Browsing as a demographic bottleneck in a semi-arid savanna: The effect of size and age on compensatory responses of *Vachellia karroo* seedlings after simulated herbivory

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By

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Abstract

Savannas are characterised by a continuous grass layer with scattered trees at varying densities. This vegetation structure is determined by several interacting factors, including fire, herbivory, resource competition and atmospheric CO_2 concentration. The preservation of savanna biomes is important and a shift towards a woody biome threatens savannas globally. Bush encroachment which describes the shift towards domination of savannas by C_3 woody plants, is especially acute in southern Africa. In semi-arid rangelands, encroachment progresses to dense thickets dominated by thorny and unpalatable bushes and trees.

There is evidence that bush encroachment is driven by a reduction in fire and browsing events as well as their interaction. Despite browsing having strong effects on African savannas its isolated role in maintaining tree-grass coexistence has not received as much attention as the role of fire. Therefore the overall aim of this study was to examine the effects of browsing on seedlings of a commonly encroaching species, *Vachellia karroo*. Browsing was hypothesized to be a demographic release bottleneck for bush encroachment in a semi-arid (MAP ~550mm) savanna in the Eastern Cape of South Africa, where fire has been historically rare.

In a single study I explored the fate of *V. karroo* seedlings (less than a year old) following browsing in sub canopy and inter canopy microhabitats. Additionally, I explored how the fate of a seedling changed under high and low tree cover. Firstly, I investigated the type, intensity and frequency of herbivory, from both small and large herbivores, which seedlings were subjected to. Results revealed that browsing was severe and frequent with the majority of seedlings browsed more than twice over a 12 month period. Large browsers such as kudu and impala caused high seedling mortality (46%) while smaller browsers such as invertebrates were more effective at suppressing growth. Microhabitat had little impact on seedling survival, but significantly influenced plant compensatory growth. Reduced seedling growth following browsing was observed in the sub-canopy in comparison to seedling growth in full sunlight in the intercanopy, suggesting *V.karroo* may be shade intolerant. Secondly, the effect of tree cover on browsed seedlings was determined by quantifying browsing frequency and intensity at high and low tree cover. No differences in browsing intensity and frequency were observed between high and low tree cover. However, high tree cover due to bush encroachment limited seedling above ground growth.

The aim of the second study was to investigate how *V.karroo* survival and growth were influenced by its age and size following simulated browsing. I explored this aim through field and greenhouse experiments. I was particularly interested in testing how plant sensitivity to varying defoliation intensities of repeated browsing varied with plant age (known ages of 6, 12, 16 and 30 weeks). There were large differences in mortality between the different age groups. Furthermore, age interacted with repeated browsing and negatively influenced seedling survival and regrowth. Older seedlings (16 and 30 week old) had greater survival and higher browsing frequencies resulted in greater mortality and reduced growth. The threshold age after which seedlings become more tolerant to herbivory occurs at an age of 28 weeks. Seedlings less than six weeks old experiencing intense (100 % defoliation) browsing had a very low probability (33%) of survival following just a single defoliation. Interestingly, all 16 week old seedlings regrew most of their foliage following a moderate (50%) defoliation with some plants overcompensating for leaf loss. The field study revealed two distinct demographic stages based on age and size (seedlings < 9 mm and saplings > 9 mm in stem basal diameter (SBD)). Browsing had a strong negative effect on seedlings, resulting in reduced investment in leaf biomass. These findings suggest plant size and age can be used as robust predictors of a plants vulnerability to browsing.

An increase in tree cover requires successful transitions of seedlings to saplings (also known as release). The results of this study suggest that in semi-arid savannas, browsing can impact tree cover through imposing a release bottleneck for tree seedlings and to lesser extent saplings. By limiting tree growth plants are kept in a disturbance trap and will therefore be exposed to not only browsers but fire. These findings also have important implications for tree-grass coexistence dynamics, suggesting that specific size and browsing thresholds should be considered in savanna management.

Table of Contents

List of Figures

List of Tables

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Declaration

This dissertation, submitted for the degree of Master of Science in the Department of Botany, Rhodes University, represents original work by the author and has not been submitted in any form to any other institution. Where mention has been made of the work of others, it has been duly acknowledged in the text.

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Lavinia Perumal

I certify that the above statement is correct.

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Prof. S Vetter

Supervisor

1. Introduction

1.1 Background

Savannas are characterised by their continuous grassy layer and variable woody cover (Scholes and Archer 1997) and are predicted to be the biome most sensitive to climate change (Sankaran *et al.* 2005; Scheiter and Higgins 2009). Future projections predict that at the expense of grassy biomes there will be significant expansions of forest and woody biomes (Sitch *et al.* 2008; Scheiter and Higgins 2009). There are many consequences of bush encroachment, which describes this shift towards unfavourable C_3 woody savanna trees (Ward 2005). These include altering microclimate, nutrient conditions, soil moisture, and the suppression of grass productivity (Roques *et al.* 2001). Additionally, there are significant impacts when biomes are transformed or biome switches occur, such consequences include disrupted agricultural practices, biogeochemical cycles and ecosystem community structure (Parr *et al.* 2012). Therefore, understanding what influences the distribution of these major biomes is necessary for progress in the field of ecology and biogeography (Sankaran *et al.* 2005).

While what defines savannas appears to be very clear, there is a debate surrounding their origin and patterns (Bond 2008). There is growing evidence suggesting that savannas are of ancient origin with molecular phylogenies dating the origin of C4 grasses, which dominate these landscapes, back to the Oligocene (Christin *et al.* 2008; Edwards *et al.* 2010; Maurin *et al.* 2014). The savanna biome however, only became prominent during the late Miocene (Cerling *et al.* 1997) and expanded during the Pleistocene glacial times about 8-10 MYA (Osborne 2008; Edwards *et al.* 2010). Today African savannas occupy approximately 40% of the land surface area, mostly within sub-Saharan regions (Scholes and Walker 1993) and are important for several reasons (Hoffmann 1996). Savannas support a significant portion of the world's population and their livelihoods (Scholes and Archer 1997) and have substantial commercial value (Kristensen and Lykke 2003). Savannas are biologically important because they support several large mammalian herbivores. These include small browsers, medium-sized mixed feeders, large browsers and non-ruminants such as zebras (Hempson *et al.* 2015a). African savannas also have high levels of floristic diversity and richness and savannas act as habitats for a variety of faunal and floral species. For instance, canopy cover provides a suitable microclimate for fauna and provides a food source for invertebrates and larger mammalian herbivores.

Several terms exist within the literature which refer to the phenomenon of increasing woody vegetation within savannas, often used interchangeably, including bush encroachment (Meik *et al*. 2002), shrub invasion (Noble 1997) and woody thickening (van Auken 2000). In this thesis I use the term bush encroachment which Van Auken (2009) defines as_an increase in cover, biomass and density of indigenous woody plants. Bush encroachment by woody species affects wildlife and natural resources, livelihood sustainability of pastoralists, commercial and subsistence grazing within savannas (Archer 1995). This is generally realised through a reduction in carrying capacity in savannas (Ward 2005). The seriousness of the problem is increased since savannas coincide with areas of expanding human populations. (van Auken 2000; Roques *et al*. 2001). Although individual trees may have positive effects on herbaceous production and diversity, increases in canopy density adversely affect herbaceous productivity (Scholes and Archer 1997; Riginos *et al*. 2009).

A large increase in tree cover requires successful release of woody seedlings in semi-arid savannas. By understanding the factors that influence tree seedling transitions we can provide valuable insight into the phenomenon of bush encroachment within savannas and how to manage it with means available to land users, such as fire and herbivory. This review addresses the various models explaining tree-grass coexistence, with a focus on demographic models, possible drivers of bush encroachment and its effect on previously effective bottlenecks to tree establishment. The objective of this study was to investigate the role of browsing as a possible seedling release bottleneck; hence a significant portion of the literature review is dedicated to examining the role of browsing in savannas.

1.2 Understanding tree-grass coexistence and bush encroachment in African savannas

Edaphic, climatic and disturbance factors all interact in ways that maintain the co-existence of grasses and woody plants in savannas. However, a shift in any of these or related factors can alter the grass to tree ratio, allowing the exclusion of one (Coetzee *et al.* 2008). At present, the predominant change is towards higher woody cover and biomass in savannas (Sankaran *et al*. 2005; Scheiter and Higgins 2009). Sankaran *et al.* (2005) modelled vegetation shifts in response to resources and disturbance and made projections for Africa's biomes for between 1850-2100. Their results indicated that deserts would become grasslands,

grasslands become savannas and forests replace savannas. The model predicted a change of C_4 dominated grassy regions to C_3 dominated woody ecosystems, with savanna regions decreasing by 9% in Africa. Several field studies confirm this projection, showing an increasing density of woody vegetation at the expense of savannas (Langeveld *et al.* 2003; Ward 2005; Bond 2008; Midgley *et al.* 2010; Ward *et al.* 2014).

Although we now see a strong effect of atmospheric CO_2 on C_3 woody plant growth increasing woody biomass occurring in savannas is not a simply explained phenomenon (Kgope *et al.* 2010; Buitenwerf *et al.* 2012; Donohue *et al.* 2013). Therefore understanding the conditions under which CO_2 fertilisation alters the savanna dynamics along environmental gradients are important (Buitenwerf *et al.* 2012; Donohue *et al.* 2013). For instance, in a semi-arid savanna rainfall and herbivory are likely to be dominant mechanisms limiting woody biomass and cover, and the effect of increasing CO_2 may change the dynamics of these mechanisms. Therefore we need to investigate these disturbance and resource drivers individually before attempting to understand their combined effects with elevated atmospheric CO_2 on tree-grass coexistence (Sankaran *et al.* 2005; Midgley *et al.* 2010). These studies are essential if we wish to predict thresholds underlying vegetation change, and ultimately biome shifts, in savannas.

Global vegetation models and meta-analysis show that at least half of the savanna and grassland ecosystems owe their existence to perturbations by fire and herbivory, otherwise they would be forests (Gignoux *et al.* 2009; Staver *et al.* 2011; Lehmann *et al.* 2014). Fire and herbivory can suppress the establishment and growth of trees and can kill adult trees. While these two factors have been demonstrated to play an important role in suppressing tree dominance, research suggest that the structure of a savanna is a result of many interacting factors, including herbivory, fire, resource availability, competition and climate, all functioning at particular temporal and spatial scales (Scholes and Archer 1997; Higgins 2000; Sankaran *et al.* 2005; Bond 2008; Midgley *et al.* 2010; Sankaran *et al.* 2013).

1.2.1 Tree-grass coexistence in savannas

Generally, savanna tree-grass co-existence explanations have been divided into two categories: competition-based models and demographic bottleneck models (Sankaran *et al.* 2004). Competition-based models explain grasses and trees in their established phase as coexisting in a stable equilibrium, where primary determinants of savanna structure are water and nutrients, and disturbance having indirect effects on the acquisition of these limiting resources. Demographic bottleneck models assume that trees would dominate savannas above a certain threshold of mean annual rainfall, and that disturbance and/or climatic variability are required to prevent the transition from tree seedlings and saplings to the mature size classes (Higgins 2000; Sankaran *et al.* 2004).

The first effort at a general understanding for bush encroachment was that of Walter's two-layer hypothesis (Walter *et al.* 1981 in Ward 2005). The hypothesis described the coexistence of trees and shrubs as resulting from root niche separation and thus reduced inter-life form competition. The coexistence of these two life forms is reliant on the availability of nutrients and soil water in the various rooting zones. According to this hypothesis, grasses out-compete woody plants for available nutrients and water within the topsoil, while woody plants have a competitive advantage in the subsoil. Hence, the removal of grasses by overgrazing allows for the percolation of water to the subsoil and favours trees (Dougill 1999). However overgrazing along with rooting niche separation is not required for bush encroachment, since the process sometimes occurs on shallow soils where trees and grasses occupy the same rooting zone (Wiegand *et al.* 2000). There is sufficient evidence (Belsky 1994; Le Roux *et al.* 1995; Seghieri 1995; Mordelet *et al.* 1997) challenging the validity of this model, and this has prompted alternative grass-tree coexistence theories (Higgins *et al.* 2000; Midgley *et al.* 2010). Savanna demographic bottleneck models have received increased research attention and are now generally favoured over competition-based models (Scholes and Archer 1997, Jeltsch *et al.* 2000, Higgins *et al.* 2000, Sankaran *et al.* 2004).

Understanding how different demographic processes translate into either tree-grass co-dominance or bush encroachment is important in addressing bush encroachment dynamics in savannas (Higgins *et al.* 2007; Bond 2008; Riginos 2009; Hoffman *et al.* 2009). Demographic research addresses changes in population structure and size (Midgley and Bond 2001; Midgley *et al.* 2010). Woody savanna plants are prolific and persistent resprouters, and population structure is thus more likely to change than population size (Midgley and Bond 2001; Midgley *et al.* 2010; Choeni and Sebata 2014). Midgley and Bond (2001) described two distinct processes that are required for increased tree cover in a region. The first is recruitment, which refers to an increase in the density of seedlings that germinate and survive to become saplings. This process results in an increased population size, but has little effect on savanna structure and function. The second is release, which refers to an increase in existing stem size that leads to a recruitment of a sapling (often no taller than the grass layer) into the adult tree size class. This process does not change the size of the tree population, but results in a greater density of adult trees, which can alter ecosystem structure and function.

Transitioning into a reproductive adult is an important component in the life history process of woody savanna species and may provide insight into the causes of bush encroachment (Midgley and Bond 2001; Mampholo 2006). Demographic bottlenecks limiting release into the adult size class are influenced by various disturbances, including fire and herbivory. These disturbances act as mechanisms for killing young seedlings and trapping established seedlings and saplings in the immature size class. Continuous intense browsing may suppress sapling growth and thus trap them in the flammable grassy layer, delaying escape into adulthood. Hence the success of "Gullivers" - those saplings that are trapped within the flammable zone, sensu Bond and van Wilgen (1996) - can be determined by top-down controls in the form of herbivory or fire. Saplings may eventually escape the flammable grassy layer through bolting. This entails accumulating carbohydrate resources in their lignotubers over several years, followed by rapid upward growth to a height where the branches and leaves are no longer susceptible to fire and/or browsing. Once trees have reached this height, their probability of survival is high, and they are able to reproduce (Bond 2008; Balfour and Midgley 2008).

The effectiveness of demographic bottlenecks in suppressing tree cover in savannas changes along resource gradients (Bond 2008). These bottlenecks include rainfall, soil content, and disturbances such as fire and herbivory. Stable savannas occur in regions with rainfall below ~650 mm, where water availability directly limits tree cover (Sankaran *et al.* 2005). In these semi-arid savannas, disturbances such as herbivory, fire and soil conditions are thought to play a modifying role in reducing and maintaining tree cover. Rainfall is an important determinant of tree recruitment in arid regions, where fires are infrequent and less intense. An increase in woody population sizes thus requires seedling recruitment and release, and is facilitated by an absence of disturbance (Higgins *et al.* 2000; Trollope *et al.* 2002) Hence in arid regions, where wet season droughts occur, seedling establishment is seen as a key bottleneck due to seedlings requiring sufficient rainfall events in order for surface soil layers to be kept moist to allow for growth and survival (Higgins *et al.* 2000). In semi-arid regions, where plant growth is severely limited by soil moisture, soil texture has a strong effect on tree recruitment and density. Higher infiltration rates on sandy soils enhance the growth of continuous vegetation during years with sufficient rainfall (Kgosikoma *et al.* 2012). Rangelands within the Kalahari sandveld thus have significantly higher woody cover than areas with finer-textured soils at the same rainfall (Kgosikoma *et al.* 2012).

CO_2 fertilisation, brought about by increases in atmospheric CO_2 (Farquhar 1997), enables woody plants to take in more carbon and lose less water (Donohue 2013). Atmospheric CO_2 levels are expected to exceed 700ppm by the end of the 21st century, and Higgins and Scheiter (2012) predict that atmospheric CO_2 will be a primary determinant of vegetation change in Africa by increasing the growth and water-use efficiency of C3 savanna trees. Elevated CO_2 causes plants in arid environments to increase the number of leaves they produce and may lead to a decreased probability of mortality in plants (Donohue 2013). An increased WUE is because plants operate at lower stomatal conductance and have higher photosynthetic rates will be more likely to benefit woody savanna trees growing in arid areas by increasing growth rates and overall plant productivity and thus increasing the rate of transition to adulthood (Polley *et al.* 1997; Morgan *et al.* 2004; Donohue *et al.* 2013).

