their influence on temperatures observed is highly probable. For example, Breshears et al. (1998) asserted that trees can lower temperatures through evaporative cooling, underscoring the importance of moisture in temperature regulation. In addition, interception of incident solar radiation by tree canopies could also be the reason for lower temperatures.

The absence of Acridids (Orthoptera) from invaded stands is an indication of the impacts of *R. pseudoacacia* on microhabitats. While their exclusion might be due to invasion mediated vegetation changes which places limitations in food resources (Andersen et al. 2019), there is also a likelihood that environmental conditions influenced their absence, especially considering the generalist nature of their feeding habit. For example, Highland et al. (2013) found that habitat structure rather than plant diversity affected arthropod

diversity in woodlands while Pawson et al. (2010) tied variation in arthropod herbivores to physical habitat characteristics such as light availability and stand structure. Thus it is plausible that changes in arthropod communities observed in this study were influenced in part by changes in the structure of the grassland habitat as well as a shift in microclimatic conditions as a result of *R. pseudoacacia* invasion. Furthermore, Samways (1990) observed that Orthopterans are more susceptible to cold conditions hence they seek refuge on mountain slopes receiving more sunlight and ruled out vegetation type and cover as determinants of the distribution of this taxa. Similarly, Rada et al. (2015) found a strong correlation between temperature and Acrididae life history traits in central Europe, emphasizing the importance of microclimates on the taxa. While grazing by ungulates has been proven to alter community composition, particularly trophic cascades of grassland arthropods (Farrell et al. 2015), in our study both habitats were equally open to grazing by cattle. Therefore, differences in taxa across habitats could be a result of invasion mediated habitat modifications.

Our results also showed that there were more Coleopterans in invaded habitats than open grassland. This taxon is comprised of a wide range of detritivores, hence, their abundance might have been influenced by the quantity and quality of litter from *R. pseudoacacia*. In corroboration with these findings, Alerding and Hunter (2013) found that *Alliaria petiolata* M. Bieb. (Brassicaceae) invasion was associated with an increase in the depth of litter which increased the abundance of detritivores. Although social arthropods such as ants can regulate their internal and surrounding environments through elaborate nest designs and physical mechanisms (Kadochova and Frouz 2014), our study showed that habitat had a significant effect on their abundance. Although there was a possibility of some trapping positions located close to ant nests and foraging grounds, the role of microclimates cannot be ruled out. For example, Silva et al. (2011) reported that the choice of ant nesting sites is also influenced by microclimatic conditions, quality and quantity of leaf litter thereby mediating ant distribution. Even though we did not measure these parameters in our study, there is a possibility that *R. pseudoacacia* leaf litter makes understory habitats unfavourable for ants.

In conclusion, invasion by *R. pseudoacacia* alters thermal and illumination environments of grassland ecosystems. Through canopy characteristics, microclimatic conditions within tree stands vary significantly from immediate local environments.

Conservation implications

The main goal of tackling biological invasions 'should be the conservation or restoration of ecosystems to preserve or re-establish native biodiversity and functions' (Hulme

2006). Countries are under increasing pressure to mitigate the negative effects of invasive species on ecosystem dynamics. However, in the absence of baseline data on how invasive species are directly or indirectly changing the ecosystems, it is difficult to measure a return to a functioning state following management (Richardson and Van Wilgen 2004; Gallardo et al. 2019). Due to the significant impacts of *R. pseudoacacia* in South African grasslands (Martin 2019; Chikowore et al. 2021), there is a need for its sustainable management. Motitsoe et al. (2020) showed recovery of an aquatic ecosystem following control of *Salvinia molesta* D.S. Mitch. (Salvinaceae) through monitoring algae and macroinvertebrate communities. Thus a similar approach might be taken for *R. pseudoacacia* using arthropod community assembly. While not all arthropod taxa observed in this study were affected by the physical and structural changes induced by the IAP, some showed enough sensitivity to enable their monitoring as indicators. For these taxa, before and after abundances can be used to assess the success of any control programme against *R. pseudoacacia*.

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Declarations

Conflict of interest The authors declare that there are no competing financial interests or personal relationships that could have influenced the work reported in this paper.

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ABSTRACT

The growing invasion of ecosystems by invasive alien plants (IAPs) has substantially affected biodiversity worldwide, compromising provision of ecosystem services. In this study, we present evidence of the impacts of an IAP, *Robinia pseudoacacia* L., on native plant diversity in montane rangelands of South Africa and its threats to grazing, an ecosystem service. We assessed stand characteristics, understory vegetation composition and rangeland condition similarities in invaded and uninvaded sites. We observed a shift in grass communities after invasion by *R. pseudoacacia* as invaded communities differed by 96% from uninvaded rangeland. Invaded habitat was dominated by nitrophilous, shade-tolerant alien ruderals that follow the primitive C₃ carbon fixation pathway. Nitrogen fixation and light-demanding properties of *R. pseudoacacia* are likely to be the main factors driving these changes. As a result, range condition was significantly lower in invaded habitats with smaller and dense trees (180 ± 24.3) (mean $\pm$ standard error) when compared with adjacent uninvaded habitat (401 ± 24.3). These preliminary findings support an urgent need for sustainable control of *R. pseudoacacia* as an effective approach to stop further reduction in grazing capacity and losses in livestock production.

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Introduction

Despite social and economic benefits, globalization has brought new challenges, notably the proliferation of transboundary movement of invasive species (Pysek and Richardson 2010). Some of the invasive alien species are ecosystem transformers and drivers of environmental change (Rilov et al. 2012), severely disrupting ecosystem integrity and provision of services necessary for human well-being (Pysek and Richardson 2010). For instance, invasive alien plants (IAPs) can substantially impact native vegetation (Hejda et al. 2009; Vitkova et al. 2020), thereby threatening biodiversity (Gaertner et al. 2009).

Upon establishment, IAPs disrupt complex ecosystem interactions, leading to changes in the structure and function of ecosystems (Wardle and Peltzer 2017). Hence, IAPs ultimately lead to shifts in the composition of native communities due to loss of

habitats or environmental modifications (Rilov et al. 2012). For example, invasion by nitrogen-fixing trees enriches soil nitrogen levels filtering out non-nitrophilous native plants in historically nutrient-poor ecosystems (Benesperi et al. 2012). This has been shown in South Africa, where Australian acacias have been widely associated with a decline in native vegetation communities due to nitrogen enrichment in nutrient poor soils of the fynbos biome (Witkowski 1991; Musil 1993; Le Maitre et al. 2011).

The high-elevation rangelands of South Africa defined by Mucina et al. (2006) as herbaceous, relatively short and simple structured vegetation are dominated by Poaceae graminoids. This biome is regarded as a key biodiversity hotspot with high plant endemism (Clark et al. 2014). However, biodiversity is increasingly affected by IAPs (Carbutt 2012). Although native species diversity has been identified as a barrier to invasion (Kennedy et al. 2002), rangelands present reduced resistance to invasion by tall woody species, which often dominate and cover native species (Hejda et al. 2009). Van Wilgen et al. (2008) identified the rangelands as one of the terrestrial ecosystems that require alien plant control primarily to conserve biodiversity. The biome is also an important resource supporting livestock production where invasion-driven changes in native flora have in some cases altered the grazing capacity of rangelands (Yapi et al. 2018).

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Despite an increasing body of knowledge on the impacts of IAPs and their management, (Richardson and van Wilgen 2004; van Wilgen et al. 2012; van Wilgen et al. 2020), the spatial extent of invasion and number of invading plants continues to evolve. One of the emerging IAPs in the high-elevation rangelands of South Africa is *Robinia pseudoacacia* L. (Fabaceae, Papilionoideae) (Henderson 1991; Martin 2019). The tree is native to North America and has now been declared invasive in 21 countries worldwide (CABI 2020). It can proliferate under a variety of environmental conditions (Nicolescu et al. 2018) and transforms the environment through shading (Haerdtle et al. 2003; Cierjacks et al. 2013) and soil nitrogen fixation (Dzwonko and Loster 1997; Vitkova et al. 2017; Campagnaro et al. 2018). Its ability to fix nitrogen often leads to the proliferation of nitrophilous understory vegetation (Benesperri et al. 2012), and as the soil nitrogen increases over time, transient colonization, followed by competitive displacement, occurs (Dzwonko and Loster 1997; Lazzaro et al. 2018). In South Africa, *R. pseudoacacia* is listed as a category 1b invasive alien plant under the National Environmental Management: Biodiversity Act (NEM: BA) (DEA 2016). Category 1b invasive alien plants are species whose importation and propagation are prohibited.

Increasing invasion of rangelands by *R. pseudoacacia* in South Africa has the potential to reduce the availability of grazing. Potential economic losses from invasion of rangelands by this IAP in South Africa alone were estimated to be approximately US\$39 million (Humphrey et al. 2019). Woody IAPs create novel conditions in rangelands that are unfavorable for native species (O'Connor and Van Wilgen 2020). This can lead to species displacement and a reduction in the grazing capacity of rangelands, especially when low-palatability forage species take over (Bankovich et al. 2016; O'Connor and Van Wilgen 2020).

The impacts of *R. pseudoacacia* on native biodiversity in South Africa remain poorly understood as evidence of its impacts is mainly anecdotal. A paucity of data on the tree was also noted in the southern hemisphere as compared with the northern hemisphere (Martin 2019). However, extrapolation of findings from studies of *R. pseudoacacia* in the northern hemisphere is difficult as there are often variations in the extent of invasion, the environment, and taxonomic groups affected (Clusella-Trullas and Garcia 2017).