In more mesic savannas, rainfall does not limit tree cover and disturbances are critical in maintaining savanna structure (Sankaran *et al.* 2005; Staver *et al.* 2011). Fire, if occurring at sufficient intensity and frequency, can suppress the transition from seedling to adult (Higgins *et al* 2000; Bond and Keeley 2005). Unlike arid savannas, wet savannas generally have sufficient grass loads to fuel regular fires. Additionally woody plant growth is affected by elevated atmospheric CO_2 concentration which allows for greater rates of carbohydrate accumulation in savanna saplings. This has the potential to facilitate earlier release of saplings from the flammable grass layer, and a shorter interval between fires is required to prevent tree dominance (Midgley and Bond 2000). However, once tree cover is greater than ~ 40%, grass biomass and fuel continuity is significantly reduced and fire no longer spreads (Riginos 2009; Archibald *et al.* 2009). Thus there is a critical threshold of tree cover beyond which bush encroachment becomes irreversible and further bush encroachment is inevitable in mesic savannas – a "point of no return" and of great management concern.

1.3 Context of the thesis: the role of herbivory

Herbivores in African savannas include wildlife, livestock and some many invertebrates. The mammal herbivore fauna in African savannas comprises a great diversity of functional types, including a wide range of body size and feeding types (Owen-Smith 2002; Hempson *et al.*

2015a). Only the influence of mega herbivores which are described as "plant-feeding mammals that typically attain an adult body mass in excess of 1000 kg" (Owen-Smith 1988) especially elephants are well documented (Shaw *et al.* 2002; Staver and Bond 2014). Few studies have examined at the combined effects of several herbivore functional types on savanna tree-grass co-existence. Additionally research on the isolated effects of invertebrates and smaller herbivores such as rodents is scarce (Shaw *et al.* 2002). Although there is sufficient evidence confirming the ability of browsers to limit tree cover in some regions research examining the role of herbivory on different demographic stages, especially seedlings and saplings are poorly documented (Staver *et al.* 2009). There is also a lack of information on the ability of browsers to regulate tree cover in the absence of other disturbances such as fire (Shaw *et al.* 2002; Staver *et al.* 2009; Staver and Bond 2014; Sankaran *et al.* 2013).

When compared to the effects of fire on savanna vegetation, the effect of herbivory poses several additional complexities due to the range of body size and feeding types (Staver and Bond 2014). Grazing can decrease grass fuel loads as well as grass-on-tree competition, thus facilitating tree recruitment (Archibald *et al.* 2005; Holdo *et al.* 2009). Browsing, on the other hand, removes leaf and twig biomass from woody plants, and can reduce their seedling and sapling growth, survival and reproduction (Augustine and McNaughton 2004; Staver *et al.* 2009). Browsing can also interact with fire and negatively affect plant growth, especially for plants within a flammable grass layer (Barnes 2001; Staver *et al.* 2009). Diverse browser assemblages may have unique impacts on growth and population structure (Olff *et al.* 2002; Staver and Bond 2014). Arid and semi-arid regions moreover have variable rainfall patterns as well as variations in herbivore populations where herbivore populations may increase during periods of high rainfall and decrease during drought years (Boone and Wang 2007). Similar patterns are observed for livestock and wild herbivore populations in arid and semi-arid conditions (Illius and O'Connor, 2000; Hempson et al 2015b).

Despite browse having strong effects on African savannas its role in maintaining in tree-grass coexistence has not received as much attention as the role of fire. Bond and Keeley (2005) suggested that the effects of fire and herbivory on plants are similar. Both fire and herbivory differ from other types of disturbance such as floods, and are similar in that they "consume" plant organic molecules and convert them to mineral products. One noticeable difference is that fire generally consumes both living and dead material while herbivory requires protein from live plant material from growth and has several broad dietary preferences. The implication is that inedible plants will provide fuel for fire. Repeated top kill events from fire can cause juvenile plant suppression and generally result in a demographic bottleneck known as the “fire trap” (Bell 1984). Fire free intervals can however result in released into adult size classes (Midgley and Bond 2000). When plants become resistant to topkill from fires they are referred to as having reached a fire resistant threshold. Resistance is generally associated with a specific plant size (Hoffmann et al. 2012). Staver and Bond (2014) proposed a similar “browse trap” model, where browsing can limit the release of saplings and trees in savanna environments.

Browsing has the ability to significantly reduce tree cover (Levick *et al.* 2009; Holdo *et al.* 2009; Moncrieff *et al.* 2013). In African savannas, herbivory by both grazers and browsers is considered a significant filter for savanna plant species composition and acts as an agent of tree mortality (Scholes and Archer 1997; Shaw *et al.* 2002; Sankaran *et al* 2005; Asner *et al.* 2009). Mega herbivores such as elephants are able to kill trees while smaller browsers can kill seedlings and are effective at suppressing seedling and sapling growth (Sankaran *et al.* 2014), thus trapping them within the flammable grassy layer (Midgley *et al.* 2010). By limiting tree growth plants can be kept in the browse trap and will therefore be exposed to not only browsers but fire (Higgins *et al.* 2000; Staver and Bond 2014).

Traits enabling woody plant species to escape the browse trap will differ along a resource gradient. In resource-rich environments, woody plants are likely to experience fast growth rates and can obtain heights beyond the reach of herbivores quicker than their counterparts in nutrient-poor or arid regions. In resource-rich environments woody plants are thus likely to employ tolerance strategies such as compensatory growth to quickly replace lost biomass (Hester *et al.* 2006). Woody plants have also evolved various chemical and structural defence strategies for avoiding herbivory (Strauss and Agrawal 1999). These avoidance strategies are more prevalent in resource-poor environments where tissues lost to herbivory are slow to replace and loss of leaves and twigs to herbivores carries a greater fitness cost. Investing in defence is costly and plants are faced with the dilemma of where to invest its resources (Hean and Ward 2012). For instance investment in structural defence may compromise plant growth resulting in suboptimal plant growth rates. Hence while chemical and structural defences may decrease herbivory, investment in growth and reproduction allow them to escape the herbivory trap sooner (Hanley *et al.* 2007).

1.4 Thesis overview

Over the past few decades there have been extensive efforts to better understand and quantify herbivory and its effects on compensatory growth (Reich *et al.* 1993; Eyles *et al.* 2009). Following damage from herbivores regrowth of plant biomass may be greater (overcompensation), less than (under compensation) or equal to (full compensation) undamaged plants (Strauss and Agrawal 1999). Tree resilience to damage from herbivory is common in adults, but saplings and particularly seedlings are vulnerable to herbivory. Identification of size and browsing of encroaching savanna tree species at which herbivory no longer suppresses their release into bigger size classes is important in understanding the dynamics of bush encroachment in savannas, especially at the more arid end of the rainfall spectrum (Hoffmann *et al*. 2012; Staver and Bond 2014). Size variables such as height and stem basal diameter (SBD) can be used as strong predictors of the probability of being browsed or killed and can explain whether these plants have become less susceptible to herbivory (Midgley and Bond 2000; Holdo 2005; Hoffmann *et al*. 2012).

The overall aim of this study was to determine the effectiveness of browsing as a demographic bottleneck, using *Vachellia karroo* in a semi-arid (MAP ~550mm) savanna in the Eastern Cape of South Africa as a case study. This was accomplished by 1) determining how browsing may limit tree cover by preventing release of smaller plants into adults, and 2) how browsing impacts survival and growth and how did this effect may change with plant size and age?

These specific aims were addressed through conducting field and greenhouse experiments. Field experiments were conducted to investigate what intensities and frequencies of herbivory seedlings were exposed to. Additional field experiments involved simulating browsing. Experiments were conducted to determine specific plant size thresholds that were necessary for seedlings and saplings to regrow and survive following browsing. These dynamics entail reaching a certain size and or height where browsing can no longer keep the sapling suppressed. While field sampling gave an indication of the extent of damage seedlings endured, greenhouse experiments provided controlled environments in which the effect of several repeated herbivory treatments on growth and survival could be tested. These experiments allowed me to look specifically at how seedlings of different ages responded, both above and below ground, to varying frequencies and intensities of herbivory. By combining the analysis from field and greenhouse experiments I was able to determine age and size thresholds at which seedlings become “immune” to herbivory .Specific thresholds for plant mortality and suppression, where plants are able to sufficiently compensate for biomass loss were addressed in chapters.

Chapter 2 describes the study site. I reviewed a series of satellite images looking at how tree cover at the study site has increased with time. I also quantified the level of encroachment within specific sampling sites on the farm. Additionally the chapter provides details such as farming practices, stocking densities and rainfall records that are assumed to have an effect on the fate of *V karroo* seedlings.

Chapter 3 explores the fate of *V. karroo* seedlings and saplings of different sizes at the study site. Here I investigated the intensity, type and frequency of herbivory, from smaller herbivores and invertebrates, as well as larger herbivores. From this I determined specific intensities and frequencies of herbivory that resulted in mortality or suppression of *V karroo* seedlings within this region.

Chapter 4 reports on experiments simulating browsing and determining how big seedlings have to be to become “immune” to browsing. In the field I simulated varying intensities of herbivory on different sized seedlings and saplings. This allowed me to examine how mortality and compensatory response are influenced by plant size. In the greenhouse I experimentally investigated the effect of seedling age on mortality and compensatory responses following simulated browsing. By investigating the responses of a range of seedling ages I was able to predict an age threshold at which the probability of mortality following defoliation drops off. Accompanying an age threshold was a specific level of intensity and frequency of herbivory.

In Chapter 5 I provide a synthesis of how the findings of the study contribute to our understanding of the role of herbivory in tree-grass coexistence and bush encroachment in semi-arid savannas. Identifying a size and age threshold at which seedlings are less susceptible to herbivory is useful information for management initiatives aimed at halting bush encroachment in these systems.

2. Study site and species

This chapter describes the study site and species. The chapter provides an overview of the region's faunal and floral diversity as well as other characteristics such as farming practices and rainfall patterns. Additionally a brief description of *V. karroo* and plant demographic phases is provided. Finally, the chapter addresses tree cover density on the farm. To determine whether tree cover regulates browse intensity and frequency and thus plant growth, as explained in Chapter 3, I quantified tree cover of specific sites on the farm.

2.1 Study site

The study was conducted at the farm Endwell located near Adelaide, Eastern Cape, South Africa (32°44'S, 26°28'E) (Fig 2.1). The farm forms part of the Smaldeel conservancy which stretches from Somerset East to Fort Beaufort. The most common land use within the region is livestock farming, particularly beef cattle (D. Painter, pers. comm).

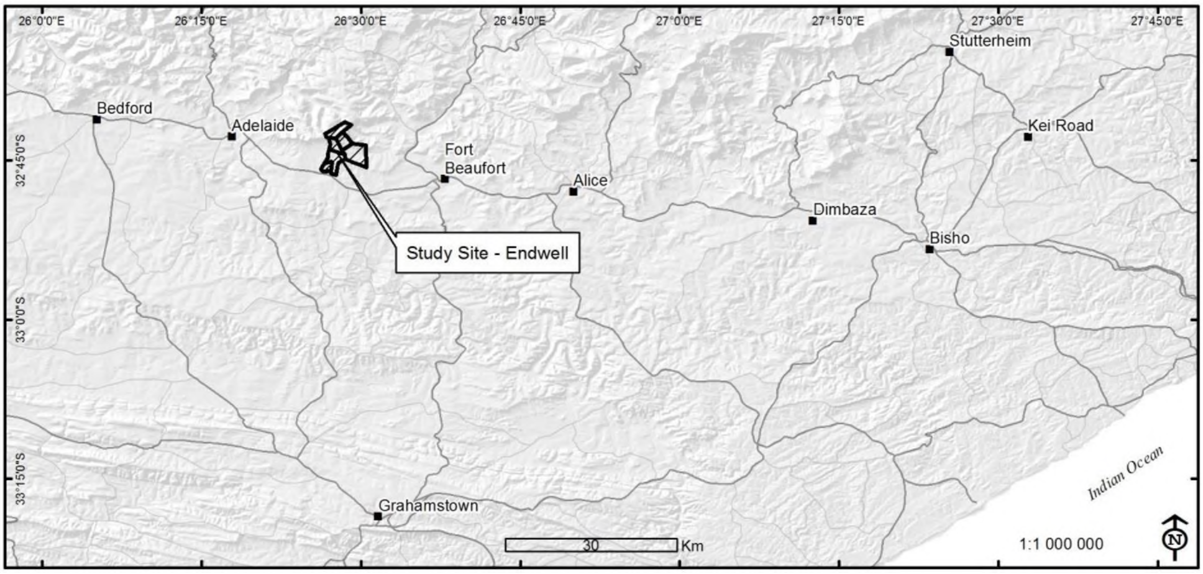


Figure 2.1: The location of the study site (Endwell farm) within the Eastern Cape. Source Andrew Skowno

Acocks (1953) classified the region as False Thornveld of the Eastern Cape while Mucina and Rutherford (2006) reclassified it as Bedford Dry Grassland. The woody vegetation on the farm is dominated by *Vachellia karroo*, while other woody species, occurring at much lower densities, include *Olea europaea* subsp. *africana* and *Scutia myrtina*. Common grass species include *Themeda triandra, Sporobolus africanus, Cymbopogon caesius, Eragrostis curvula, Panicum aequinerve* and *Panicum maximum*.

The farm has cattle at relatively low stocking rates (4ha LSU^{-1}) with a presence of game. Common game includes bushbuck (*Tragelaphus sylvaticus*), kudu (*Tragelaphus strepsiceros*), impala (*Aepyceros melampus*), common duiker (*Sylvicapra grimmia*) and warthog (*Phacochoerus africanus*).The Smaldeel has distinct wet and dry seasons. Rainfall is bimodal with peaks in March and October (Martens *et.al.* 1996). Rainfall at the study site has been measured since 1952 with a standard rain gauge positioned on the farm. Mean annual rainfall on the farm is 450 – 650 mm and is generally high for the study area (Fig 2.2). This is possibly due to the farm's location at the foot of the Smadeel Mountains. The mean annual rainfall for the study period (May 2014 – May 2015) was 848 mm. An unusual spike in rainfall during June and July 2015 increased the average rainfall for the region. This however did not influence the experiment since sampling was completed in April 2015. For analysis purposes seasons were defined by the amount of rainfall they received over the study period. The region has a distinct dry season (May-July, ~4.1 mm rainfall) while the other months receive moderate (Aug-Dec, ~52.8 mm rainfall) and high rainfall (Jan-Apr, ~118.5 mm rainfall).

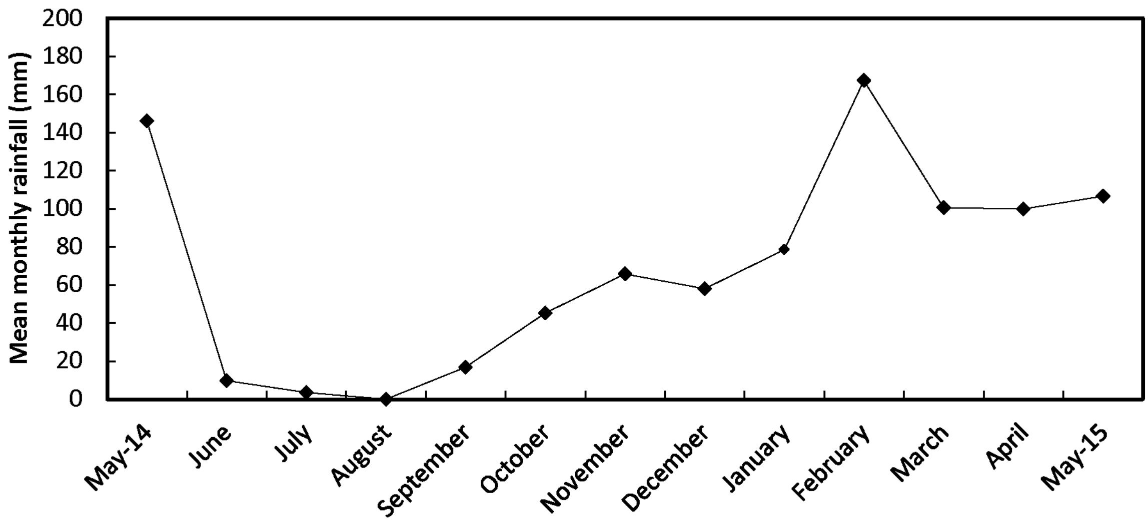


Figure 2.2: Mean monthly rainfall (mm) for Endwell farm (32°44'S, 26°28'E) during the study period (May 2014-May 2015). Source: Tony Painter

2.2 Study species

Figure 2.3: Image of an adult *V.karroo* at Endwell farm

Vachellia karroo (Hayne) Banfi & Gallaso (Fabaceae), previously known as *Acacia karroo* is widely distributed in its native region of southern Africa. The adult may range from shrub to tree and can range from 1 m to 20 m in height. A distinct feature of the species is its paired white spines which can be as long as 25 cm. While a typical form may resemble that of a slender and sparsely branched savanna tree, environmental conditions and disturbances are known to influence its shape and structure (Archibald and Bond 2003).

An increase in abundance of indigenous woody *V.karroo* plants is a widespread form of bush encroachment in the Eastern Cape and other parts of South Africa (O'Connor *et al*. 2014). The species is widespread throughout southern Africa and has drastically increased in extent and abundance within its original distribution original distribution range resulting in shifts from grass to woody dominated landscapes (O'Connor 1995).

Studies indicate that African acacias are known to produce large quantities of seeds and very often accumulate high densities of viable seeds in the soil (Coe and Coe 1987; Ross 1965; Walters and Milton 2003: O'Connor *et al.* 2014). Soil seed banks provide assurance for these woody species and allow for the persistence of populations through periods unfavourable conditions or disturbance (O'Connor *et al*. 2014). Large persistent soil seed banks of acacias guarantee a source of viable seeds (Fig 2.7-1; Midgley and Bond 2001). Many studies have assumed that seed production prior to seed predation is not limiting. From personal observation and *V. karroo* field surveys I found massive quantities of seed pods being produced, a high percentage of seed viability, and an abundance of saplings and seedlings at the study site. This suggests that seed set, dispersal and germination (Fig 2.4, processes labelled 1, 6-10, 11-12) are not limiting factors. Additionally processes labelled 1-12 in Fig 2. 4 cannot be easily influenced by farming practices and management whereas disturbances, for instance fire and herbivory, can be modified or used in bush encroachment management initiatives. Recruitment (Fig 2.4, transition in processes labelled 13-18 to 19-22), which refers to the successful transition to large size classes appears to be a strong bottleneck and may cause an increase in abundance of *V.karroo* within this semi-arid savanna (Midgley and Bond 2001).

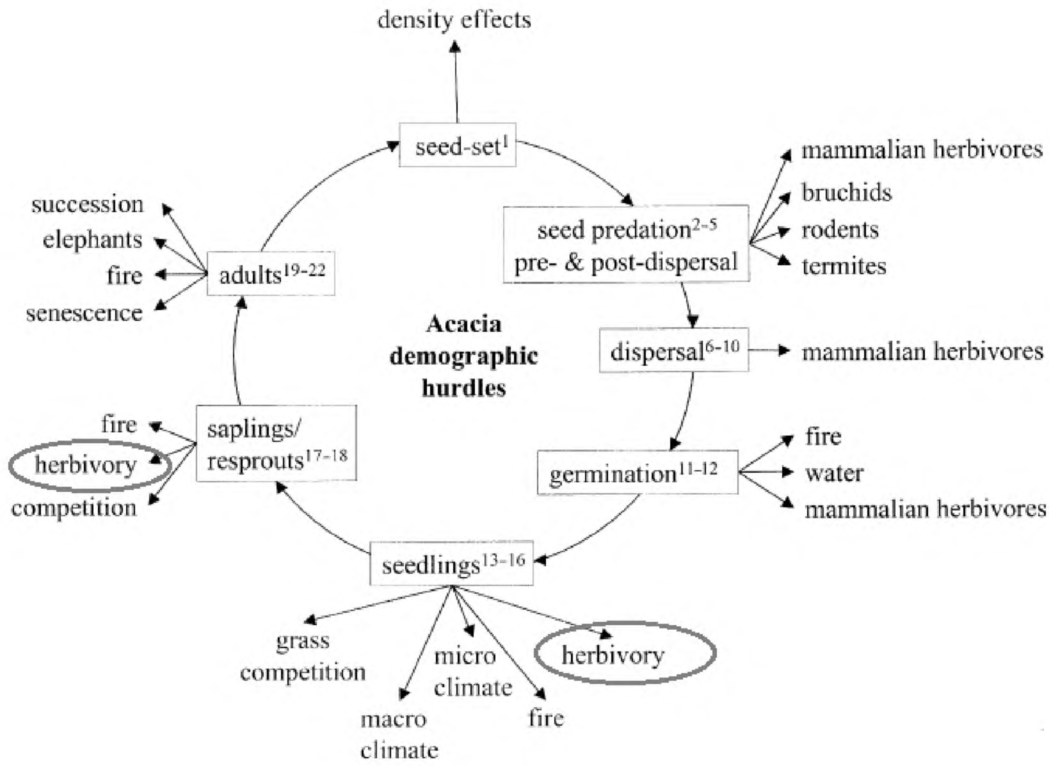


Figure 2.4: Demographic hurdles of African acacia species. Highlighted (grey ovals) represent specific demographic stages examined within this study. Source: Midgley and Bond (2001)

2.3 Woody encroachment at Endwell

Woody canopy cover, predominantly *V. karroo*, has increased by approximately 20% between 1985 and 2009 (Klopper 2015). This is clearly seen in aerial photographs (Fig 2.3 Endwell 1985 and 2014). To address woody encroachment dynamics I had to examine the study site landscape and determine the best locations for conducting sampling.

To determine the effect of encroached and open savanna states on seedling dynamics I selected, from aerial imagery, areas on the farm that represented the desired degrees of encroachment (Fig 2.6). Site visits confirmed that these areas were encroached by *V. karroo*. I confirmed that selected areas had different levels of encroachment by quantifying tree canopy cover within these regions of the farm (Fig 2.7). I imported an aerial photograph (2013) of the study site into QGIS (a free open source geographic information system). This image was georeferenced using a 2013 satellite image to give it a spatial representation. Thereafter the georeferenced image was projected using Transverse Mercator 27 WGS84 coordinate system. I used polygons to select encroached and open regions and quantified *V.karroo* tree cover within each polygon. The encroached site had ~45% tree cover whereas the selected open region had ~5% tree cover.


Endwell 1985
Author: Andrew Skowno June 2013
1:20 000
370
Meters
N
W
E
S

Figure 2.6: Aerial images of Endwell farm in Eastern Cape, South Africa (32°44'S, 26°28'E). Images) show the encroachment of V.karroo) in the region between 1985 and 2014 (the most recent available image). Marked regions (black squares) indicate the experimental locations within the farm. Sites were distinguished by canopy cover; the encroached site had ~40 % canopy cover while the open site had ~5 % canopy cover. Source: Andrew Skowno

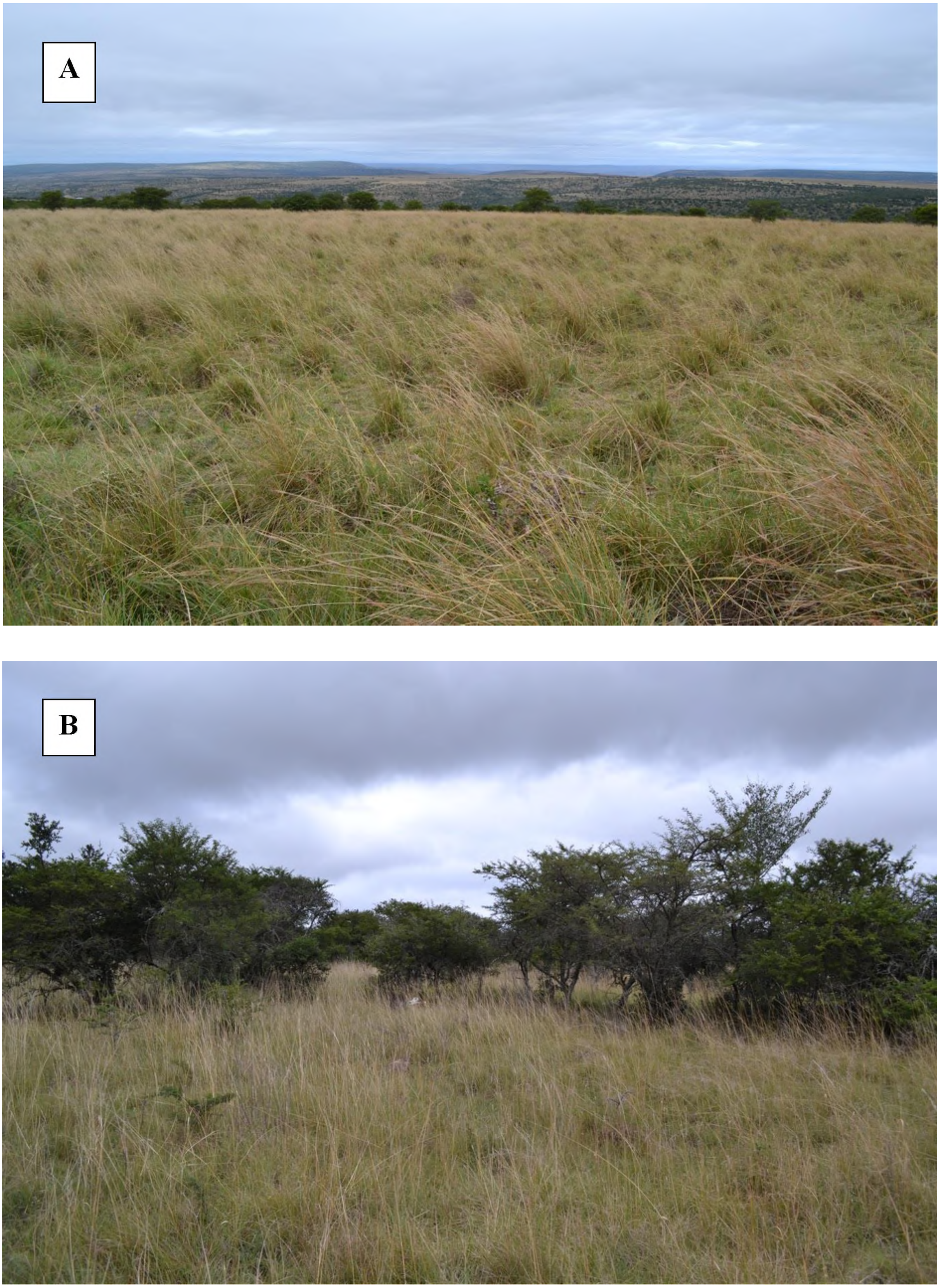


Figure 2.7: Images of sampling areas (A) open site with ~5% tree cover and (B) encroached site with ~40% tree cover at Endwell farm (32°44'S, 26°28'E). V. karroo is the dominant woody species at both sites. Source: Kyle Lloyd

3. The Fate of Acacia Seedlings in Semi-arid Savanna

3.1 Introduction

Top down disturbances such as fire and herbivory play important roles in contributing and maintaining tree grass co-dominance within savannas (Scholes and Archer 1997; Higgins *et al*. 2000; Sankaran *et al*. 2005; Sankaran *et al*. 2014). Although savanna research has progressed considerably, there is still debate on the key determinants of tree grass coexistence (Barnes 2001; Augustine and McNaughton 2004; Staver *et al*. 2009). Savannas are one of the most widespread biomes within the tropics and subtropics and support abundant herbivore assemblages (Holdo *et al*. 2013) yet the role of herbivory in regulating tree cover is very often overlooked (Holdo *et al*. 2013).

Herbivory exerts top-down effects on savanna structure (Sankaran *et al*. 2013). Although the effects of herbivory on demography are difficult to generalise, browsers are able to significantly influence mortality and growth across a range of size classes, having the most impact on the seedling stage (Staver *et al*. 2009; Midgley *et al*. 2010; Sankaran *et al*. 2013). Seedlings are more susceptible to herbivory and are easily killed by leaf/stem consumption. In savannas where herbivores are abundant, herbivory is found to limit tree establishment (Augustine and McNaughton 2004; Rooke *et al*. 2004).

Some experimental studies have shown that the exclusion of fire and herbivory have resulted in significant increases in woody biomass, sometimes in complete biome shifts (Tilman *et al*. 2001, Briggs *et al*. 2005). Other research suggests that wild herbivores, despite high browsing intensity may have minor effects on plant biomass (Guldemond and Van Aarde 2008; Kalwij *et al*. 2010) while others demonstrate a strong effect on the suppression of plant populations (Edkins *et al*. 2007, Fornara and du Toit 2008). The effect of domestic cattle, which are not generally browsers may have indirect and direct influence on plant growth, some positive (Riginos 2009) and others negative (Hejcmanova *et al*. 2009).

It is generally accepted that herbivory, through its influence on survival and growth can largely affect plant abundance and distribution (Goheen *et al*. 2010).This is especially true for the role of herbivory at the seedling and sapling demographic phases (Midgley *et al*. 2010). Despite the effects most savanna models rarely include herbivory as an important determinant and when used represent a fixed, rather than a continuous factor influencing vegetation structure (Scholes and Walker 1993; Scholes and Archer 1997; Sankaran *et al*. 2004; Holdo *et al*. 2013). This is generally the case because large herbivore populations are usually absent from most savannas across the world and largely removed and replaced with domestic livestock across Africa (Holdo *et al*. 2013). There is also too little available data on herbivore abundance and composition to feed into these models. Hempson *et al* (2015 a) have made a major, but very recent contribution to our knowledge of what herbivory regimes existed in Africa before humans reduced their populations. However, present-day abundance data over the sorts of large vegetation areas that are typically modelled is unavailable (Staver *et al*. 2011). Despite herbivory playing a crucial role in maintaining savanna structure it has not received as much attention as the role of fire. This is an important research gap in regions where fire is infrequent and browsing may act as an important demographic bottleneck in tree establishment (Staver and Bond 2014).

In Africa much research has focused on larger mammals such as elephants and giraffe (Bond and Loffel 2001; Birkette and Stevens-Wood 2005) and not as much on the role of smaller mammals and insects and their effects on seed and seedlings (Goheen *et al*. 2010). Larger mega herbivores such as elephants by killing trees, can rapidly decrease tree cover while, smaller herbivores, through supressing seedling release and growth, limit tree establishment (Augustine and McNaughton 2004; Moe *et al*. 2009). Smaller browsers are efficient at regulating seedling transition rates, which may determine the rate at which woody density increases within a region. The effect of individual herbivore species on savanna tree recruitment and release is well researched, but the effect of an array of herbivores such as invertebrates versus larger mixed feeders is largely unknown (Shaw et al 2002; Staver and Bond 2014). Another research gap concerns data on the rate at which seedlings are consumed. While research on the effects of browsing on seedlings and saplings exists, measurements of rates at which these plants are eaten in savannas are rare (Staver *et al*. 2014; Riginos *et al*. 2009).

Through field observations and herbivore exclosures, I examined the fate of seedlings at Endwell Farm (see Chapter 2). I investigated (i) the fate of seedlings following natural browsing from different herbivore guilds, (ii) how the effect of browsing on seedlings was influenced by tree cover, and (iii) the direct effects of subcanopy microhabitats on *V. karroo* survival and growth following browsing. Although field experiments were conducted over a short period of time, they provided direct insight on how browsing in a semi-arid savanna may can cause seedling mortality and suppression.

3.2 Methods

3.2.1 Experimental design

3.2.1.1 Quantifying browsing frequency and intensity at high and low woody canopy cover

To determine the fate of seedlings I monitored the frequency and intensity of herbivory on marked seedlings at the study site at monthly intervals over a 12 month period (May 2014 – May 2015). *Vachellia karroo* plants below 30cm in height and 3mm in stem basal diameter were selected and marked. This size class represents seedlings (< 1 y old) that were chosen on the basis that they represented plants that had established in the current year and largely within the grass layer. I marked 120 seedlings, 60 each at the open and encroached sites identified and characterised in Chapter 2 as having (~ 5 % and ~ 40 %) tree canopy.