To understand and adequately respond to the challenges of IAPs, there is a need to assess impacts on a continuum linking plant traits, ecosystem processes, and services (Walker and Smith 1997; Parker et al. 1999). This is critical in defining management goals that are specific and benchmarking future evaluation of interventions. The present study therefore investigated the impacts of *R. pseudoacacia* on native plant communities and cascading effects on the grazing capacity of montane rangelands in Eastern Free State Province, South Africa. The aims were to determine 1) if *R. pseudoacacia* is driving changes in the richness and composition of grass and forb communities, 2) if there are any relationships between vegetation community dynamics and *R. pseudoacacia* stand characteristics of the invading species, and 3) whether there are any changes in the grazing capacity of rangelands as a result of invasion.

Materials and Methods

Study Site

The study was conducted at Clifton (28°32' 48.08"S; 28°25'08.91"E) and Sunnyside (28°32'12.87"S; 28°31'59.60"E) properties in the eastern Free State Province, South Africa, between February 2019 and February 2020. This area falls under the subtropical highland climate (Cwb) according to the Köppen-Geiger climate classification with temperatures averaging 13.7°C

and an average annual rainfall of 693 mm (Beck et al. 2018). Vegetation in this area is mainly composed of grasses with climax indigenous species such as *Themeda triandra* Forssk. (Poaceae) and *Cymbopogon dieterlenii* Stapf ex Schweick. (Poaceae) dominating (Du Preez and Venter 1992). However, there is an increasing incidence of woody species, most of which are non-native (Carbutt 2012). Agriculture is the primary land use within this region with livestock, cereal, and fruit production as the major enterprises (Hensley et al. 2006).

Robinia pseudoacacia Stand Characteristics

Stand characteristics based on one population parameter, density (trees/hectare), and one plant level measurement, diameter at breast height (DBH), were assessed for four tree stands, two at Clifton and two at Sunnyside properties. Sampling was done within three 10 × 10 m quadrats established 10 m apart along 50-m transects randomly selected within each stand. The number of trees in each quadrat was counted, and the DBH of each tree was measured at approximately 1.3 m from the soil surface using a steel diameter tape (West 2009). For forked trees, measurements were taken just below the fork while for multitemmed trees, the base was measured. To measure variation in light penetration through *R. pseudoacacia* canopy, hemispherical images were captured from 10 randomly selected locations in each of the four stands. The images were captured using a 170-degree, Voyager Explorer 4K Ultra HD activity camera in February 2020 when *R. pseudoacacia* trees were in full leaf and on a cloudy day to avoid the effects of the sun. The images were then transferred to a Samsung Galaxy J2 Core smartphone for determination of canopy cover indices using the mobile application Gap Light Analysis Mobile Application (GLAMA) (Tichy 2016). The application distinguishes between dark and light pixels from the canopy hemispherical images, classifying them as either vegetation or sky in the process. The Canopy Cover index (CaCo), a projection of canopy cover onto the surface, is then calculated and expressed as a percentage (Tichy 2016).

Understory Vegetation Composition

These surveys were conducted between December 2019 and February 2020, when most of the grasses were flowering, for ease of identification. Sampling was done in two habitats, patches of *R. pseudoacacia* ($n = 3$) (hereafter "invaded") and adjacent open rangeland ($n = 3$) (hereafter "uninvaded"). The fourth site (Sunnyside 4) had no vegetation, so it was excluded from these surveys. Invaded and uninvaded sites 50 m apart were surveyed, while sampling within invaded sites was done at least 5 m from the edge to minimize edge effects. Pairing of sites was done to ensure similar environmental conditions. Although uninvaded sites chosen were used as pasture, care was taken to select areas where there was no history of soil disturbance based on property owners' accounts. Invaded and uninvaded sites were exposed to the same grazing pressure by livestock. The line-point intercept method was used to estimate plant species cover and composition. The method uses a length of nonstretch rope marked at 1-m intervals and a handheld rod. Starting at zero, and working from left to right and on the same side of the line each time, one moved to the first point on the line and dropped a rod from a height of 50 cm without guiding it to the ground. Once the rod reached the ground, all plant species it intercepted in descending order were recorded from top to ground cover (Herrick et al. 2005). Sampling was done at each site along 4 transects, 50 m long and 5 m apart, giving a total of 200 points per site and a total of 1 200 points in both invaded and uninvaded habitats. Plants were grouped into two functional types (i.e., grasses and forbs [nongraminoid herbaceous plants]),

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and these were identified to species level by taxonomists with the aid of guides by Moffett (1997) and Van Oudtshoorn (1999).

Invaded and Uninvaded Rangeland Condition Similarities

The total number of top canopy strikes for each species was calculated as a percentage of total observations for the species. Grazing index values (GIVs), which range from 0 (low quality) to 10 (high quality), for grasses sampled in this survey were obtained from lists compiled by Esler et al. (2006). Grazing index values were determined using volumes, amount of grazeable dry matter, and chemical composition of the specific species (Du Toit 1995). For species without listed GIVs, the average for two phylogenetically close species was used. Range condition indices were then calculated for both habitats by summing the product of the percentage cover of species and their GIVs (Du Toit 1995).

Statistical Analyses

Data analyses were carried out using STATISTICA, version 7 (TIBCO Software), R software version 3.2.1 (R Development Core Team 2015) and Paleontological Statistics Software Package (PAST) (Hammer et al. 2001). *R. pseudoacacia* canopy cover, density, and DBH were analyzed using one-way analysis of variance in STATISTICA, and Tukey-Kramer post hoc tests were used to separate statistically significant groups. Vegetation data from invaded and uninvaded transects were grouped into two functional groups, grasses and forbs, and tested for sampling saturation using the Mao tau sample-based rarefaction method at 95% confidence interval in PAST. Since the curve for forb sampling did not reach an asymptote and preliminary analyses resulted in a high stress value (> 0.2), the data were not subjected to distance-based analyses (Fig. S1, available online at ...). To visualize the separation of grass communities between invaded and uninvaded plots, nonmetric multidimensional scaling (nMDS) was performed and the Bray-Curtis distance was applied using the “vegan” package (Oksanen et al. 2019) in R. Permutational Multivariate Analysis of Variance (PERMANOVA) was then used to test for differences in grass community composition between the invaded and uninvaded sites. To explore the contribution of each species to dissimilarity between sites, similarity percentages (SIMPER) were computed also using the Bray-Curtis distance. To test variation in species composition between the two habitats, the data were tested for the assumption of homogeneity of multivariate dispersion (Anderson 2006).

Differences in Range Condition Index were analysed using a linear mixed model (LMM) specified with Gaussian errors and a log link function (Bolker et al. 2009), with site (Clifton/Sunnyside) and habitat (invaded/uninvaded) as fixed effects. Quadrat and transect were included as random effects to account for potential nonindependence between data points (Bolker et al. 2009). A global (maximal) model was specified, including an interaction term between site and habitat and both random effects. The “dredge” function from “MuMIn” R package (Barton 2009), which specifies all possible combinations of fixed effects as possible models, was then used in a model selection framework to assess the importance of predictor variables. Model selection was performed by ranking all candidate models using Akaike's information criterion corrected for small sample sizes (AICc). Moreover, Akaike weights (w_i) were calculated to determine the degree of support for each candidate model being the best model. The sign and magnitude of the effect of each model term on range condition index scores were assessed by semiparametric bootstrapping of parameter estimates, using 999 bootstrap replicates, implemented with the “bootMer” function from the “lme4” R package (Bates et al. 2015).

Preliminary analyses showed that inclusion of transect as a random effect did not improve model fit when analyzing Shannon-Weiner (H') diversity and percentage grass and forb cover variables. As such, Shannon-Weiner diversity was analyzed using a GLM with Gaussian errors and a log link function, and vegetation cover was analyzed using a logistic GLM with binomial errors and a logit link function. For both models, site (Clifton/Sunnyside) and habitat (invaded/uninvaded) were specified as fixed effects.

Results

Robinia pseudoacacia Population Demographics and Canopy Characteristics

R. pseudoacacia canopy cover varied significantly between stands (Table S1, available online at ...). However, results of post hoc tests showed that there was no significant difference in canopy cover among three stands (Clifton 1, Clifton 2, and Sunnyside 1) while Sunnyside 4 had significantly higher canopy cover ($> 60\%$) (Fig. 1). Similarly, tree densities varied significantly across stands (Table 1). Sunnyside 4 had the highest density of trees while the lowest densities were recorded at Sunnyside 1 (see Fig. 1). DBH varied significantly across sites (see Table 1), and the widest tree stems were found at Sunnyside 1 while stem diameter did not differ significantly at the other three sites (see Fig. 1).

Effects of Invasion on Grass and Forb Species Community Composition

The nMDS plot showed that grass community composition differed between invaded and uninvaded rangeland with no overlaps between the two habitats (Fig. 2). The grass community in uninvaded rangeland was mainly composed of native species (e.g., *Tristachya leucothrix* and *Cymbopogon dieterlenii*) while exotic species such as *Dactylis glomerata* L. (Poaceae) and *Bromus catharticus* Vahl. (Poaceae) were dominant in habitats invaded by *R. pseudoacacia* (Fig. 3). There was a significant site (49%) and habitat (36%) effect ($P < .001$) in explaining the variation in grass community composition between the two vegetation communities (Table S2, available online at ...). Although the grass community in uninvaded habitats seemed to be more dispersed than in invaded habitats, the assumption of homogeneity multivariate dispersion was met ($F=1.16$, $P=.29$). The SIMPER analysis further showed that dissimilarity between invaded and uninvaded rangeland was 96%, of which $> 50\%$ was due to *E. biflora* (14.3%), *B. catharticus* (13.4%), *Tristachya leucothrix* (12.3%), and *Cymbopogon dieterlenii* (10.2%) (see Table 1). The two native grasses common to both habitats, *C. dieterlenii* and *T. triandra*, contributed significantly to differences in community composition between invaded and uninvaded habitats ($P < .05$), both being more abundant in uninvaded than invaded plots.