On a monthly basis, from May 2014 to May 2015, marked seedlings were visually inspected. If a seedling had been subject to any browsing not previously recorded, or showed increased intensity of browsing since the previous month, this was recorded as a browsing event. In this study I refer to browsing frequency as the number of times a seedling experienced browsing events over the 12 month study period. For instance, if in April the intensity of a particular seedling was recorded as low, then in May, browsing intensity on the same seedling was found to be severe, and in June the seedling had been entirely eaten, I quantified total browsing frequency endured by the seedling as 3 per 12 month period. Browsing intensity was determined by the amount of above ground biomass removed each month. I initially recorded morphological characteristics of the plant and the section of the plant that was browsed. This was done so by the next month I could identify whether there was any new regrowth or browse. In terms of biomass, I estimated the amount of biomass removed in relation to plant size. This was classified as no damage, low (~ 25 %), medium (~ 50 %), severe (~ 75 %) and eaten (~ 100 %, complete removal of above ground biomass). Cumulative intensity was recorded for the entire sampling period (1 year) and was calculated by summing the approximate percentage above ground biomass lost in each feeding event.

To determine how browsing changed with season I divided the 12-month study period into three seasons defined by the mean amount of rainfall they received over the study period (low, medium and high). Average monthly rainfall data was used to generate seasonal averages. The region has a distinct dry season (May-July, 4.1 mm) while the other months received medium (Aug-Dec, 52.8 mm) and high rainfall (Jan-Apr, 118.5mm). Browsing intensity was quantified for each season and the entire study period. This was done by calculating the cumulative amount of above ground biomass removed over time. Browsing frequency was also summed for each season.

At the end of the experiment all above ground plant material was removed. Plant material was dried for 72 h before determining dry weights.

3.2.1.2 The effect of canopy cover on the probability and effect of seedling damage by different herbivore assemblages

The aim of the field experiment was to evaluate the influence of different herbivore assemblages on seedling survival and growth. I also examined whether the frequency and effects of herbivory differed in the subcanopy and intercanopy. This was done by excluding different herbivore guilds based on herbivore size class.

I used a paired design experiment to examine the probability and effects of seedlings being browsed by vertebrate and invertebrate herbivores in subcanopy and intercanopy microhabitats at the open (~ 5 % canopy cover) site on Endwell Farm. Herbivore damage was compared between seedlings inside and outside 20 exclosures designed to exclude all vertebrates including rodents. Exclosures were covered in bird wire mesh (0.6 x 0.6 m, 12 mm hole netting), and I placed 10 under *Acacia* canopies, and 10 in the open at least 5-7 m from the edge of the nearest tree canopy. Exclosures were positioned at least 15 m apart in the same grazing paddock and were anchored to the ground with steel pegs. While excluding all vertebrate herbivores, they were permeable to most invertebrates.

Only seedlings with no cotyledon or foliage damage were selected. I marked 204 seedlings, with a minimum of 4 individuals each inside and outside per exclosure. Seedlings were marked on the 1st May 2014, and were thereafter monitored at the beginning of every month for 12 months. I recorded any evidence of herbivory and repeated measures of herbivory, regrowth and mortality each month (as described above).

3.1.1.3 The effect of canopy cover and different herbivore assemblages on plant size and growth parameters

At the end of the 12 month period, all above and below ground plant material was harvested. Above and below ground material was separated and dried for 72 h before determining dry weights. Several plant traits were measured and compared between treatments within this exclosure experiment. These included total above ground biomass (g), below ground biomass (g), plant lignotuber length (mm), increase in height over the study period (cm) and stem basal diameter (SBD, mm).

3.2.2 Statistical analysis

3.2.2.1 Quantifying browsing frequency and intensity at high and low woody canopy cover

I was interested in how browsing affects time to mortality. I used survival analysis because, unlike logistic regression and generalised linear modelling, it accounts for unexpected seedling mortality during the study period. Survival analysis using the survival package in R (R Core Team 2014) focuses on survival distribution and is used when the time to mortality is of interest. Data was entered for each individual seedling in the format *start date of experiment*, *end date of experiment* (i.e. after 12 months), *status* (1= mortality occurred, 0= mortality did not occur), *encroachment level* (treatments: encroached or open), *browsing frequency* (number of defoliation events throughout study period) and *browsing intensity* (cumulative browsing intensity for the study period). Browsing frequency and intensity were added to the model to determine whether it had any influence on survival patterns.

Additionally Kaplan-Meier estimates were generated using the survival package in R. The Kaplan-Meier method (Kaplan and Meier 1958) was used to estimate the probability of survival past a certain point in time by calculating a survival distribution. The calculation was made from the change in the number of individuals that died and those that survived at monthly intervals over the period of the study.

A GLM with quasipoisson errors was run to determine the effect of season on browsing in the encroached and open site. When there was a significant effect of season on browsing, Tukey *post hoc* tests were conducted. This allowed me to examine differences in the effect of various seasons on browsing within encroached and open sites. General linear models with quasipoisson errors were also performed to determine if there was any effect of site (encroached vs open) on browsing frequency and intensity over the study period. I also tested the effect of site on plant above ground biomass, and whether this plant-site relationship was modified by an effect of browsing frequency and intensity.

3.2.2.2 The effect of canopy cover on the probability and effect of seedling damage by different herbivore assemblages

Survival analysis (as described above) was performed to determine the effect of browsing, of different herbivore guilds (all herbivores vs. invertebrates only) on seedling survival. In this analysis *treatments* represented microhabitats (subcanopy vs intercanopy), exclosure treatments (exclosure: all vertebrates excluded; no exclosure – all herbivores present) and their interactions. Treatment combinations were subcanopy, exclosure (SE), subcanopy, no exclosure (SNE), intercanopy, exclosure (IE) and intercanopy, no exclosure (INE).

To determine the influence of season on browsing I ran GLMs with quasipoisson errors. I then tested for an added effect of microhabitat and the exclusion of vertebrate herbivores as sub-treatments on browsing frequency and intensity. Where significant differences were observed, Tukey *post hoc* analysis was performed. This allowed me to determine whether sub-treatments interact with season to influence browsing frequency and intensity.

3.2.2.3 The effect of canopy cover and different herbivore assemblages on plant size and growth traits

GLMs were performed to test the effects of microhabitat and the exclusion of vertebrate herbivores on above and below ground biomass, lignotuber length and plant growth in height. I also tested whether there was any additional effect of browsing frequency and intensity on the above-mentioned plant traits within and between each treatment (microhabitat and exclusion of vertebrate herbivores). Where significant differences were observed, Tukey *post hoc* analysis was performed. Since all seedlings were of the similar size (in SBD), I tested for an effect of browsing frequency and intensity on plant growth traits.

To determine the bivariate relationships between the above-mentioned plant growth traits I fitted standardized major axis (SMA) lines as implemented in SMATR (Falster *et al.* 2003).This type of analysis allowed me to quantify and define the biological relationships between growth traits. SMA analysis, which is similar to linear regressions, determines how one plant variable may scale against another. I was specifically interested in the strength of relationships between measured plant growth traits. This allowed me to investigate possible growth trade-offs between growth in height, above and below ground biomass and lignotuber length. SMA analysis was also performed to test the effect of plant size on plant growth traits (i.e. the relationship between final stem basal diameter (SBD) and plant traits). All plant trait variables were log transformed.

3.3 Results

3.3.1 Quantifying browsing frequency and intensity at high and low woody canopy cover

All marked seedlings showed some evidence of browsing throughout the study period. At both sites, the majority of seedlings were browsed 2 or 3 times during the 12 month study. There was no significant difference in the number of times seedlings were defoliated between sites. There was also very little difference in browsing intensity between the open and encroached sites ($F = 2.88$; $df = 1$; $p = 0.09$). At the encroached site, the probability of survival was 0.4 by the end of the study period (1 year) while within the open site survival probability was 0.35 by May 2015 (Fig3.1). The coxph survival model revealed no significant effect of site (open vs. encroached) on seedling survival ($z = -0.50$; $p > 0.05$), with the probability of survival decreasing with time for both sites (Fig 3.1). There was a significant influence of browsing frequency ($z = -6.50$; $p < 0.001$), browsing intensity ($z = 2.45$; $p < 0.05$,) and their interaction; ($z = 2.03$; $p = 0.04$) on seedling survival.

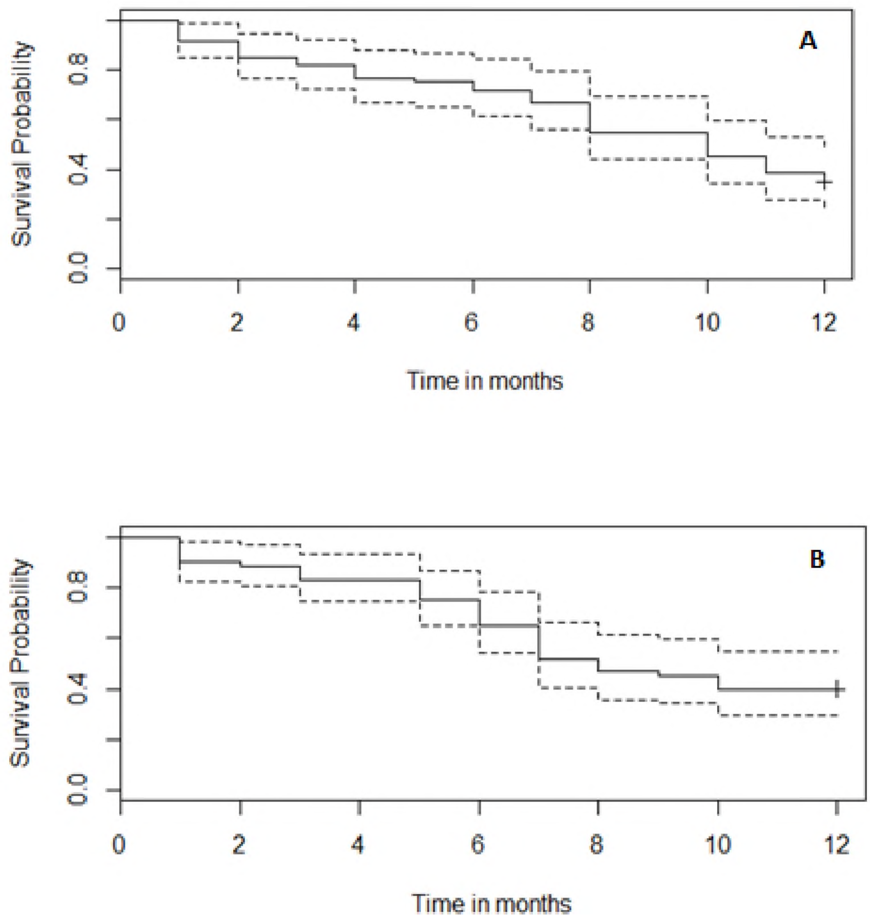


Figure 3.1: Kaplan-Meier estimates generated using the survival analysis. Estimates indicate the probability of plants surviving past a certain point in time (in months) following browse in the open (A) and encroached (B) sites. Dotted lines represent 95% confidence intervals generated from survival analysis.

The number of seedlings browsed differed markedly between months. At the open site, the highest proportion of seedlings browsed (78%) occurred in May 2014 and the lowest (4.1%) in September 2014 (Fig 3.2). A similar pattern was observed in the encroached site; however the initial browsing proportion was lower at 63%.

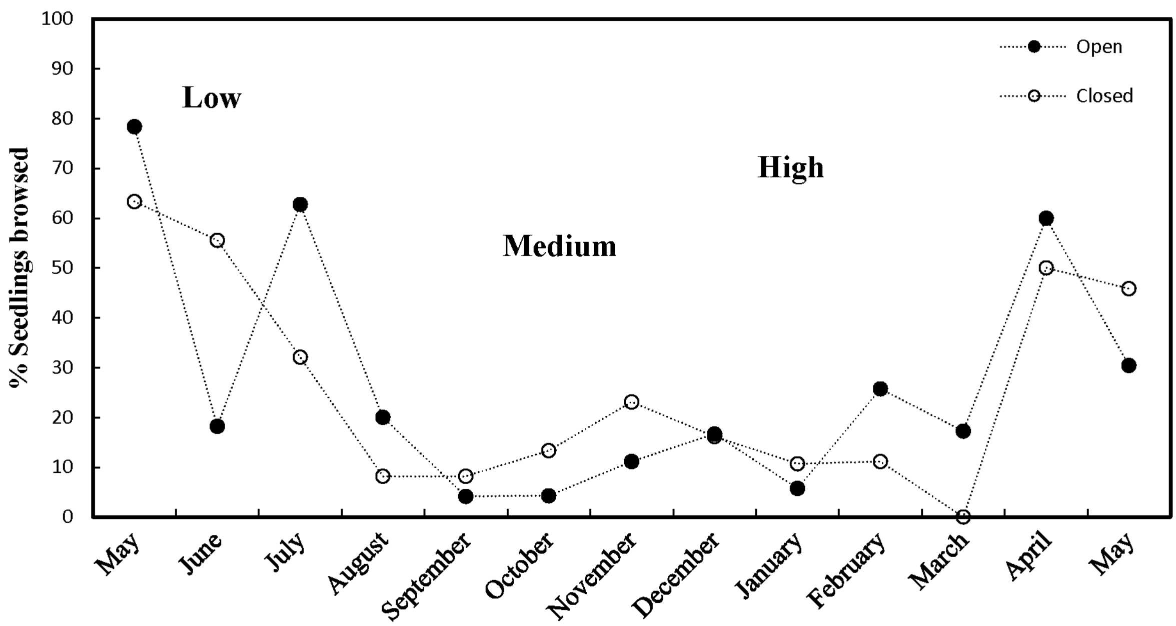


Figure 3.2: Total percentage of seedlings showing new browse damage during the month preceding sampling at the open (~5% canopy cover) and encroached (~40% canopy cover) sites on Endwell Farm. Seedlings were monitored every month from the 1st May 2014- 1st May 2015. There was no significant difference in browsing between sites. Seasons are indicated on graph (Low= low rainfall May-July; Medium= medium rainfall August-December and High= high rainfall January- April).

Although there was a strong effect of season on browsing frequency ($F = 60.42$; $df = 2$; $p < 0.001$) and intensity ($F = 22.9$; $df = 2$; $p < 0.001$), there was no significant effect of site (open or encroached) on either frequency or intensity of browsing. At the open site, season had a strong influence on both frequency ($F = 32.30$; $df = 2$; $p < 0.001$) and intensity ($F=15.0$; $df = 2$; $p<0.001$) of browsing. Browsing frequency and intensity differed between all seasons, with the smallest difference seen between high and low rainfall periods (Table 3.1). At the encroached site, season also had a strong effect on browsing frequency ($F = 28.33$; $df = 2$; $p < 0.001$) and intensity ($F = 8.33$; $df=2$; $p < 0.001$). While browsing frequency differed significantly between all seasons, browsing intensity only differed between the wet and dry season (Table 3.1).

Table 3.1: Results from Tukey HSD post hoc analysis showing pairwise comparisons between the three seasons to examine the influence of rainfall season on browsing frequency and intensity at the encroached and open site.

	Encroached site				**Open site**			
Season (rainfall)	**Browse frequency**		**Browse intensity**		**Browse frequency**		**Browse intensity**	
	z value	p value	z value	p value	z value	p value	z value	p value
High vs Low	-2.86	**< 0.01**	-2.02	> 0.05	-2.68	0.02	-2.28	0.06
Medium vs Low	-7.80	**< 0.001**	-4.25	**< 0.001**	-9.09	**< 0.001**	-6.11	**< 0.001**
Medium vs High	-3.55	**< 0.001**	-1.47	> 0.05	-5.13	**< 0.001**	-2.99	**< 0.01**

Although there was no effect of either browsing frequency or intensity on above ground biom ass, total plant above-ground biomass was significantly lower at the encroached site (0.2 g ± 0.036) than the open site (0.74 ± 0.19; $F = 11.61$; $df = 1$; $p < 0.01$).

3.3.2 The effect of canopy cover on the probability and effect of seedling damage by different herbivore assemblages

At the end of 12 months, the probability of survival in the intercanopy had decreased to 0.84 inside exclosures and to 0.61 outside of exclosures (Fig 3.3 SNE and SE). The probability of surviving past 12 months in the subcanopy was 0.79 inside exclosures and 0.48 outside of exclosures (Fig 3.3 INE and IE). Fig 3.4 shows only a slight difference in effect of exclosure treatments on seedling survival in the intercanopy ($z = 1.66$; $p = 0.09$) and subcanopy ($z = 2.36$; $p < 0.05$). Results from the coxph model revealed no significant difference in seedling survival between subcanopy and intercanopy microhabitats ($z = -0.38$ $p < 0.05$; Fig 3.3). Browsing frequency ($z = -3.83$; $p < 0.001$) and intensity ($z = 4.85$; $p < 0.001$) had a significant negative effect on seedling survival.

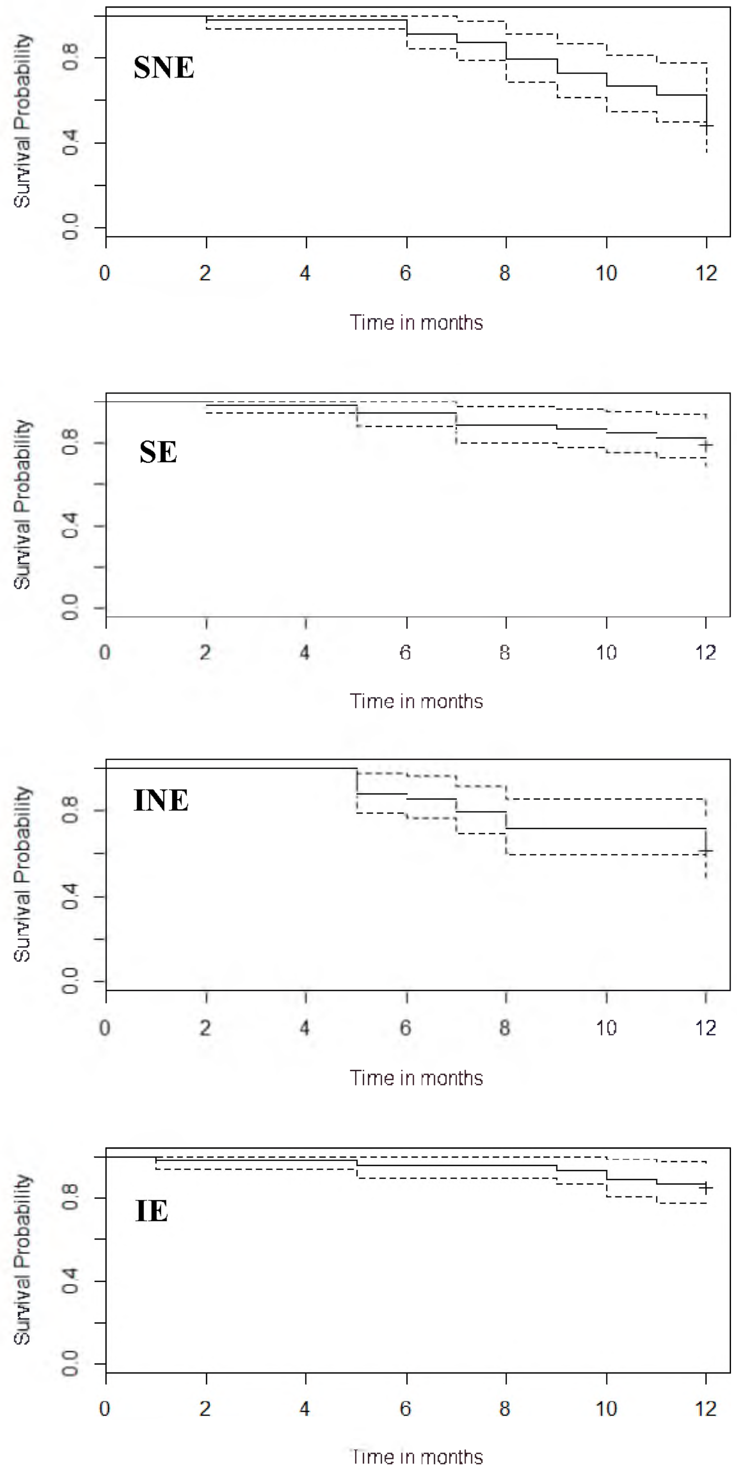


Figure 3.3: Kaplan-Meier (KM) estimates generated using the survival analysis. Estimates indicate the probability of plants surviving past a certain point in time (in months) following browse from small and large herbivores in subcanopy and intercanopy microhabitats. Several KM curves and estimates were generated for each treatment (**SNE**= subcanopy no exclosure; **SE**= subcanopy exclosure; **INE**= intercanopy no exclosure and **IE**= intercanopy exclosure). Dotted lines represent 95% confidence intervals generated from survival analysis.

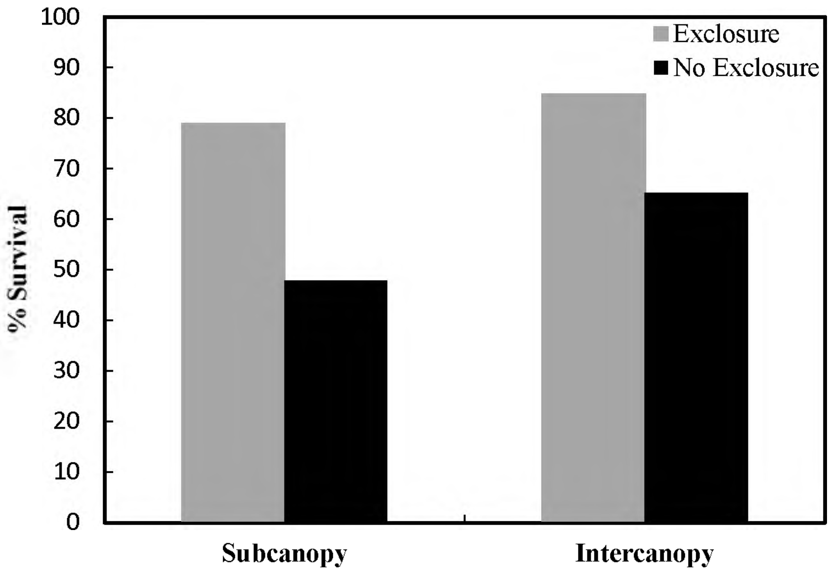
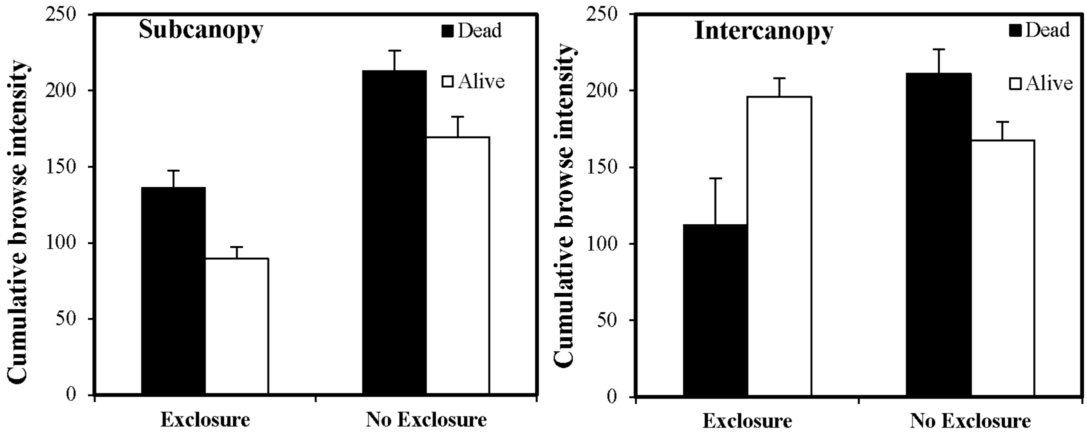


Figure 3.4: Percentage survival of marked seedlings monitored for a year (May 2014-May 2015) in response to herbivore assemblage (exclosure: all vertebrates excluded; no exclosure – all herbivores present) and microhabitat (subcanopy vs. intercanopy). Survival was calculated at the end of the study period.

Fig 3.5 illustrates the overall effects of browsing frequency and intensity within the two microhabitats. Seedlings still alive after 12 months had significantly lower intensity and frequency of browsing inside than outside exclosures (the exception being intensity in the intercanopy, where no significant effect was found). Thus exclosures reduced damage and damage reduced survival. Dead plants had more cumulative browse damage (i.e. browse intensity) inside exclosures, but no such pattern was observed for browse frequency (no significant difference in the subcanopy, and higher frequency inside exclosures in the intercanopy). There was thus no clear relationship between browsing frequency and likelihood of death among those plants that had died, suggesting other causes of death may have played a role.

There was an overall effect of browsing intensity ($F = 35.6$; $df = 1$; $p < 0.001$) and frequency ($F = 6.2$; $df = 1$; $p < 0.05$) on seedling survival within both microhabitats. There was no difference in the effect of browsing frequency on seedling survival between exclosure treatments in either microhabitat. While the effect of browsing intensity was slight in the intercanopy ($z = -1.85$; $p = 0.06$) there was a stronger effect in the subcanopy ($z = -2.485$; $p < 0.05$).

A



B

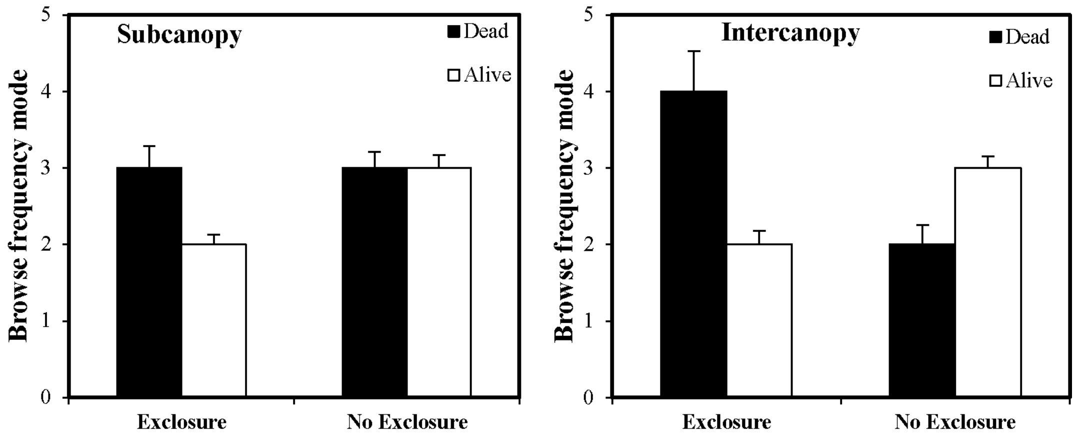


Figure 3.5: The effect of browsing frequency and intensity on seedling survival. Cumulative browsing intensity (**A**) and browse frequency (most common frequency) (**B**) are shown for seedlings that were dead and alive at the end of the study period inside and outside exclosures. Seedlings were monitored for the period May 2014- May 2015.

The cumulative browsing intensity did not differ between the intercanopy and subcanopy microhabitats (Fig 3.6). However there was a significant influence of exclosures on browsing intensity ($F = 31.5$; $df = 1$; $p < 0.001$) with seedlings outside of exclosures having approximately 68% more above ground biomass removed. Hence, differences in browsing intensity were observed between exclosures and no exclosures in the subcanopy ($z = 4.72$; $p < 0.001$) and intercanopy sites ($z = 3.50$; $p < 0.01$).

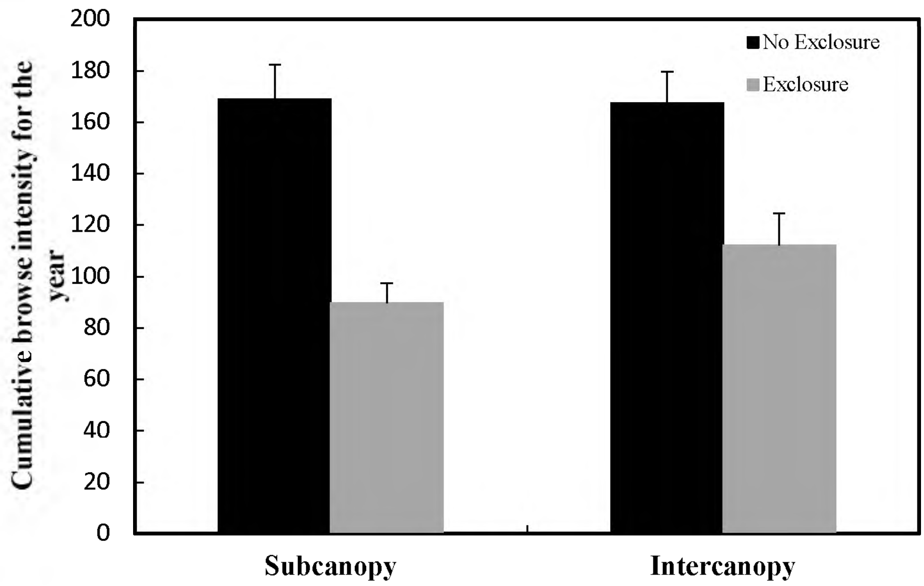


Figure 3.6: Cumulative browse intensity (total above ground biomass removed) was calculated for the study period (May 2014- May2015). Cumulative browse intensity is shown for seedlings in the subcanopy and intercanopy regions (within and outside of exclosures) in a semi-arid savanna.

There was no difference in observed browsing frequency between subcanopy and intercanopy microhabitats ($F = 0.32$; $df = 1$; $p > 0.05$), but there was an effect of exclosures on browsing frequency ($F = 19.1$; $df = 1$; $p < 0.001$; Fig 3.7). Seedlings in the subcanopy within exclosures experienced fewer browsing events than those outside of exclosures ($z = 3.58$; $p < 0.01$). For seedlings in the intercanopy there was a lesser, but significant, effect of exclosures on reducing browsing frequency ($z = 2.81$; $p < 0.05$).

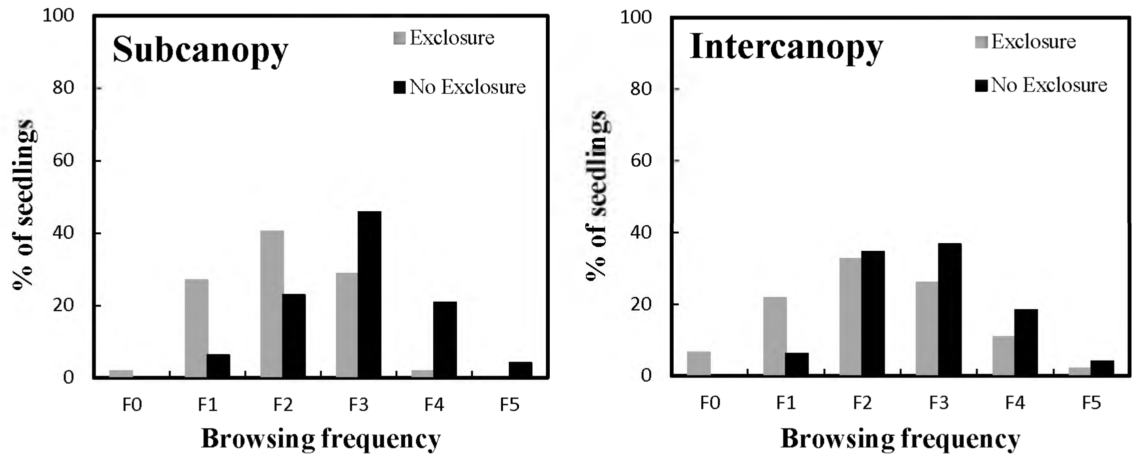


Figure 3.7: Percentage of marked seedlings exposed to different browsing frequencies (F0-F5) during a year. There was no significant difference in the browsing frequency between the subcanopy and intercanopy. For both subcanopy and intercanopy sites there was an effect of exclosures ($p < 0.05$).

Season had a strong influence on browsing intensity ($F = 16.6$; $df = 2$; $p < 0.001$) and frequency ($F = 23.1$; $df = 2$; $p < 0.001$) in the subcanopy. There was however a slight difference in browsing intensity between intercanopy and subcanopy sites over the low rainfall season ($z = 2.82$; $p = 0.05$). Vertebrate exclusion only had a significant effect on browsing frequency ($z = 4.57$; $p < 0.001$) within the low rainfall season browsing intensity was however affected within both the dry ($z = 6.62$; $p < 0.001$) and very wet season ($z = 3.96$; $p < 0.01$). Dry season browsing intensity was affected by vertebrate exclusion in the intercanopy ($z = 3.08$; $p < 0.05$). Despite an overall effect of season on frequency ($F = 41$; $df = 2$; $p < 0.001$) there was no effect of vertebrate exclusion ($F = 1.2$; $df = 2$; $p > 0.05$) in the intercanopy.