Grass species diversity varied significantly between uninvaded and habitats invaded by *R. pseudoacacia* ($F=29.41$, $P < .001$), but not sites ($F=2.10$, $P=.152$). There was no significant interaction effect between habitat and site ($F=1.06$, $P=.366$). Results of post hoc tests showed that grass species diversity was significantly higher in uninvaded rangeland at Clifton (Fig. 4). While grass species diversity at Sunnyside was marginally higher in uninvaded than in invaded sites, the differences were not statistically significant. On the contrary, forb species were more diverse in sites invaded by *R. pseudoacacia* at Clifton than uninvaded and invaded sites at Sunnyside (see Fig. 4). The effect of site contributed significantly to variation in forb diversity ($F=11.71$, $P=.001$). All forb species in invaded sites were early successional invasive alien species as compared with natives in uninvaded habitats.

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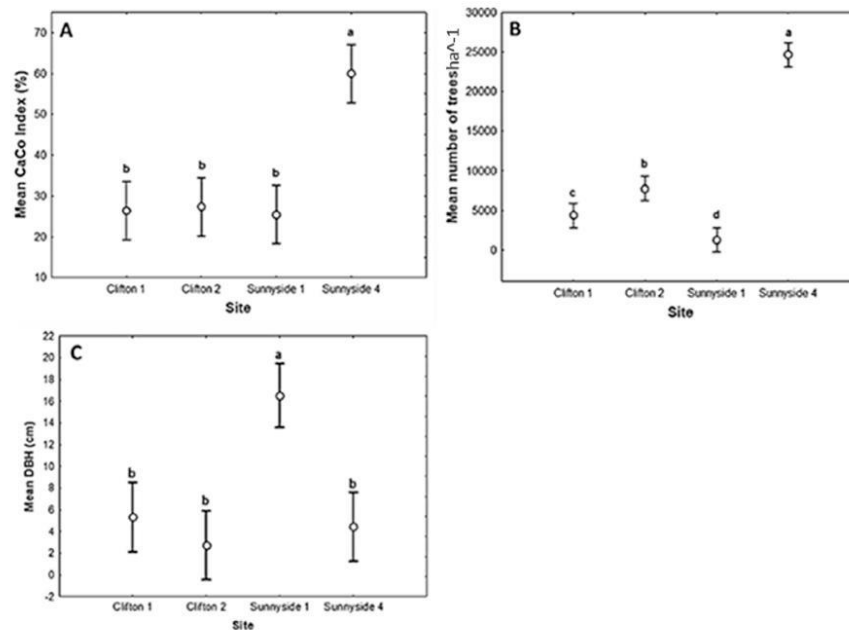


Figure 1. Differences in canopy cover (CaCo) (A), densities (B), and diameter at breast height (DBH) (C) of *Robinia pseudoacacia* across sites (mean $\pm$ 95% confidence limits). Means with the same letter are not significantly different from each other.

Table 1

Similarity percentages for grasses between open rangeland and under *Robinia pseudoacacia* stands based on Bray-curtis distance

Species	Average	SD	Ratio	Mean Veld	Mean Invaded	Cumulative sum	p-Value
<i>Eragrostis biflora</i> Hack. ex Schinz (Poaceae)	0.14	0.10	1.34	0	10.67	0.14	0.001
<i>Bromus catharticus</i> Vahl. (Poaceae)*	0.13	0.10	1.32	0	10.33	0.28	0.001
<i>Tristachya leucothrix</i> Trin. (Poaceae)	0.12	0.14	0.86	10.08	0	0.40	0.005
<i>Cymbopogon dieterlenii</i>	0.1	0.12	0.80	8.00	0.08	0.50	0.003
<i>Dactylis glomerata</i> L. (Poaceae)*	0.09	0.14	0.66	0	8.25	0.60	0.083
<i>Themeda triandra</i>	0.07	0.06	1.33	6.67	2.17	0.68	0.044
<i>Heteropogon contortus</i> L. (Poaceae)	0.05	0.05	0.90	4.00	0	0.73	0.003
<i>Aristida diffusa</i> Trin. (Poaceae)	0.04	0.07	0.57	3.08	0	0.76	0.018
<i>Brachypogon distachyon</i> (L.) P. Beauv. (Poaceae)*	0.04	0.07	0.54	0	3.17	0.80	0.158
<i>Elionurus muticus</i> (Spreng.) Kuntze. (Poaceae)	0.03	0.06	0.54	2.67	0	0.83	0.012
<i>Helictotrichon turgidulum</i> (Stapf) Schweick. (Poaceae)	0.03	0.02	1.24	2.25	0	0.86	0.001
<i>Eragrostis capensis</i> (Thunb.) Trin. (Poaceae)	0.03	0.04	0.58	2.17	0	0.89	0.005
<i>Aristida adscensionis</i> L. (Poaceae)	0.02	0.04	0.62	1.50	0	0.91	0.001
<i>Eragrostis curvula</i> (Schrader) Nees. (Poaceae)	0.02	0.05	0.42	0	1.42	0.93	0.140
<i>Eragrostis chloromelas</i> Steud. (Poaceae)	0.02	0.03	0.61	1.33	0	0.95	0.031
<i>Harpachloa falk</i> (L.f.) Kuntze. (Poaceae)	0.01	0.02	0.80	0.92	0	0.96	0.001
<i>Setaria pallide-fusca</i> (Schumacher) Stapf and C.E. Hubb. (Poaceae)	0.01	0.03	0.43	0	0.83	0.97	0.364
<i>Miscanthus capensis</i> (Nees) Andersson. (Poaceae)	0.01	0.02	0.44	0	0.75	0.98	0.420
<i>Microchloa caffra</i> Nees. (Poaceae)	0.01	0.01	0.37	0.42	0	0.99	0.074
<i>Eragrostis plana</i> Nees. (Poaceae)	0.01	0.01	0.44	0.42	0	0.99	0.088
<i>Penisetum sphacelatum</i> (Nees) T.Durand and Schinz (Poaceae)	0.01	0.01	0.55	0.33	0	1.00	0.001
<i>Paspalum dilatatum</i> Poir. (Poaceae)*	0.00	0.01	0.30	0.17	0	1.00	0.130
<i>Andropogon appendiculatus</i> Nees. (Poaceae)	0.00	0.01	0.30	0.17	0	1.00	0.138

* Non-native grasses

Effects of Invasion on Rangeland Grazing Index Values

Site, habitat, and the interaction between site and habitat were all statistically significant in explaining range condition index values (Table 2). Range condition was significantly lower in invaded habitats (180 ± 24.3) than uninvaded rangeland (401 ± 24.3) at Clifton. However, these differences were less noticeable at Sunnyside, where range condition in both invaded (432 ± 34.4) and un-

invaded habitats (450 ± 34.4) was better than both invaded and uninvaded habitats at Clifton. *Dactylis glomerata*, which had the highest grazing index value of 7.59, occurred under *R. pseudoacacia* at Sunnyside while in all uninvaded sites *T. triandra*, which was most abundant, had a grazing value of 6.81.

Vegetation (grasses and forbs) cover also followed a similar trend as it was significantly higher in uninvaded than invaded habitat at Clifton while at Sunnyside rangeland had slightly higher

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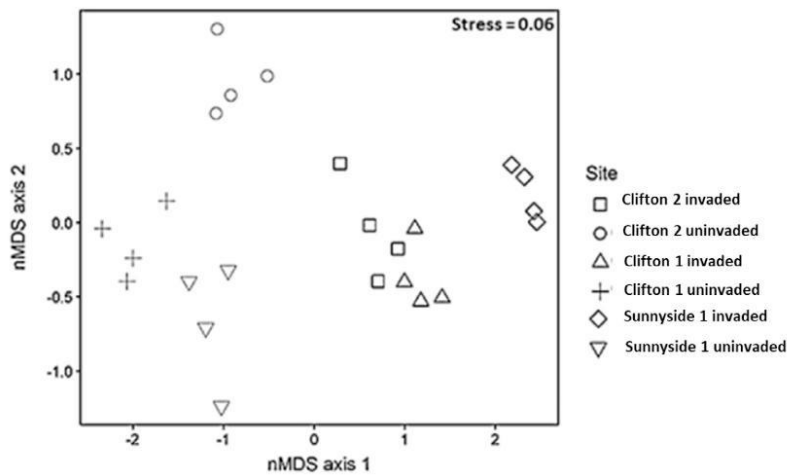


Figure 2. Two-dimensional nonmetric multidimensional scaling (nMDS) plot showing grass species composition in plots invaded by *Robinia pseudoacacia* and adjacent uninvaded rangeland based on Bray-Curtis distance.