3.3.3 The effect of canopy cover and different herbivore assemblages on plant size and growth traits

Plant total above ground biomass was much higher in the intercanopy ($F = 26.22$; $df = 1$; $p < 0.001$; Fig 3.8A). There was no significant effect of exclosure treatment on above ground biomass ($F = 0.03$; $df = 1$; $p > 0.05$). Although there was a slight effect of browsing frequency ($F = 2.30$; $df = 1$; $p = 0.08$) on above ground biomass, it was strongly influenced by browsing intensity ($F = 4.30$; $df = 1$; $p < 0.05$).

Below ground biomass ($F = 31.01$; $df = 1$; $p < 0.001$), with seedlings in the intercanopy having significantly higher biomasses (Fig 3.8B). However there was no difference in below ground biomass between exclosure treatments. While there was an effect of browsing frequency ($F = 6.12$; df = 1; $p < 0.01$), intensity only had a slight influence on below ground biomass ($F = 3.68$; df = 1; $p = 0.05$). Although there were moderate correlations between lignotuber length and below ground biomass (R^2 averages 0.62) outside of exclosures (See Appendix Table 1), within exclosures there appeared to be weak relationships between the two growth traits (R^2 averages 0.42). Additionally there was an effect of subcanopy on lignotuber length ($F = 25.44$; df = 1; $p < 0.001$; Fig.8C) but no differences were found in lignotuber length between exclosures and no exclosures ($F = 1.16$; df = 1; $p > 0.05$). Lignotuber length was significantly correlated with below ground biomass (Table 3.2). The relationship was generally stronger outside exclosures (mean R^2 was 0.62) than inside exclosures (mean R^2 was 0.42).

Microhabitat had no significant effect on the root: shoot ratio (RS) of seedlings outside exclosures ($F = 2.54$; df = 1; $p > 0.05$). There was some effect of vertebrate exclusion on root/shoot ratio ($F = 3.24$; df = 2; $p < 0.05$). In the subcanopy, seedlings within exclosures had higher RS than those outside exclosures (0.97 and 0.46, respectively; $z = -2.55$; $p = 0.05$).

Seedling growth in height was significantly lower in the subcanopy ($F = 40.83$; df = 1; $p < 0.001$; Fig 3.8 D). Seedlings outside exclosures had significantly less growth ($F = 45.45$; df = 1; $p < 0.001$), both in the subcanopy ($z = -5.42$; $p<0.001$) and in the intercanopy ($z = -3.51$; $p < 0.01$). Although browsing frequency had no effect on seedling growth, browsing intensity had a strong negative influence on growth ($F = 15.25$; df = 1; $p < 0.001$

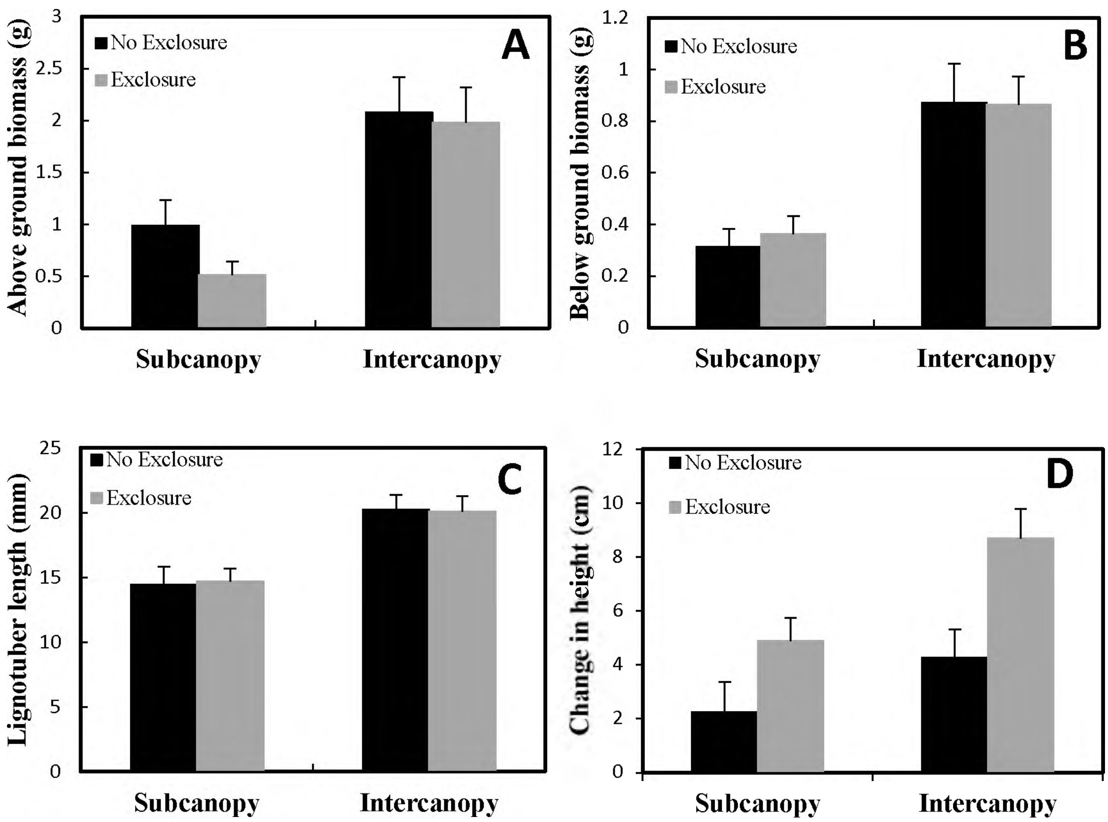


Figure 3.8: The effect of natural herbivory (exclosure vs no exclosure) was monitored for a year under a canopy and in the open regions of a semi-arid savanna. Below ground biomass (g) (A), above ground biomass (B), lignotuber length (C) and change in height (D) were recorded for each seedling.

To test whether the amount of compensatory plant growth (Fig 3.8) may be attributed to plant size, I tested the strength of the relationships between change in plant height and plant size. All measures of plant size (lignotuber length, above and below ground biomass, and final stem basal diameter, SBD) were significantly correlated (Table 3.2). Height increase was significantly but weakly correlated with above and below ground biomass for most treatments (Table 3.3). The relationships between plant growth and final stem basal diameter (SBD) were generally weak and only significant in the intercanopy (treatments IEN and IE, Table 3.2).

Table 3.2: The bivariate relationships between several plant traits were analysed. Relationships were analysed using Standardized Major Axes (SMA) techniques. Each slope estimate is associated with a confidence interval (CI) limit and R^2 (correlation with p value).

Regression	Treatment	Slope	CI Lower	CI Upper	R-squared	P-value
Height Change ~ Below ground biomass	SNE	0.52	0.33	0.81	0.068	> 0.05
	SE	0.72	0.53	0.98	0.103	**0.04**
	INE	0.42	0.29	0.60	0.197	**0.02**
	IE	0.42	0.32	0.56	0.256	**< 0.001**
Height Change~Above ground biomass	SNE	1.10	0.72	1.67	0.208	**0.03**
	SE	0.96	0.70	1.30	0.086	0.06
	INE	0.57	0.40	0.80	0.276	**< 0.01**
	IE	0.70	0.51	0.95	0.161	**< 0.05**
Lignituber length~ Below ground biomass	SNE	0.84	0.65	1.09	0.655	**< 0.001**
	SE	1.19	0.93	1.51	0.431	**< 0.001**
	INE	0.57	0.45	0.73	0.605	**< 0.001**
	IE	0.65	0.51	0.84	0.426	**< 0.001**

Table 3.3: The bivariate relationships between several plant traits were analysed to determine the influence of plant size, in stem basal diameter (SBD) on growth. Relationships were analysed using Standardized Major Axes (SMA) techniques. Each slope estimate is associated with a confidence interval (CI) limit and R^2 (correlation with p value).

Regression	Treatment	Slope	CI Lower	CI Upper	R-squared	P-value
Height Change~SBD	SNE	0.92	0.59	1.44	0.074	> 0.05
	SE	1.04	0.76	1.42	0.064	> 0.05
	INE	0.91	0.62	1.3280120	0.136	**0.05**
	IE	1.02	0.76	1.37	0.246	**< 0.01**
Below ground biomass~SBD	SNE	1.78	1.23	2.60	0.369	**< 0.01**
	SE	1.45	1.10	1.91	0.266	**< 0.001**
	INE	2.18	1.62	2.93	0.474	**< 0.001**
	IE	2.42	1.90	3.14	0.402	**< 0.001**
Above ground biomass~ SBD	SNE	0.84	0.57	1.23	0.337	**< 0.01**
	SE	1.09	0.85	1.40	0.409	**< 0.001**
	INE	1.60	1.30	1.97	0.733	**< 0.001**
	IE	1.46	1.10	1.94	0.284	**< 0.001**

3.4 Discussion

This study explored the role of different herbivore guilds in causing a seedling release bottleneck in a semi-arid savanna. My results show that in addition to vertebrate herbivores, invertebrates and possibly rodents account for a significant portion of seedling mortality and release into adulthood. Additionally, the subcanopy microhabitat negatively affects seedling growth, lending support to the argument that *V. karroo* may be shade intolerant. My research also demonstrates that increased canopy cover in the form encroachment may limit seedling above ground growth.

3.4.1 Browsing at high and low woody canopy cover

Although browsing negatively influenced survival (~ 38%) there was no difference between the encroached and open site suggesting that the effect of browsing on mortality was unaffected by encroachment level. The probability of survival within both sites also decreased to less than half following browsing implying that browsing levels within this semi-arid savanna may negatively influence transitions to saplings. The majority of seedlings within both sites was browsed twice and had approximately 169% cumulative above ground biomass removed over the study period. This high intense repeated browse resulted in low survival rates and reduced growth rates. These results complement previous research and imply that while browse may lead to mortality of seedlings the majority (~ 60%) of *V. karroo* seedlings are affected through reduced rates of transition (release) to adulthood (Sankaran *et al*. 2013; Shaw *et al*. 2002; Staver and Bond 2014).

Although there was a strong influence of season on browsing frequency and intensity during the study period, there was again no difference in effect of encroachment level. Research suggest the late dry season as the intense browse period, however, I found browsing intensity and frequency was greatest during the dry season and lowest during the late dry and wet season. By the very wet season browsing activity started increasing again. The majority of browsed seedlings started recovering biomass mid-way through the wet season and had regrown sufficient above ground biomass only by the end of the wet season. Hence seedlings were not attractive to herbivores and therefore experienced less browsing during the wet season.

Despite all seedlings subjected to natural browsing been similar in size, above ground biomass within the open site was at least 0.5 g higher. *V. karroo* is known to be heliophytic and may thrive under high irradiance conditions, which may explain enhanced growth in the open site (O'Connor 1995). These findings suggest that *V. Karroo's* shade intolerance may limit further encroachment in already-encroached regions. Although the encroached region has lower irradiance due to increased tree cover it may still receive sufficient light from tree gaps since there is only 40% canopy cover in the site. This suggests that 40% canopy cover which interacts with browsing has the ability to restrict growth.

3.4.2 The effect of canopy cover on the probability and effect of seedling damage by different herbivore assemblages

While several studies suggest browsing can influence growth, there is little evidence suggesting that herbivory (apart from mega-herbivores) can directly cause seedling mortality (Barnes 2001; Midgley *et al.* 2010; Sankaran *et al.* 2013; Staver and Bond 2014). I found in both microhabitats the probability of seedlings surviving past 12 months was much higher within exclosures (0.81) when all vertebrate herbivores were excluded than outside of exclosures (0.54) where all herbivores were present. This implies that the exclusion of vertebrate herbivores results in a dramatic increase in survival. Survival within subcanopy and intercanopy sites were also largely impacted by browsing intensity and frequency over time.

While the effect of herbivory, on plant growth, is common in arid and semi-arid savannas mesic sites have increased growth rates and experience reduced browsing activity (Ritchie and Olff 1999; Sankaran *et al.* 2013). I found browsing intensity was much higher (~90% more removed) outside of exclosures, however there appeared to be no difference between subcanopy and intercanopy sites. Although there is evidence suggesting herbivore activity increases in the sub-canopy (Treydte *et al.* 2010) the lack of difference between sub and intercanopy may be explained by several factors. For example the reduced presence of herbivores including rodents in the region, the slow regrowth un the sub-canopy which may have resulted in reduced susceptibility to further damage or the presence of smaller trees in the sub-canopy which make the area less accessible to browsers. Those seedlings within exclosures also experienced significantly less browsing events than outside of exclosures. These results explain that although browsing from invertebrates and possibly rodents negatively influence growth the effect of larger vertebrate herbivores is stronger. In the absence of additional disturbance and increased browser biomass, seedlings within mesic savannas are assumed to escape the browse trap faster (Sankaran *et al.* 2013). Current browsing levels within this semi-arid savanna appear to trap seedlings within the browse layer through preventing seedling release to adulthood (Midgley and Bond 2001; Sankaran *et al*. 2013; Staver and Bond 2014).

Evidence testing the effects of canopy shade on growth is varied, while some research explains that shade enhances growth and survival (O Connor 1995; Hoffmann 1996, Salazar *et al.* 2012) there are studies suggesting shade negatively influences (Scholes and Archer 1997) or does not have any apparent influence on growth and survival (Vadigi and Ward 2012, 2013). While shade from established canopies appeared to have less impact on browsing frequency and intensity, compensatory growth responses following browsing was directly impacted. Subcanopy had a strong effect on below ground biomass, with seedlings in the intercanopy having higher below ground biomasses. This implies that *V.karroo* seedlings growing under shade and exposed to browsing may experience reduced regrowth following browse events. There was no difference in the effect of browsing from different herbivore guilds on below ground biomass, but there was a strong effect of frequency and not intensity. These findings suggest that intense browsing from vertebrates is not as effective as the number of times seedlings are browsed in reducing *V.karroo*'s regrowth. These repeated browse events presumably deplete starch reserves within plants and thus contribute to a reduced below ground biomass (Schutz *et al*. 2011). Subcanopy also affected the length of lignotuber of seedlings, with those in the intercanopy having longer lignotubers. Shorter lignotubers may be attributed to a plant in the subcanopy allocating fewer resources to below ground biomass (Chirara 1999). Consequentially deeper roots will promote survival and allow seedlings to cope with drought stress and prolonged dry seasons (Hoffmann 2004).

According to the functional equilibrium hypothesis plants in the shade should have increased allocation to stems and leaves and not roots (Poorter *et al*. 2012). However within my study above ground biomass was much higher in the intercanopy site with no effect of exclosures on biomass. I can therefore stipulate that the effect of browsing may have triggered a change in resource allocation. This then suggests that herbivory in the form of browsing may be an overriding driver of allocation to above ground biomass. Reduced regrowth seen in plants growing under shade may explain reduced plant above ground biomass. Additionally, the interactive effect of shade and browsing appears to have a strong negative effect on growth. Irradiance is important for plant growth and survival and there is evidence to suggest shade may reduce biomass production of *V. karroo* (Scholes and Archer 1997; Chirara *et al*. 1999).

For instance Australian Acacia species had lower growth under reduced irradiance, suggesting a strong effect of shade (Milton 1982).

Shade should select for more resource allocation to plant leaves resulting in lower root/shoot ratios (Chirara 2002; Poorter *et al*. 2012). I found no effect of subcanopy on the root: shoot ratio, there was however an effect of vertebrate herbivory, where seedlings within exclosures had almost doubled R/S ratios (0.79). Higher root: shoot ratios of woody savanna trees have important consequences for disturbance tolerance. Increased root: shoot ratios are generally accompanied by increased root non-structural carbohydrate reserves and allow plants to replace above ground biomass following disturbance (Hoffmann 2004). A decreased RS ratio generally results in reduced energy storage necessary for survival during dry seasons and reduced plant compensatory growth responses (Aarssen 1995; Barnes 2001). Change in height following browsing was also significantly influenced by tree canopy, possibly due to direct removal of plant material by browsers on growth. Despite the effect of vertebrate exclusion, growth in height was limited in the subcanopy zone. Although an encroached (previous field trial) region differs in its abiotic and biotic conditions, both seem to have a similar shade effect where above ground growth was limited. These results again support previous findings within this study and other research suggesting *V. karroo* may be prefer environments with increased irradiance (Chirara *et al*. 1999; Scholes and Archer 1997; Hoffmann 2004; Vadigi and Ward 2012). As expected seedlings within exclosures (vertebrate exclusion) grew much more than those exposed to all herbivores, this was probably because both browsing frequency and intensity were higher outside the exclosures. These findings imply herbivory, through reducing growth can directly reduce seedling release rates (Staver and Bond 2014). Although herbivory from invertebrates and rodents which access exclosures negatively influence seedling growth they were not as efficient as vertebrate herbivores in reducing transition rates of *V. karroo* seedlings in a semi-arid savanna.