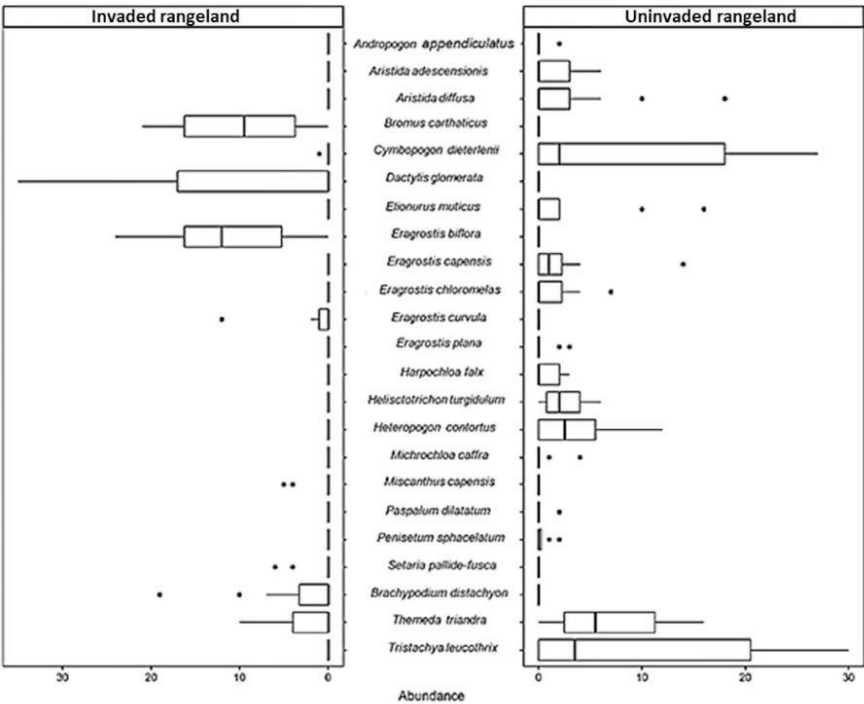


Figure 3. Grass species abundance in uninvaded rangeland and habitats invaded by *Robinia pseudoacacia* in eastern Free State, South Africa.

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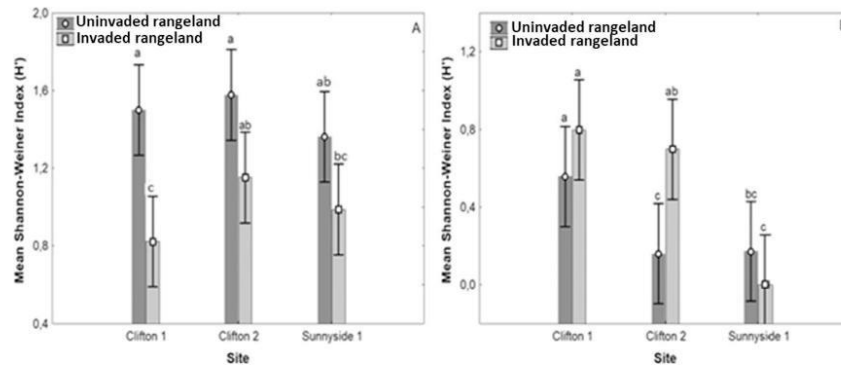


Figure 4. Differences in grass (A) and forb (B) diversity (Shannon-Weiner index) (mean $\pm$ 95% confidence limits) between uninvaded rangeland and habitats invaded by *Robinia pseudoacacia* in eastern Free State, South Africa. Means with the same letter are not significantly different from each other.

Table 2

Linear Mixed Model selection matrix based on semi-parametric bootstrapping of parameter estimates for Veld Condition Index (VCI) values in open rangeland and habitats invaded by *Robinia pseudoacacia* at Clifton and Sunnyside properties in eastern Free State, South Africa.

Model structure	K	AICc	Δ AICc	LogLik	w_i
Site * Habitat	7	244.7	0.00	-111.85	1.00
Site + Habitat	6	264.1	18.40	-123.58	0.00
Habitat	5	275.0	30.30	-130.83	0.00
Site	5	286.6	41.90	-136.65	0.00
Random effects only	4	297.8	53.10	-143.82	0.00
Null model ^a	2	304.58	59.88	-150.00	0.00

^a Null model = ~1 (random intercept model)

but insignificant cover than invaded habitat (Fig. 5). Habitat had a significant influence on grass cover ($F = 24.03$, $P < .001$).

Discussion

This study highlights the impact of *R. pseudoacacia* on plant community dynamics in rangelands. There was a reduction in abundance and diversity of native grasses followed by succession by alien grasses and forbs, thereby reducing the grazing capacity of the rangeland. Emergent communities following invasion were dominated by nitrophilous and shade-tolerant species, demonstrating the impact of *R. pseudoacacia* on microclimates. To our knowledge, this is the first study assessing the impacts of this species on native plant community composition and associated ecosystem services in South Africa and the southern hemisphere.

Robinia pseudoacacia Population Demographics and Canopy Characteristics

Overstory vegetation characteristics influence understory vegetation communities (Ali et al. 2019). In this study, we observed an influence of canopy cover on light availability and subsequently understory vegetation communities. Other studies noted the influence of the density of invasive alien species on understory vegetation locally (Ndhlovu et al. 2011; Yapi et al. 2018) and elsewhere (Benesperi et al. 2012). To further affirm this, we did not find any significant undergrowth at Sunnyside 4, which had the highest canopy cover and density, demonstrating the importance of light availability on understory vegetation dynamics. Tree diameter can be used as a proxy for tree age (McElhinny et al. 2005). Hence, we suggest that stand age influences successional dynamics in understory vegetation. Campagnaro et al. (2018) also found

an influence of stand successional dynamics and other stand characteristics, such as stand basal area, tree diameter, and stand vertical structure, on understory vegetation communities. It is therefore plausible that vegetation dynamics in our study were a result of invasion.

Effects of *R. pseudoacacia* Invasion on Grass Species Composition

In the present study, *R. pseudoacacia* invasion led to a marked reduction in native grass and forb diversity, consistent with studies on the same species in other geographical areas (Benesperi et al. 2012; Sitzia et al. 2012), as well as other invasive alien species (Hejda et al. 2009; Paterson et al. 2011). These changes could be a result of its active nitrogen fixation activity and deposition of nitrogen-rich leaf litter (Cierjacks et al. 2013), which leads to the recruitment of nitrophilous grasses and forbs. In nutrient-poor soils, such nitrogen enrichment gives *R. pseudoacacia* a competitive advantage over natives adapted to nutrient-poor soils (Rawlik et al. 2018). Indeed, grass species found underneath *R. pseudoacacia* stands in our study, *D. glomerata*, *B. catharticus*, and *E. biflora*, reportedly have a high affinity for nitrogen (Van Oudtshoorn 1999), underlying its role in understory composition. By contrast, other studies, despite linking *R. pseudoacacia* to nitrophilous understory vegetation, suggested that the tree contributes to biodiversity after association with equally species-rich vegetation communities (Campagnaro et al. 2018; Slabejova et al. 2019). However, in our study, although understory vegetation at Sunnyside appeared to be as diverse as in uninvaded habitat, it was composed of alien instead of native species.

Our study showed that invasion of rangelands by *R. pseudoacacia* leads to the succession of native grass communities by mainly alien taxa. This indicates that invasive alien species can act as

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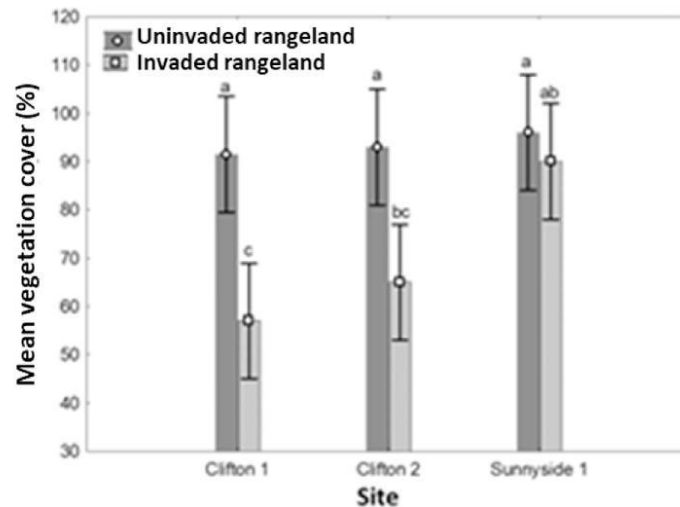


Figure 5. Differences in vegetation cover (mean $\pm$ 95% confidence limits) between uninvaded rangeland and habitats invaded by *Robinia pseudoacacia* in eastern Free State, South Africa. Means with the same letter are not significantly different from each other.

drivers for further invasion by other alien species. Even though the sources of the alien understory vegetation in this biome are not clear, we posit that some of the grasses could have escaped from planted pastures while the rest of the forb species are generally weedy species transported inadvertently through various vectors, primarily birds. Tecco et al. (2006) also recorded higher richness of exotic species under an invasive alien tree, *P. angustifolia*, than *Condalia montana*, a native plant. These results also indicate approximately 100% species turnover due to invasion, and in keeping with this observation, Vitkova et al. (2020) recorded a 20% decline in the frequency of native species under *R. pseudoacacia* stands in xeric rangelands of southern Europe. Although there may be variations in the composition of understory vegetation, our results are consistent with findings by Sitzia et al. (2018) in Mediterranean lowlands of Europe that generally *R. pseudoacacia* stands are associated with aliens, ruderals, and habitat specialists. Invasion by *R. pseudoacacia* therefore creates conditions necessary for the recruitment of these alien species from incoming invaders due to creation of enabling niches. Moreover, there was a shift in composition of grass species that follow the C_4 carbon fixation pathway, which are common in this biome as the case with uninvaded plots, to those following the C_3 pathway in this study. C_4 grasses are adapted and highly competitive under high light and temperature intensities and also thrive best in low nitrogen soils (Taylor et al. 2010). All 16 native grasses recorded in this study are adapted to the C_4 pathway while exotic species, found under *R. pseudoacacia* all follow the C_3 pathway (Milton 2004), suggesting an influence of the tree in the recruitment of species with special adaptations different from natives.

R. pseudoacacia is a light-demanding and fast-growing species that creates light-deficient conditions suitable for the shade-tolerant species in the understory (Cierjacks et al. 2013). It is therefore possible that shade tolerance could have played a role in the grass assemblages underneath *R. pseudoacacia* in our study. For example, the *D. glomerata* that we recorded was previously associated with shade tolerance in a silvi-pastoral agroecosystem, where its nutritional value increased with shading (Kyriazopoulos

et al. 2012), indicating its adaptation to low-illumination conditions. Shading was also found to shape grass communities underneath another invasive alien plant, *Chromolaena odorata* (Te Beest et al. 2015). Our results therefore suggest that, in all likelihood, the interplay between shading and nitrogen fixation influences the emerging communities underneath *R. pseudoacacia* stands and the reduction of native grass and forb diversity.

Effects of Invasion on Grazing Quality

Invasion of rangelands by alien taxa has been associated with a corresponding reduction in grazing capacities, affecting livestock enterprises, which are largely dependent on rangelands (Palmer and Ainslie 2005; Suttie et al. 2005). In this study, there was a marked reduction in range condition at one of the properties. Consistent with these findings, several studies have linked the reduction in grazing capacity to invasive alien species locally and globally (Duncan et al. 2004; Ndhlovu et al. 2011; Yapi et al. 2018) and potential reduction in gross margins (Humphrey 2019). Through quantification of the increased losses in grazing capacities of the invaded rangelands, our study further demonstrates how invasive species can compromise livestock production. While overstocking can also drive rangeland degradation and alter grass community composition (Van der Merwe et al. 2018), our habitats were subjected to the same grazing intensities. Hence, we suggest that differences in community composition and grazing capacity observed in this study were a result of invasion by *R. pseudoacacia*. High range condition indices under *R. pseudoacacia* at Sunnyside were likely influenced by the dominance of *D. glomerata*, a species with high grazing value, high understory vegetation cover, and low tree densities. However, this good condition might be offset if considered together with Sunnyside 4, one of the invaded sites on the property without understory vegetation possibly due to high tree density and low light penetration. *R. pseudoacacia* is capable of reducing grazing capacity through alteration of grass communities, takes up grazing space, and is also poisonous to livestock (Humphrey 2019).

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Implications

The current study highlights the negative impacts of *R. pseudoacacia* in rangelands. Invasion-mediated replacement of native grass communities by alien grasses and forbs reduces the grazing capacity of the rangelands. Given these adverse effects, urgent control measures together with ecological restoration are required. With property owner reports indicating failure of chemical and mechanical control due to the vigorous coppicing capabilities of *R. pseudoacacia*, biological control offers better prospects for successful management of this invasive species. Furthermore, to allow for predictable successional trajectories as suggested by Sheley and Krueger-Mangold (2003), long-term management approaches should be adopted. While integrated approaches that promote desirable species and suppression of the proliferation of undesirable ones are recommended (DiTommasso et al. 2017), there are no prescribed methods that are effective against *R. pseudoacacia*. Moreover, control of the IAP through utilization as a browse species is curtailed by its toxicity to animals. Elimination of *R. pseudoacacia* might be possible through aggressive mechanical techniques that involve the complete destruction of the rooting system. However, this form of management, besides its practical limitations at larger scales, introduces further disturbance leading to invasion by secondary weeds. Overall, there is a need to develop a sustainable management regime for *R. pseudoacacia* and other invasive species with similar functional traits. Meanwhile, rangeland managers should focus on early detection and prevention while those with existing infestations should minimize mechanical disturbance to limit rapid spread.

Declaration of Competing Interest

None.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.rama.2021.02.006.

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Thermal tolerance of the biological control agent *Neolema abbreviata* and its potential geographic distribution together with its host *Tradescantia fluminensis* in South Africa

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ABSTRACT

In this era of global climate change, understanding how climate influences species distributions together with their subsequent interactions is a major priority for managers of both natural and managed ecosystems. Using the predictive algorithm Maximum Entropy (MaxEnt), we projected the current and potential distribution of *Tradescantia fluminensis* Vell (Commelinaceae) before using thermal tolerance indices of its biological control agent *Neolema abbreviata* (Coleoptera: Chrysomelidae) to simulate its potential distribution in South Africa. The mean temperature in *T. fluminensis* microhabitats, recorded using data loggers, was 24.7 ± 0.1 °C (mean $\pm$ SE) whilst temperatures ranged from -1 to 29.7 °C. Following 2 h exposure at low temperatures, *N. abbreviata* survival from 0 to 100% fell between -13 and -6 ± 0.2 °C with 50% mortality occurring at -9.8 ± 0.2 °C. For heat assays, temperatures above 45.8 ± 0.2 °C resulted in 100% mortality whilst temperatures below 43 ± 0.2 °C resulted in 100% survival with 50% mortality occurring at 44.2 ± 0.2 °C. The mean critical thermal limits were 1.8 ± 0.4 °C (CT_{min}) and 48.8 ± 1.3 °C (CT_{max}). The derived warm and low temperature tolerance largely matched with current and projected climate in *T. fluminensis* microhabitats. Model evaluation for *T. fluminensis* distribution was based on mean Area Under the Curve (AUC), which was 0.85 ± 0.017 , suggesting good predictive performance of the model. Our results showed a limited capacity for geographic range expansion by *T. fluminensis* under future climate scenarios. This indicates good prospects for biological control of *T. fluminensis* using *N. abbreviata* in South Africa.

1. Introduction

Tradescantia fluminensis Vell (Commelinaceae), commonly known as Brazilian spiderwort, is a monocotyledonous groundcover plant with soft hairless leaves and succulent stems. Native to South East Brazil and northern Argentina, it has since naturalised and been declared an environmental weed in many countries including Australia, New Zealand and South Africa (Standish et al., 2001; Standish, 2004; Standish et al., 2004; Fowler et al., 2013). In South Africa, it is largely in its incipient phases of invasion and has been classified as a category 1B invader under the National Environmental Management: Biodiversity Act (NEMBA). The growth of *T. fluminensis* is largely restricted to forest understorey where it forms dense swaths of vegetation smothering any low-lying vegetation. Thus, poor native seedling recruitment, which leads to poor forest regeneration, is the main adverse attribute of *T. fluminensis* invasions (Standish et al., 2001; Standish, 2004; Standish

et al., 2004). Mechanical control of large infestations of the plant are difficult as stem fragments can easily develop roots and form new plants. Chemical control is largely unsuitable due to potential non-target effects on sensitive ecosystems such as riparian habitats where *T. fluminensis* also grows (Mbande et al., 2019, 2020). Biological control using arthropods and pathogens has therefore been employed as a sustainable measure to combat invasive populations (Fowler et al., 2013).

Despite the prospective benefits of classical biological control of weeds, its success remains sporadic due to a diverse range of operational and non-operational constraints (Schwarzländer et al., 2018). For example, climate incompatibility has been cited as one of the major constraints hampering establishment of insect biological control agents in their introduced range (Chidawanyika et al., 2017; Ramanand et al., 2017; Sun et al., 2017; Paterson et al., 2019), and temperature is often the most important environmental factor determining the activity and

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geographic distribution of insects (Azrag et al., 2018).

To enhance biological control, species distribution models (SDMs) have been used to determine the potential suitability of biological control agents together with identifying areas for agent collection based on the match in climate between native and introduced ranges (Sun et al., 2017; Muskett et al., 2020). Species distribution models have also been used to project the spatial extent of invasive alien plants (IAPs) (Sun et al., 2017).

Invasive species are projected to extend their geographic ranges in response to climate change due to their superior tolerance to environmental stressors, phenotypic plasticity and rapid adaptive evolutionary responses (Seebacher et al., 2015; Kingsolver and Buckley, 2017; Mutamiswa et al., 2017, 2018; Chidawanyika et al., 2019). Similarly, climate change may have a direct impact on the distribution of biological control agents (Coetzee et al., 2007; Allen et al., 2014). However, the responses of insects and their host plants to climate change in both biogeography and phenology can differ (Forrest, 2016; Lehmann et al., 2020) making it difficult to make generalisations about what will happen. SDMs are foreseen as being increasingly important because they can project the potential mismatches between biological control agents and their target weeds under future climate scenarios. In addition, dispersal capacity of biological control agents, depending on environmental variability, can mediate demographic patterns and ultimately population persistence, as high dispersal propensity may be antagonistic to population persistence through depletion of initial metapopulations (Kean and Barlow, 2000a, 2000b; Jonsen et al., 2007). It is therefore important to understand the potential distribution of both the agents and their weeds to better predict their performance under variable environments. This in turn will help managers and policy-makers to develop mitigating measures (Kriticos et al., 2003; Wang and Wang, 2006; Allen et al., 2014; Terera and Wood, 2014).

Neolema abbreviata Lacordaire (Coleoptera: Chrysomelidae) is native to Brazil and was first used for biological control of *T. fluminensis* in New Zealand (Fowler et al., 2013). Plant damage by the beetles is inflicted on young shoot-tips and mature leaves by the feeding action of both adults and larvae resulting in stunted plant growth and ultimately death (Fowler et al., 2013; Mbande et al., 2019, 2020). In South Africa, *N. abbreviata* was first released in 2018 following host range testing. Despite apparent extensive field damage on *T. fluminensis*, little is known about the beetle and its host's biogeography in introduced habitats such as South Africa despite the implications for the efficacy of biological control in both current and future climate scenarios. To address this, we sought to project the current and potential distribution of *T. fluminensis* together with its agent *N. abbreviata* in South Africa. We specifically asked if there was any enemy-free space for the weed in the current and potential distribution of the agent and its weed based on the climatic requirements as indicated by the agent's thermal tolerance and the currently-observed global climate envelope of *T. fluminensis* habitats.