3.4.3 Conclusion

Although individual trees may have positive effects on herbaceous production and diversity within subcanopy habitats increases in canopy density are undesirable and adversely affect semi-arid savanna performance (Scholes and Archer 1997; Riginos *et al*. 2009). An increase in tree cover within savannas requires successful transitions of *V.karroo* seedlings to saplings. This study demonstrates that invertebrates and rodents as well as vertebrate herbivores act as a major top down disturbance influencing a seedlings transition within a semi-arid savanna.

The effects of herbivory were far greater on mortality than compensatory growth responses. Growth responses were however, significantly influenced by shade from established trees.

At local landscape scale I found smaller browsers (rodents and invertebrates) were effective at decreasing the rate of transition from seedling and eventually to adulthood while larger vertebrates such as kudu and impala were more effective at causing mortality. Therefore browsing from small and large herbivores will both probably have long term negative effects on growth and reproduction of the species (Augustine and McNaughton 2004; Sankaran *et al.* 2013). Research suggests that despite the effectiveness of individual top down disturbances, predominantly fire and herbivory, as ecological bottlenecks within semi-arid savannas the combined effect of both disturbances is much stronger. Although there is a general understanding of how these individual factors may facilitate bush encroachment, research on how underlying mechanisms interact is necessary.

Additionally the interaction of browsing and shade had a stronger negative influence on seedling compensatory responses. This then provides support for the hypothesis that *V.karroo* seedlings are shade intolerant. Further research into this may provide valuable insight into the role of shade and its interactive effect with browsing in preventing further tree cover in a semi-arid savanna. It may also be useful for future studies to investigate the relationships between specific canopy traits, such as canopy density and height and role as facilitators in woody plant growth.

4. The effect of *Vachelia karroo* size on mortality and compensatory responses following herbivory

4.1 Introduction

Despite the large volume of literature on the effects of the various drivers of savanna dynamics, there is a lack of understanding about the conditions of the environment in which savannas may demonstrate threshold behaviours that have been referred to as tipping points (Sankaran and Anderson 2009). Savanna trees are exposed to several disturbances and their ability to survive these disturbances depends strongly on their size. Small seedling and establishing saplings are often top killed by fire and herbivory and a threshold size which allows saplings to survive fires has been determined (Bond 2008). However, a similar survival threshold that allows seedlings to survive single or repeated herbivory events has not been established (Hanley 2004).

In addition to disturbance more research is suggesting that increasing CO_2 may have and is still contributing to this environmental phenomenon (Idso 1992, Polley 1997 and Kgope *et al.* 2010; Buitenwerf *et al.* 2012). This is due to the fact the increasing CO_2 will favour C_3 savanna trees over C_4 grasses (Ainsworth and Long 2005 and Kgope *et al.* 2010), resulting in faster C_3 growth, and decreasing the time required for trees to escape the browse and fire trap (Bond 2008). Survival and growth of seedlings and saplings is a key demographic process (and potentially bottleneck) in bush encroachment, and changes in browsing and possibly atmospheric CO_2 are potential drivers of the increase in tree cover in the study area in the 1980s.

Identifying key drivers within savannas is important, but the response of demographic stages of growth within these systems are just as essential and may offer a reliable explanation for tree-grass coexistence (Higgins *et al* 2000; Bond 2008). Demography refers to both changes in population size and structure (Midgey *et al* 2010) and demographic bottlenecks are necessary for maintaining and reducing tree cover in savannas. The success of these bottlenecks will change along environmental gradients (Bond 2008). For instance we know rainfall is an important determinant in arid regions where fires are infrequent and less intense (Higgins *et al* 2000, Bond and Keeley 2005) and here, in addition to drought, surviving herbivory is assumed to be a major hurdle in seedling establishment (Midgley and Bond, 2000). Herbivory, which has a bigger effect on seedlings in arid regions, can also be effective at causing mortality or seedling suppression within a region (Wiegand *et al.* 2006).

Woody plants need to survive herbivory and have sufficient compensatory growth between browsing events to escape the browse trap. Evidence suggests that the success of seedlings that are trapped within the flammable zone can be determined by the frequency and intensity of top-down disturbance of herbivory and fire (Bond and van Wilgen 1996). Research has shown that the exclusion of disturbance has resulted in increases in woody tree cover and even biome shifts (Tilman *et al.* 2001, Briggs *et al.* 2005; Staver and Bond 2014). The presence of browse lines, in regions with high browser density indicates that acacia trees below certain size face substantial herbivory (Trollope and Tainton 1986; Hoffmann 1999; Bond and Loffell 2001; Hoffmann *et al.* 2009). Seedlings and smaller saplings are less tolerant to browsing while mature saplings and adult trees have increased tolerance to damage from herbivores (Midgley *et al.* 2010). In response to herbivory and a reduction in biomass, less tolerant plants have reduced growth rates, altered branching patterns and root: shoot ratios, and have modified photosynthetic activity (Kolb *et al.* 1999). These response strategies have important implications for the rate at which plants escape the disturbance trap.

Woody plants also use various defence and tolerance mechanisms to prevent herbivory (Hester *et al.* 2006; Strauss and Agrawal 1999). Investing in defence is costly and plants are faced with a trade-off of where to invest resources since defences require a larger allocation of stored reserves, and as a result divert resources from plant growth and reproduction (Hanley *et al.* 2007). While chemical and structural defences may decrease herbivory, investment in growth allow them to escape the herbivory trap sooner (Hanley *et al.* 2007). This resource allocation patterns may change with plant size and available resources (Poorter *et al.* 2012). Smaller seedlings may invest in growth to secure establishment while larger plants or saplings may allocate more resources to defence, but these relationships require further research.

In the central Eastern Cape, herbivory is predicted to limit seedling establishment because fires were historically rare, while wild ungulate browsers such as kudu were abundant (D. Painter, pers. comm). Surprisingly studies of the causes of bush encroachment in the area have focused very little attention on the seedling and sapling stages (Midgley *et al.* 2010). It is important to understand the fate of woody plants exposed and vulnerable to herbivory and how various stages of a tree's life cycle are impacted by disturbance (Bond and Midgley 2001). By identifying specific size and browsing thresholds we are more equipped to determine what may suppress seedling growth or cause mortality.

Given the lack of understanding of the size thresholds that determine whether seedlings survive herbivory or not, I conducted greenhouse and field experiments investigating the effects of seedling size and age, and the intensity and frequency of simulated browsing, on survival and growth of acacia seedlings and saplings. The main objectives of the study were to determine: (i) whether there is there a critical size and age above which a seedling or sapling has a higher chance of surviving browsing, (ii) is there a critical frequency or intensity of browsing that can kill an acacia seedling or suppress it long-term, (iii) do compensatory responses vary with plant size and age following browsing, (iv) does the ability to recover following browsing depend strongly on below ground biomass, and finally (v) does browsing interact with age to influence seedling investment in spinescence.

4.2 Methods

I conducted field and greenhouse trials to determine the effect of plant age and size on the survival and compensatory responses of *V. karroo* seedlings and saplings following simulated browsing under controlled and natural conditions.

4.2.1 Greenhouse experiment

4.2.1.1 Study plants

V. karroo (Hayne) seeds were collected during growing seasons from Endwell, the study site (see Chapter 2). Collected seeds were scarified and germinated in petri dishes on damp cotton wool. Once seedlings showed evidence of successful germination and the plumule had emerged, they were transplanted into 7 l pots filled with topsoil. The experiment was conducted in a naturally lit polythene tunnel at Rhodes University and seedlings were planted at different dates, such that on the 19th of January a collection of seedlings aged 6 (1.5 months), 12 (3 months), 16 (4 months) and 30 (7.5 months) weeks were available for defoliation experiments.

4.2.1.2 Experimental Design

I investigated the effects of seedling age on their ability to survive and regrow after various frequencies of simulated browsing (Experiment 1). This was done in a two-factor, fully randomized factorial design, with age of seedlings (hereafter referred to as age in weeks: 6W, 12W, 16W and 30W) and frequency (hereafter referred to as 1, 2 or 3 defoliations over a 12 month period) of browsing being the two factors. Browsing was simulated by defoliating seedlings, but branches were left intact.

At the start of the experiment, all seedlings of each age category (N=21) were subjected to a 100 percent defoliation, except controls, which were kept intact for the entire duration of the experiment (N=7). Seedlings were then allowed to recover for 4 weeks after which 14 of the seedlings were subjected to a second defoliation. After another 4 weeks, 7 of the seedlings that had already been defoliated twice were subjected to a third defoliation and a further 4 week recovery period. This protocol was applied to each of the four age classes of seedlings. At each defoliation event, the removed leaves were collected, dried and weighed to determine leaf biomass.

In a second experiment (Experiment 2), I determined the effects of repeated browsing and differing intensities of defoliation on seedlings that were 16 weeks old. This was conducted using a fully randomized factorial design. Half of the seedlings underwent 50% defoliation (N=21) while the rest underwent 100% defoliation (N=21). For each defoliation treatment, I subjected the seedlings to varying frequencies of herbivory (1, 2 and 3 defoliations, N=7 at each frequency). Seedlings were also left to recover for 4 weeks between defoliations with total experiment duration of 12 weeks

The 50% defoliation treatment was implemented by removing leaflets from one half of each leaf. An additional subset of 15 plants was used to ensure that this method did in fact represent 50% leaf biomass defoliation. Here half the leaflets were removed, dried and weighed and compared to the weight of the leaflets from the other half of the leaves. I performed an ANOVA on a GLM model to determine whether the removed half of leaflets differed from other remaining half. No significant difference [F (1, 28) = 0.918, p=0.346] was observed between predicted leaf biomass (1.1±0.1 g) and actual lead biomass (1.33±0.18 g).

At the end of each experiment, 12 weeks from the start date, all above and below ground plant material was harvested. Above and below ground material was separated, and then dried for 72 hrs before determining dry mass. Leaf biomass recovered was calculated for seedlings within each defoliation frequency and plant age treatment and was calculated as: final leaf biomass at end of experiment/initial leaf biomass at start of experiment*100. Stem basal diameter (SBD) was also calculated for each seedling within each plant age treatment. Final SBD (mm) minus initial SBD at the start of the experiment represented SBD regrowth.

To quantify spinescence, the main stem was divided into 3 equal regions from the apex to the base. At the final harvest the total number of spines was counted on all individual plants to get total number of spines produced per an individual plant. I also measured the length and diameter of spines at their midpoint using Vernier calipers on freshly harvested plants prior to drying. This was done on 9 spines per plant, 3 from each designated region. The accuracy of predicted length and diameter measurements against actual whole plant measurements was tested statistically. I found no statistical difference in values predicted from subsets (thorn length = 15.07 ± 0.8 mm; thorn diameter = 0.96 ± 0.05 mm) and actual (thorn length = 15.2 ± 0.65 mm; thorn diameter = 0.98 ± 0.05 mm) measurements for lengths [F (1, 18) = 0.001, p = 0.98] or diameters [F (1, 18) = 0.089, p = 0.77].

4.2.1.3 Statistical analyses

The aim of Experiment 1 was to investigate whether the risk of mortality increased with increased frequency of herbivory and how this was influenced by plant age. Mortality of seedlings occurred at various time intervals during the experiment. To incorporate this effect of time on mortality as well as the distribution of survival times throughout the experiment, I used the survreg function from the survival package in R (R Core Team 2014). Survival analysis (also known as event history analysis) modelled the relationship between survival and its predictors, which were age of seedlings and browsing frequency. Data was entered in the format *start time*, *stop time* (time of death in weeks), *status* (1= mortality occurred, 0 = mortality did not occur), *seedling age* (6, 12, 16 and 30 weeks) and *frequency* (0, 1, 2 or 3 defoliation events).

To investigate how browsing affected those seedlings that did survive, GLM and Tukey HSD *post-hoc* tests were run in R (R Core Team 2014). Several measured plant traits were used as response variables, these included above and below ground biomass, SBD regrowth, final leaf biomass, spinescence and seedling regrowth. I fitted a GLM with quasipoisson errors to determine whether seedling response was influenced by age, browsing frequency (number of defoliations) and their interaction in Experiment 1. In Experiment 2, I determined the effect of different browsing intensities and frequency on the response of 16W old seedlings. Where GLM results revealed significant differences between age and browsing intensity and frequency treatments, *post-hoc* Tukey HSD analyses were performed.

To determine how leaf biomass regrowth (final-initial leaf biomass) was influenced by below ground biomass I fitted a GLM with quasipoisson errors. Tukey HSD *post-hoc* results revealed combinational effects within and between browsing frequency and age treatments in experiment 1 and browsing intensity and frequency in experiment 2.

I tested for possible influences of the different size and growth traits on each other, particularly whether there was any evidence for a growth-defence trade-off, by using linear model analyses to predict relationships. Specific growth characteristics measured include SBD regrowth, plant biomass, number of spines and spine size.

4.2.2 Field experiment

4.2.2.1 Experimental Design

The aim of this experiment was to determine the effect of browsing on the survival and growth of *V. karroo* seedlings and saplings of different sizes. To define life-history stages and determine what range in plant size was indicative of immature acacia saplings, I had to first determine a size threshold of reproductive adults under natural conditions. I measured SBD and observed the plant's reproductive status over a range of plant sizes on 30 selected plants at various microsites in the Endwell farm. All reproductive plants (with flowers or pods present) were scored as 1 and those that did not have any were scored as 0. Individuals above 15 mm (in SBD) were able to reproduce and produce seeds. I therefore worked on individuals mostly with SBD < 15 mm for the field defoliation trial. Some individuals (SBD > 15mm) were selected to examine differences in growth responses between younger adults and saplings and seedlings. Defined size classes included seedlings, immature non-reproductive saplings (<1.5m height) and larger reproductive saplings (> 1.5m height). This allowed me to more accurately determine a size threshold of browsing resistance of *V. karroo*. More importantly these size classes could be related to plant age. I therefore measured a robust relationship between SBD and plant age.

In a pre-experiment I determined that stem basal diameter (SBD) was a good predictor of below ground biomass ($r^2 = 0.78$; $p < 0.001$). Below-ground biomass reflects seedling or sapling age on the basis that it reflects both underground carbohydrate reserves and plant age (Skowno *et al.* 1999). Seedlings growing under controlled conditions were also useful for determining plant age (Fig 4.1). Under controlled growth conditions *V. karroo* reached 9 mm in SBD after 24 weeks. Field growth rates were expected to be lower than rates of plants growing under controlled conditions. Seedling growth rate monitored under field conditions grew, on average 0.5 ± 0.1 mm per 3 month. This means seedlings growing under field conditions grew at approximately half the rate of seedlings under controlled conditions suggesting it will take seedlings in the field ~ 48 weeks to reach 9 mm in SBD. Hence larger seedlings < 9mm SBD were defined as saplings while those < 9 mm were termed seedlings. I thus used SBD as a predictor variable of age for the effect of herbivory on seedling and sapling growth.

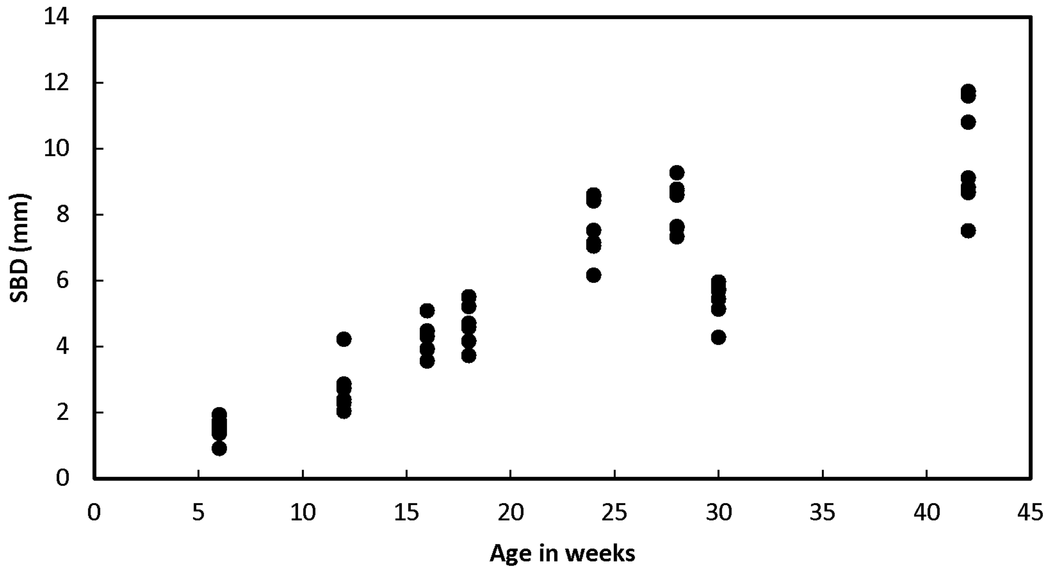


Figure 4.1: The relationship between *V. karroo* age (in weeks) and stem basal diameter (mm). SBD growth was measured under controlled greenhouse conditions.