2. Materials and methods

2.1. Test insects and rearing conditions

A founder colony of *N. abbreviata* was obtained from field-collected beetles from Auckland, New Zealand in 2010. The beetles were maintained in mesh-covered, steel-framed cages (50 × 50 × 82 cm) in a quarantine insectary at the Agricultural Research Council station at Cedara, South Africa. The beetles were maintained for several generations but were kept in high numbers (~100 per cage) in each cage to promote heterozygosity (Charlesworth and Willis, 2009). Despite being released in the field after completion of host range assessments, a colony of *N. abbreviata* was retained in quarantine. The entire colony was maintained under controlled conditions, ranging between 25 and 28 °C and 60 to 80% relative humidity (RH). At all times, the beetles were regularly provided with fresh intact *T. fluminensis* plants that were

propagated in plastic pots (18 × 10 cm) under drip fertigation for ~5 weeks before being introduced into the cages. The damaged plants that were replaced following defoliation were held in different cages to allow for adult eclosion from pupae that had accumulated in the soil. Sub samples of these newly-eclosed adults were used for thermal tolerance assays.

2.2. Field microhabitat temperatures

Environmental microhabitat temperatures were recorded using Hygrochron iButton dataloggers (DS1923-F5, ± 0.5 °C accuracy, Maxim Integrated Products, San José, USA). The iButtons were placed within two *T. fluminensis* sites at Hilton, KwaZulu Natal Province, South Africa. The iButtons (N = 4) were placed on the ground and in the *T. fluminensis* canopy (~40 cm above the ground). After twelve months, the mean, maximum and minimum temperatures were calculated for the entire duration.

2.3. Determination of lethal temperature limits

A programmable Grant TX150 water bath (Grant Instruments, UK) was used to expose *N. abbreviata* adults to a range of high (42, 43, 44, 45, 46 °C) and low (-4, -6, -8, -10, -12, -14 °C) temperatures for 2 hrs using a direct plunge protocol (e.g. Stotter and Terblanche, 2009; Allen et al., 2014). In all cases, 10 live newly emerged adults were collected from the main culture and placed in five 60 ml screw top polypropylene vials to yield a sample size of N = 50 for each treatment. Lids of the vials were perforated to allow air circulation and moistened strips of filter papers were placed in each vial to maintain RH above 80%. The five vials were then placed in a Ziploc bag (33 × 38 cm) before immersing in the water bath. A mixture of propylene glycol and water 1:1 was used in the water bath to enable operation at lower temperatures without freezing. In all cases, vials containing the assayed beetles were placed in a 26 °C, 65% RH climate chamber for 24 h before assessing survival. Here, survival was regarded as the ability to respond to stimuli such as mild prodding and resumption of locomotory behaviour (e.g. Stotter and Terblanche, 2009). Generalised Linear Models (GLMs) in R version 3.3.0 (R Development Core Team, 2019) assuming a binomial error distribution (dead/alive) with a logit link function were used to determine the effect of different temperatures on survival.

2.4. Determination of critical thermal limits

Critical thermal maximum (CT_{max}) and minimum (CT_{min}) were assayed using the same water baths following standard protocols (Stotter and Terblanche, 2009; Mutamiswa et al., 2018, 2019). Beetles were individually placed into a series of borosilicate glass tubes in double jacketed Perspex chambers connected to the water bath. Thereafter, temperatures were increased (CT_{max}) or decreased (CT_{min}) starting from 26 °C using a pre-determined environmental rate of temperature change of 0.06 °C min⁻¹ from microsite data recordings. Critical thermal maximum and CT_{min} were regarded as the upper and lower temperatures, respectively at which the beetles were incapacitated losing any form of coordinated muscle function such as movement of limbs (Stotter and Terblanche, 2009). Chamber temperatures were verified using a type T copper-constantan thermocouple connected to a digital thermometer (Fluke Cooperation, Australia Ltd. Sydney).

2.5. Projected temperature tolerance

Due to limited *N. abbreviata* presence data for use in SDMs, the critical thermal limits (CTLs) were used to determine warming tolerance (WT) and cold tolerance (LTT) as sensitivity of *N. abbreviata* relative to mean environmental temperature of both current and projected future climatic conditions (e.g. Allen et al., 2014). The mean environmental temperature in the warmest and coldest quarter was

regarded as T_{max} and T_{min} , respectively. Thus, the WT was regarded as a measure of the insect's sensitivity to climate warming whilst LTT measured cold tolerance (e.g. Deutsch et al., 2008; Allen et al., 2014).

Temperatures of warmest and coldest quarters for both current and 2050 climate scenarios were downloaded from WorldClim version 2.0 (Fick and Hijmans, 2017) and masked in ArcGIS version 10.3 (ESRI, Redlands, CA, USA) using the national boundary for South Africa. The WT and LTT were calculated using the raster calculator function from ArcToolbox in ArcGIS. The following formulae were used to calculate thermal tolerances:

$$WT = CT_{max} - T_{max}$$

$$LTT = CT_{min} - T_{min}$$

Thereafter, WTs were mapped onto South African maps for both current and future average temperatures using ArcGIS.

2.6. Plant occurrence data

Over 3000 occurrence records of *T. fluminensis* were downloaded from the Global Biodiversity Information Facility (GBIF, <http://www.gbif.org/>), the Southern African Plant Invaders Atlas (SAPIA) database (Henderson, 2007) and from physical surveys we conducted in South Africa from 2015 to 2019. The data were cleaned thereby avoiding duplicate records and obviously erroneous records before being analyzed for spatial clustering in ArcGIS. Data that fell in managed environments such as botanical gardens were also removed to minimize sampling bias (Elith et al., 2006; Yackulic et al., 2013). Pseudo-replication was avoided by only using one sample per 2.5-min pixel during calibration. The remaining presence locations were used to build the model.

2.7. Model environmental data

Predictor climatic variables, which are based on temperature and precipitation were downloaded from WorldClim at a spatial resolution of 2.5 arc minutes (~5 km). The raster datasets were clipped for South America, the native range of *T. fluminensis* and South Africa, the invaded range, and converted to binary ASCII format using ArcGIS. The 19 variables were analysed for collinearity using Pearson's correlation in R and variables that best described the ecology of *T. fluminensis* were selected from pairs with a correlation coefficient (r) > 0.9. Consequently, only 8 bioclimatic variables and the Digital Elevation Model (DEM) from the NASA Shuttle Radar Topography Mission (SRTM) (National Aeronautics and Space Administration, 2000) were used to run the model (Table 1).

The same bioclimatic variables selected for current climatic conditions were also downloaded from the Beijing Climate Center Climate System Model (BCC-CSM1-1) Representative Concentration Pathway 6.0 (RCP 6.0) for the 2050 climate change scenario in WorldClim (Wu et al., 2019; Fick and Hijmans, 2017). The RCP 6.0 represents radiative forcing values pathway to 6 W/m² (ca. 850 ppm CO₂) stabilizing

without overshoot after 2100 with temperatures predicted to rise by 1.3 ± 0.5 °C between 2046 and 2065 (van Vuuren et al., 2011; IPCC, 2013). This model was chosen as it is a more realistic representation of the global warming trajectory given on-going efforts to cut back on emissions of greenhouse gases which might result in the gradual stabilization of temperatures by 2100 (Hijjoka et al., 2008).

2.8. Model building

MaxEnt, a machine learning algorithm that applies the principle of maximum entropy to predict the potential distribution of species from presence-only data and environmental variables (Phillips et al., 2006) was used to model potential distribution of *T. fluminensis*. The model was run on South America, the native range of the species and South Africa, the invaded range. Model training was done using 70% of the presence records whilst the remaining 30% were used to test and validate the model through subsampling. Subsampling repeatedly splits presence points into random training and testing subsets. A default background (a sample of points from the landscape) of 10 000 pixels was selected from a bias file created in ArcGIS. To model future distribution under changing climate, a projection file containing predictor variables for the 2050 climate scenario was uploaded. Performance of the model was then assessed using the Receiver Operated Characteristic (ROC) and Area under the Curve (AUC) statistic. The ROC curve is a plot of true positives against false positives with AUC values between 0 and 1. An AUC closer to 1 indicates high predictive capability of the model. The ASCII outputs from Maxent was converted to the GEOTIFF format in ArcGIS and predictive maps depicting projected habitat suitability on an index of 0 (least suitable) to 1 (most suitable).

The maps for current and 2050 distributions were further converted to binary maps using the 10-percentile training presence threshold, which uses suitability associated with the presence record that occurs at the 10th percentile of presence records. The resultant output was a map depicting areas where suitable habitat will be lost and areas which will become suitable with changes in climatic conditions.

3. Results

3.1. Microhabitat environmental temperature

The mean temperature recorded in *T. fluminensis* microhabitats was 24.7 ± 0.1 °C (mean $\pm$ SE). The maximum and minimum temperatures recorded were 29.7 and -1 °C respectively. These data covered all the months of the year 2017 in Hilton, KwaZulu Natal.

3.2. Lethal temperatures, critical thermal limits

Thermal exposure for 2 hrs at various static temperatures significantly influenced survival at low (LT: $z = 10.12$ SE = 0.13, $p < 0.0001$) and high temperatures (ULT: $z = 9.17$, SE = 0.31, $p < 0.0001$). At low temperatures, survival from 0 to 100% ranged between -13 and -6 ± 0.2 °C with 50% mortality occurring at -9.8 ± 0.2 °C (Fig. 1). For heat treatments, temperatures at 43 ± 0.2 °C and below resulted in 100% survival whilst temperatures above 45.8 ± 0.2 °C resulted in absolute mortality, with 50% mortality (ULT) recorded at 44.2 ± 0.2 °C (Fig. 1). The mean CT_{min} of the beetles was 1.8 ± 0.4 °C (mean $\pm$ SE, $n = 30$) and CT_{max} was 48.8 ± 1.3 °C ($n = 30$).

3.3. Projected temperature tolerance

Warming tolerance values for *N. abbreviata* ranged from 19 to 46 °C under both current and future climate scenarios (Fig. 2). In the current scenario, WT values between 28 and 32 °C were the most frequent within the geographic range of the host plant. However, the 2050 s future climate scenario showed decline in warming tolerance with the

Table 1
Bioclimatic variables used to model areas suitable for *Tradescantia fluminensis*.

Variable	Name
Bio1	Mean annual temperature
Bio3	Isothermality
Bio7	Temperature annual range
Bio11	Mean Temperature of coldest quarter
Bio12	Annual precipitation
Bio16	Precipitation of wettest quarter
Bio17	Precipitation of driest quarter
Bio18	Precipitation of warmest quarter
Elevation	Height above sea level

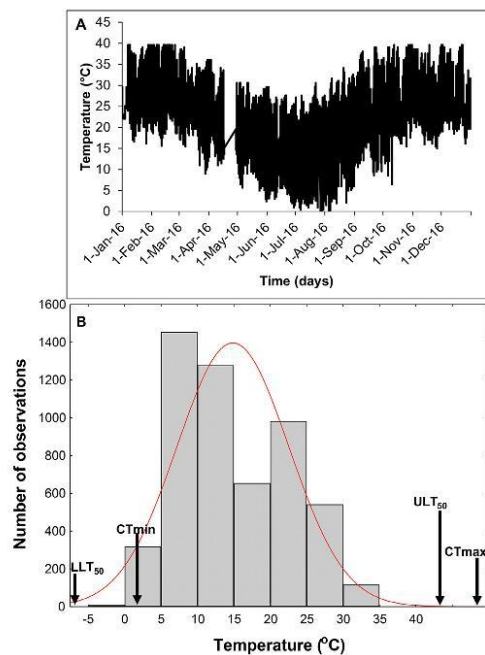


Fig. 1. (A) Microhabitat temperatures recorded using Thermocron iButtons (Model DS1923-F5; Maxim Integrated Products, San José, USA) at 1 h sampling frequency on the ground and canopy of *Tradescantia fluminensis* at Hilton KwaZulu Natal Province, South Africa in 2016 (A). Temperature frequency distribution in the same sampling period was plotted where arrows indicate the points and how often temperatures that compromise fitness of *Neolema abbreviata* are reached (B). CTmin and CTmax denote Critical thermal Minima and Critical thermal Maxima, respectively. LLT50 and ULT50 denotes where 50% mortality was realised following 2 h exposure to cold or heat, respectively.

most frequent WT being 24 to 26 °C (Fig. 2). There was an increase in T_{min} between current and projected LTTs with number of sampling points falling below the CT_{min} decreasing (Fig. 3).

3.4. Habitat suitability model

The species distribution model for *T. fluminensis* had an average test AUC of 0.85 ± 0.017 SD for ten replicate runs. (Fig. 4). This model performance matrix was close to 1, which is an indication that the

model performs better than random (AUC: 0.5). Amongst the nine bioclimatic variables used, temperature annual range (Bio7) had the highest contribution of 38.5% whilst precipitation of wettest quarter had the least contribution of 2.2% (Table 2). A jackknife test of variable importance also indicated that temperature annual range (Bio7), contained the most important information as it had the highest gain when it was used in isolation and the least gain was realized when it was omitted.

A few areas were predicted to be highly suitable with Habitat Suitability Indices (HSI) between 0.8 and 1 under prevailing climatic conditions (Fig. 5). However, the HSI in these areas is expected to decline by 2050, becoming moderately suitable ($HSI > 0.5$). Some lowly suitable areas are also expected to improve during same period, as the most frequent indices in 2050 s will be ranging between 0.6 and 0.8.

The spatial extent of habitat suitable for *T. fluminensis* in South Africa was predicted to be approximately 23 900 km² and is expected to expand to about 26 000 km² if temperature increases by 1.3 ± 0.5 °C by 2050. For that expansion, areas bordering Gauteng, North West and Free State provinces had the highest habitat gain, the Western Cape, KwaZulu Natal and Limpopo provinces were intermediate whilst the Eastern Cape had the least (Fig. 6). The Northern Cape and mainland North West provinces remained unsuitable for *T. fluminensis* under current and future climate scenarios. During the same period, there was also minimal contraction of suitable habitats in Eastern Cape, Limpopo, Western Cape and KwaZulu Natal provinces (Fig. 6). In terms of land area, this translated to approximately 1800 km² changing from being suitable to unsuitable whilst 3 900 km² will become favorable for the species. Hence, the currently suitable habitats in all provinces largely remain unchanged (Fig. 6).

3.5. Discussion

Plant and insect geographic distributions are highly responsive to climate variability. In sub-Saharan Africa, temperatures are projected to increase where the southern region will, in all likelihood, experience warmer mean environmental temperature in the warmest quarter due to increased frequency and magnitude of extreme heat events (Kruger and Sekele, 2013; Allen et al., 2014). The range of microhabitat temperatures that were recorded in the patches of *T. fluminensis* were well within the range that is tolerated by *N. abbreviata*. Moreover, prior exposure to sub-lethal temperature can confer resistance to thermal stress in otherwise lethal environments (Stotter and Terblanche, 2009; Mutamiswa et al., 2018) in what is referred to as hardening, for acute exposure and acclimation for relatively longer time scales (Bowler, 2005; Sinclair and Roberts, 2005). This therefore further suggests that the targeted microhabitats provide conditions that ensure population persistence through both non-lethal exposure and sub-lethal exposure.

Our study showed a projected increase in mean temperature in the warmest quarter from current scenario to the 2050 s. This suggests a corresponding decline in warming tolerance for *N. abbreviata* within

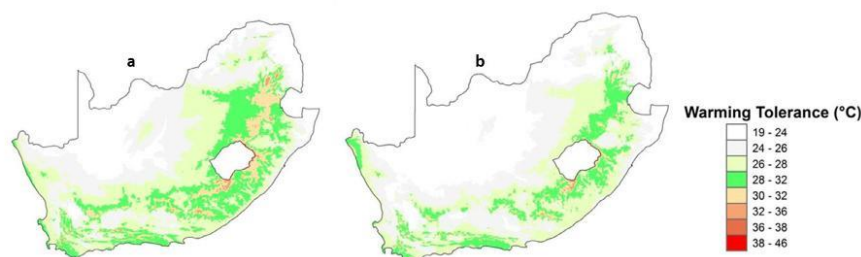


Fig. 2. Warming Tolerance (WT) for *N. abbreviata* under current (a) and 2050 temperatures of the warmest quarter (b).

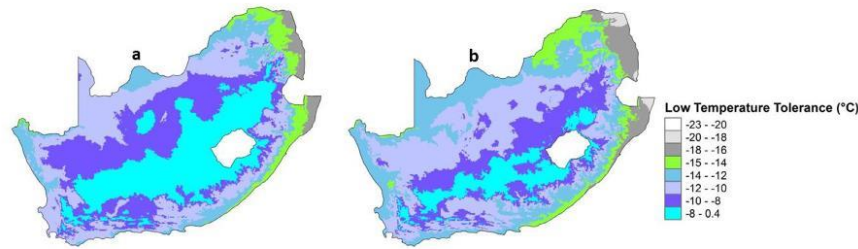


Fig. 3. Low Temperature Tolerance (LTT) for *N. abbreviata* under current (a) and 2050 temperatures of the coldest quarter (b).

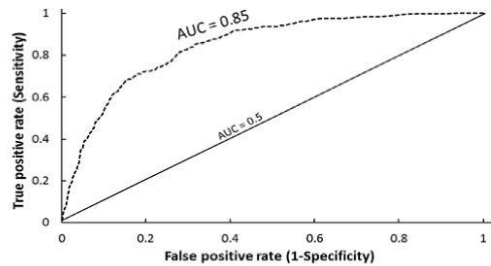


Fig. 4. ROC curve and AUC of the Maxent model for *Tradescantia fluminensis*.

Table 2
Contribution of environmental variables to the potential distribution of *Tradescantia fluminensis* Vell (Commelinaceae).

Variable Name	Contribution (%)	Cumulative contribution (%)
Temperature annual range	38.5	38.5
Mean annual Temperature	19.2	57.7
Precipitation of driest quarter	12.7	70.4
Isothermality	8.6	79
Mean Temperature of coldest quarter	5.8	84.8
Height above sea level	5.7	90.5
Annual precipitation	3.7	94.2
Precipitation of warmest quarter	3.6	97.8
Precipitation of wettest quarter	2.2	100

South Africa. There was also an increase in mean temperature in the coldest quarter between current and projected cold tolerance with number of sampling points falling below the CT_{min} . These results

therefore indicate vulnerability of *N. abbreviata* under future climate scenarios. Since we did not investigate the role of phenotypic plasticity in *N. abbreviata* thermal tolerance, the scope of our conclusion is limited to the basal responses to thermal stress in *N. abbreviata*. Although somewhat limited for high temperature tolerance among subtropical ectotherms (Hoffmann and Sgrò, 2011; Araújo et al., 2013; Allen et al., 2014; Gunderson and Stillman, 2015), phenotypic plasticity and evolutionary adaptation is still widely reported to confer resilience to novel climate conditions (Huey et al., 2012; Seebacher et al., 2015; Kingsolver and Buckley, 2017). The net response to the change in climate is however dependent on the relative stability of the environmental selection pressure and the species demographic characteristics such as generation time (Seebacher et al., 2015; Kingsolver and Buckley, 2017).

Our models predicted few areas that are highly suitable for *T. fluminensis* under current climate scenarios. Furthermore, the HSI in these areas was projected to decline by 2050 ($HSI > 0.5$), thus, becoming moderately suitable with 1800 km² becoming totally unsuitable. However, some currently lowly suitable areas were projected to improve their HSI in the same period with 3900 km² currently unsuitable areas becoming suitable, consequently resulting in a net habitat expansion from 23 900 to 26 000 km².

These results have several implications on the invasion of *T. fluminensis* and subsequent biological control in South Africa using *N. abbreviata* under both current and future climate scenarios. The current and projected future habitats of *T. fluminensis* largely remain within the fundamental niche of *N. abbreviata* as reflected by the beetles' thermal tolerance. Previous studies have reported how climatic requirements of a biological control agent can pose constraints on the success of a biological control agent (Goolsby et al., 2006; Cowie et al., 2016; Sun et al., 2017). Our niche analyses did not show any mismatches between the species thereby suggesting potential successful biological control of *T. fluminensis* using *N. abbreviata* under current and future climate scenarios.

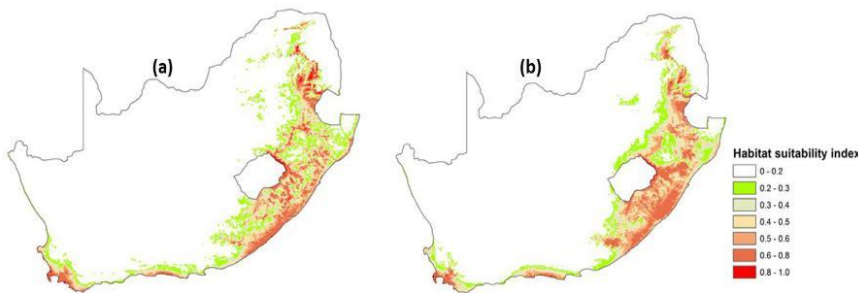


Fig. 5. Predicted habitat suitability model for *T. fluminensis* under current (a) and 2050 climate scenarios (b).

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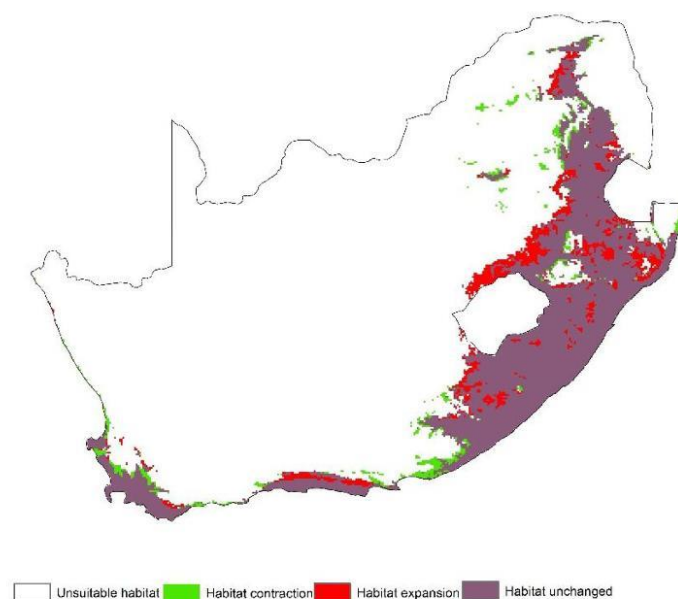


Fig. 6. *Tradescantia fluminensis* suitable habitat shifts due to changing climatic conditions.

Our study also revealed that *T. fluminensis* distribution in South Africa is highly constrained by climate with limited potential for habitat expansion under climate change. Temperature and precipitation seemed to play a key role in its distribution as indicated by the bioclimatic variables that, in order of importance, mean annual temperature (Bio7), temperature annual range (Bio1) and precipitation in the driest quarter (Bio17) contributed the most on the model (Table 2). Previous reports have indicated that *T. fluminensis* thrives best in damp habitats in its tropical native region of Brazil (Fowler et al., 2013; Mbande et al., 2019, 2020) even though it also occurs in both tropical and temperate areas in its introduced ranges (Dugdale et al., 2015). It is therefore not surprising that precipitation in the driest quarter was revealed as a limiting factor as the weed will not be able to withstand prolonged dry spells as also supported by contemporary empirical studies (Burns, 2004; Mbande et al., 2019, 2020). Furthermore, *T. fluminensis* is frost sensitive and thrives best in frost-free areas (Bannister, 1984). This further explains why most areas in South Africa had poor HSI due to harsh winter temperatures that tend to limit *T. fluminensis*. Moreover, *T. fluminensis* is sensitive to direct sunlight and requires cool shaded forest habitats making its invasion highly fragmented patches (Standish, 2004; Standish et al., 2004). This therefore suggests that it will require transporting agents for stem fragments, its main propagules, to move from one suitable habitat to another. This effectively eliminates major dispersal agents such as wind and animals (e.g. seed carrying excreta of frugivorous birds) as is the case with several invasive plants (e.g. Schiffrman, 1997; Vardien et al., 2012).

Overall, our findings imply that the threat posed by *T. fluminensis* may be highly localised due to limited suitable habitats and capacity to rapidly spread in South Africa. This is of particular importance for policy makers in the prioritisation of resources as weeds that cause severe impact on biodiversity in both spatial and temporal extent including disruption of key ecosystem services may require urgent attention. Here, *T. fluminensis* presents an interesting scenario where potential for spread is limited despite localised major impacts on

biodiversity through its disruption of native seedling recruitment (Standish, 2004; Standish et al., 2004; Mbande et al., 2019, 2020) which may lead to local extinction. For biological control, the major concern is that climate change can have negative impact on the efficacy of control efforts through differential physiological, ecological and evolutionary responses between target weeds and their agents (Sun et al., 2020). By contrast, climate change may also lead to improved biological control of invasive plants. For example, climate warming altered biotic interactions tipping the competition in favour of native species at the expense of non-native species subjected to biological control Lu et al. (2016). In addition, warming can also expand the geographic range of biological control agents leading to improved efficacy against invasive plants in otherwise unsuitable cooler habitats (Lu et al., 2013). Thus, the impacts of climate change on environmental weeds and their biological control agents are multifaceted.

Temperature was the major determinant of the distribution of *T. fluminensis* followed by precipitation in the driest quarter which matched with the areas suitable for *N. abbreviata* in both current and future climate scenarios. Thus, making biological control a viable management option. However, our insect assays were only based on adult beetles. Yet insect vulnerabilities to thermal stress may be dependent on life-stage (Bowler and Terblanche, 2008; Mutamiswa et al., 2019). We also could not account for humidity and desiccation stress resistance, a major factor mediating insect fitness and performance at various life-stages (Weldon and Taylor, 2010). Future studies should therefore endeavour to account for these physiological aspects in addition to ecological and evolutionary processes for more comprehensive conclusions. Nevertheless, our study still gives important insights into the potential geographic distribution of *T. fluminensis* and its agent.

Our study has several implications on the ecology and biological control of IAPs as a whole. We showed that plant invasions are highly responsive to climate and habitat suitability where potential geographic range can be constrained. This implies that, under limited range expansion, the impacts of IAPs may be highly localised. Control measures

should therefore be implemented at similar spatial scales despite magnitude of invasion on a national or regional scale relative to other widely prolific invaders. Limited or geographically isolated suitable habitats may lead to fragmented habitats for potential biological control agents. Agent dispersal and abundance may therefore be constrained. In particular, for specialist herbivores such as biological agents where habitat connectivity is associated with abundance and self-dispersal through increased emigration and immigration rates among connected habitats (Hambäck and Göran, 2005; Maguire et al., 2015; Rossetti et al., 2017; Chidawanyika et al., 2019).

Other studies have reported that the limited dispersal among fragmented habitats may lead to intensive plant damage and longer persistence of herbivores on smaller patches (Kean and Barlow, 2000a, 2000b, Hambäck and Göran, 2005; Hines et al., 2005; Jonsen et al., 2007) which could be beneficial for the efficacy of biological control at finer local and shorter time scales. Overtime, this may however lead to population collapse of biological control agents due to density dependent population dynamics under limited resources as posited by the resource concentration hypothesis (Grez and González, 1995). Overall, the impacts of climate and subsequent global change leads to an array of possibilities for the ecology of IAPs and their agents. It is therefore important to understand the role of climate in the biology and ecology of IAPs and their agents in order to predict and enhance the efficacy of biological control under current and future climate scenarios.

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CRedit authorship contribution statement

Frank Chidawanyika: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing. **Gerald Chikowore:** Formal analysis, Investigation, Methodology, Visualization, Writing - review & editing. **Reyard Mutamiswa:** Data curation, Investigation, Methodology, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.biocontrol.2020.104315>.

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