To determine the effect of herbivory on seedling and sapling growth, I selected plants of a range of sizes that I experimentally subjected to either 50 % or 100 % defoliation. Sixty undamaged seedlings and saplings ranging from 2-30 mm SBD were selected and equal number subject to each treatment (N=30). An additional 25 plants were similarly selected and served as the un-treated controls. Plant morphological traits such as stem basal diameter (SBD) and height were initially recorded. All seedlings were ranked by their SBD and I ensured that each herbivory treatment (0, 50 and 100 % defoliation) had a similar range of size classes. Those plants subjected to a 100% defoliation had all their leaves removed while opposite leaves were removed for plants subjected to a 50% defoliation as described in the greenhouse experiment.

Seedlings were monitored monthly, and once seedlings showed sufficient biomass recovery after 3 months (March 2015 – May 2015) their leaf biomasses were harvested. Prior to harvesting, final SBD, height and any evidence of further herbivory were noted. Since browsing herbivores were not excluded those plants that exhibited evidence of further damage from herbivores were removed from analyses. Samples (only leaf material) were oven dried and weighed for analysis.

4.2.2.1 Statistical analyses

To determine the effect of defoliation intensity and plant size on survival I ran a GLM with binomial errors (0= death; 1= survived). I also ran linear regression models to predict relationships between the measured growth parameters. This was done to investigate possible trade-offs between growth traits that may have occurred following simulated browsing disturbance. All statistical tests were run in R (R Core Team 2014).

To determine the effect of plant size on measured traits (height, basal diameter, leaf biomass and predicted aboveground biomass) I determined how growth variables scaled against size and how this relationship changed with browsing intensity (0, 50 and 100% defoliation). Since smaller seedlings were observed to have different growth responses to larger seedlings, I divided them into two main size classes on the basis of SBD: seedlings less than 9 mm (small) and those greater than 9.1 mm (large Relationships between growth variables were defined by fitting standardized major axis (SMA) lines using SMATR (Falster *et al.* 2003).This type of morphometric analysis allows a user to quantify and examine biological relationships. In this study I wanted to determine whether plant trait regressions between small and large seedlings were allometric (different). This was done by estimating a line of best fit which allowed me to test for common slope and intercept amongst several SMAs (See Appendix Fig 2) (Warton *et al.* 2006). All plant traits were log transformed during analysis.

4.3 Results

4.3.1 Greenhouse experiments

Seedling age and frequency of defoliation both influenced survival (Figure 4.2). All seedlings in the control (0 defoliation events) survived, regardless of age. Seedling survival was strongly influenced by seedling age ($z = 2.07$, $p < 0.001$), number of defoliations ($z = -2.08$, $p < 0.001$) and an interaction between the two ($z = 1.59$, $p << 0.001$). Rates of survival in the youngest seedlings (6 week) decreased with repeated browsing, with only 33% surviving the 1^{st} defoliation, and none of the seedlings surviving a 2^{nd} defoliation (Fig 4.2A). The 12-week old seedlings had 67% survival following a single defoliation. Of the seedlings subjected to a second defoliation, 86% survived by the third defoliation (Fig 4.2B). Presumably subjecting the younger plants to repeated defoliation killed the intolerant individuals and the more resistant plants had increased survival after the third defoliation. All 16 week old seedlings survived 1, 2 or three defoliations (Fig 4.2C). Of the oldest seedlings (30 week old), 14% died after the second defoliation and all plants defoliated a third time survived (Fig 4.2D).

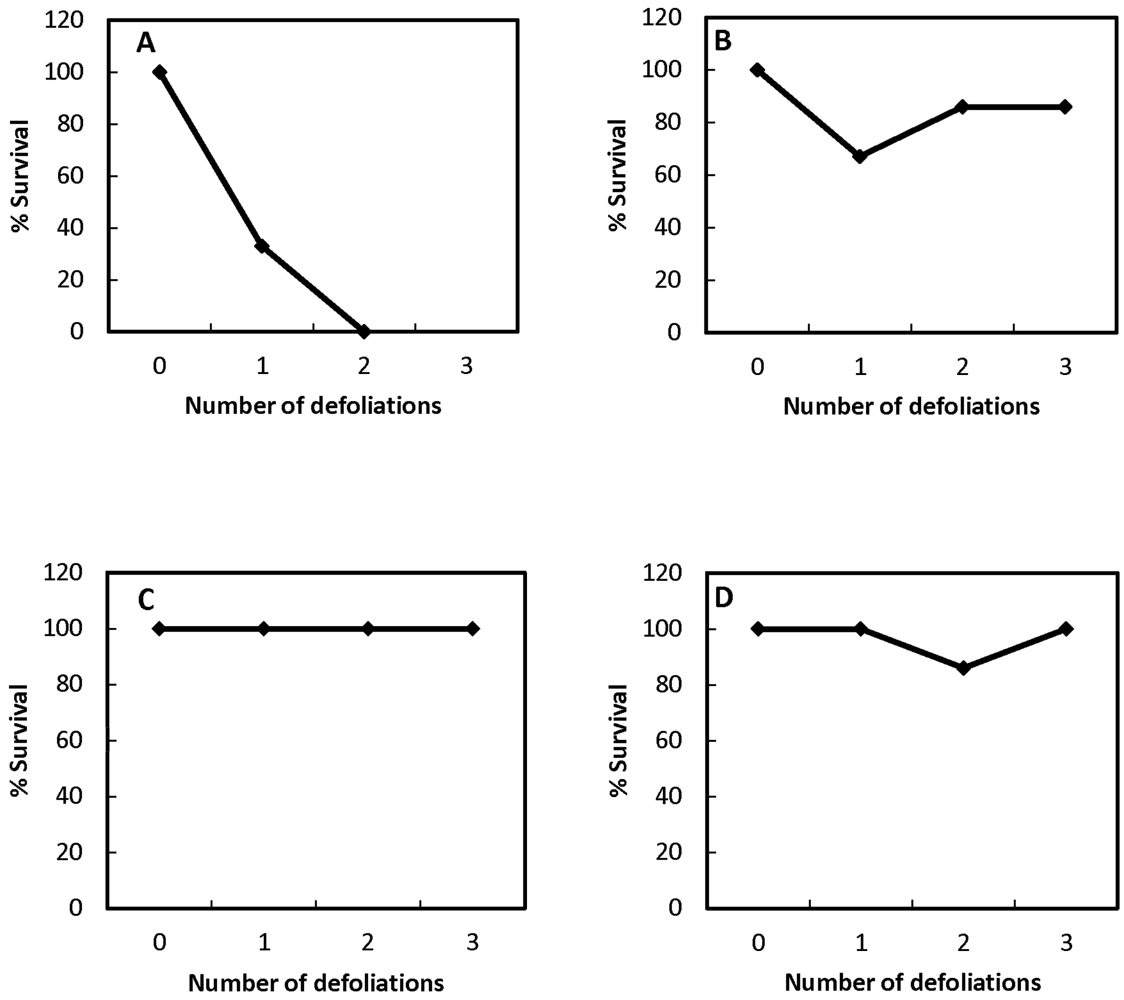


Figure 4.2: Percentage survival of seedlings of different ages: **A** (6 weeks), **B** (12 weeks), **C** (16 weeks) and **D** (30 weeks) in response to browsing and repeated browsing (0, 1, 2, 3 defoliation events). Survival analysis indicates an overall effect of age ($z = 2.07$, $p < 0.001$) and number of defoliations ($z = -2.08$, $p < 0.001$) on survival.

Fig 4.3 shows the effect of simulated browsing, of 100% intensity and varying frequencies on seedlings of different ages. Seedlings aged 6 weeks experienced 100% mortality by the 2nd defoliation, while 12 week old seedlings experienced 67% mortality after the 1st defoliation. Mortality meant that all 6 week old plants after 1, 2 or 3 defoliations and 12 week old plants following a single defoliation were not available for further plant growth analyses.

Seedling recovery was greatest for those seedlings subjected to a single defoliation (F1) and lowest following the 3rd defoliation event (Fig. 4.3 A). Percentage recovery was lower in older plants. For instance, following a 3rd defoliation, 12 week old seedlings recovered on average 30% of their initial biomass, while 30 week old seedlings recovered only 10%. Final leaf biomass, used to generate leaf biomass recovery follows a similar trend. Leaf biomass produced by plants of all ages show a significant reduction with increasing browsing frequencies (Fig 4.3 E).

Overall SBD growth was significantly influenced by age ($F = 6.30$; $df = 3$; $p < 0.001$). For seedlings of 12 - 30 weeks in age, growth in SBD was significantly reduced following repeated defoliation frequency (Fig 4.3C). Although SBD growth, for all treatments was significantly lower than that of the control plants, repeated defoliation reduced regrowth after the 2nd and 3rd defoliation events and the effect was the same for plants of all ages (See Appendix Table 1).

Above ($F = 7.30$; $df = 3$; $p < 0.001$) - and below ($F = 25.20$; $df = 3$; $p < 0.001$) - ground biomass (Fig 4.3D and E) showed a similar response to simulated browsing. Irrespective of seedling age, all browsing frequencies reduced growth relative to controls (See Appendix Table 1). Younger seedlings were most vulnerable to defoliation and the magnitude of this effect was increased with increasing frequencies of defoliation. Again there was a difference in the effect of a single and three defoliations (See Appendix Table 1).

Overall spine length ($F = 8.50$; $df = 3$; $p < 0.001$) and diameter ($F= 7.55$; $df = 3$; $p < 0.001$) were influenced by age while spine number ($F = 2.71$; $df = 3$; $p = 0.05$) was only slightly impacted by age. Two or more defoliations reduced the number of spines produced (Fig 4.3F). The effect of age on spine production was only seen after a 3rd defoliation where the number of spines produced was significantly lower, most notably in the case of 12 week old seedlings (See Appendix Table 1). For seedlings of all ages, the effect of simulated browsing on spine size did not differ from the control (Fig 4.3G and H).

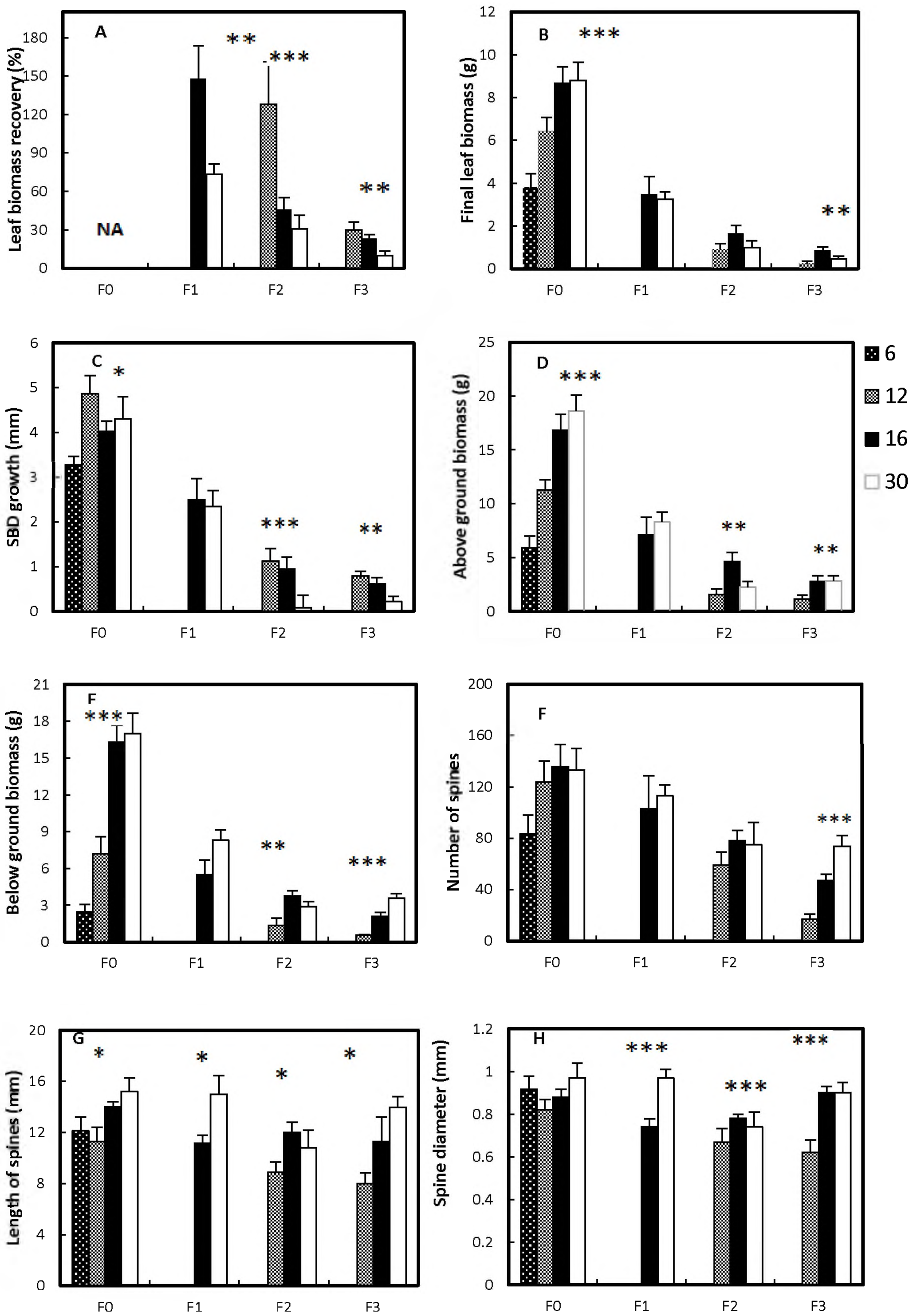


Figure 4.3 : Mean (±SE) percentage of leaf biomass recovered (**A**), final leaf biomass (B), increase in stem basal diameter (SBD) (**C**), above ground biomass (**D**), below ground biomass (**E**), number of spines (**F**), length of spines (**G**) and spine diameter (**H**) of *V karroo* different aged seedlings (6, 12, 16 and 30 weeks) subjected to various frequencies (F0 (control), F1, F2, F3) of browsing . Due to mortality no data was available for 6 (NA-after F1, F2 and F3) and 12 (after F1) week old seedlings following herbivory. Significance symbols show differences in age response within each frequency *P < 0.05, **P < 0.01, ***P < 0.001.

There was a significant difference in responses between plant ages (Fig 4.4A, B and C) in the influence of below ground biomass on a seedling's ability to regrow leaf biomass. Younger plants had smaller below-ground biomass but recovered more leaf biomass than older seedlings. Older seedlings had higher below-ground biomass but this decreased with repeated defoliation events, as did the amount of leaf regrowth (Fig 4.4A to C). For instance, 30 week old seedlings subjected to a single defoliation had at least three times more leaf biomass than seedlings subjected to a 3rd defoliation ($z = -5.792$, $p < 0.001$). There was a significant difference between a single and 3rd defoliation ($z= -4.178$, $p < 0.001$) and a 2nd and 3rd defoliation ($z = -2.466$, $p < 0.05$), but there was no difference in effect of a single or 2nd defoliation event ($z = -1.924$, $p> 0.05$) on regrowth. Within each frequency, I tested for age-related differences in the allometric relationship between regrowth and below-ground biomass. I found that following a single defoliation (Fig 4.4A), 16-week old seedlings had significantly greater regrowth for the same amount of below-ground biomass than 30 week old seedlings ($z = -3.210$, $p < 0.05$). Seedlings subjected to a 2nd defoliation generally had negative values for regrowth, which indicate that regrowth was less than initial plant biomass, and I found no significant difference in regrowth per unit below-ground biomass between older seedlings (16 and 30 weeks). Responses following a 3rd event were markedly different, with an overall decline in leaf biomass regrowth with increasing below-ground biomass.

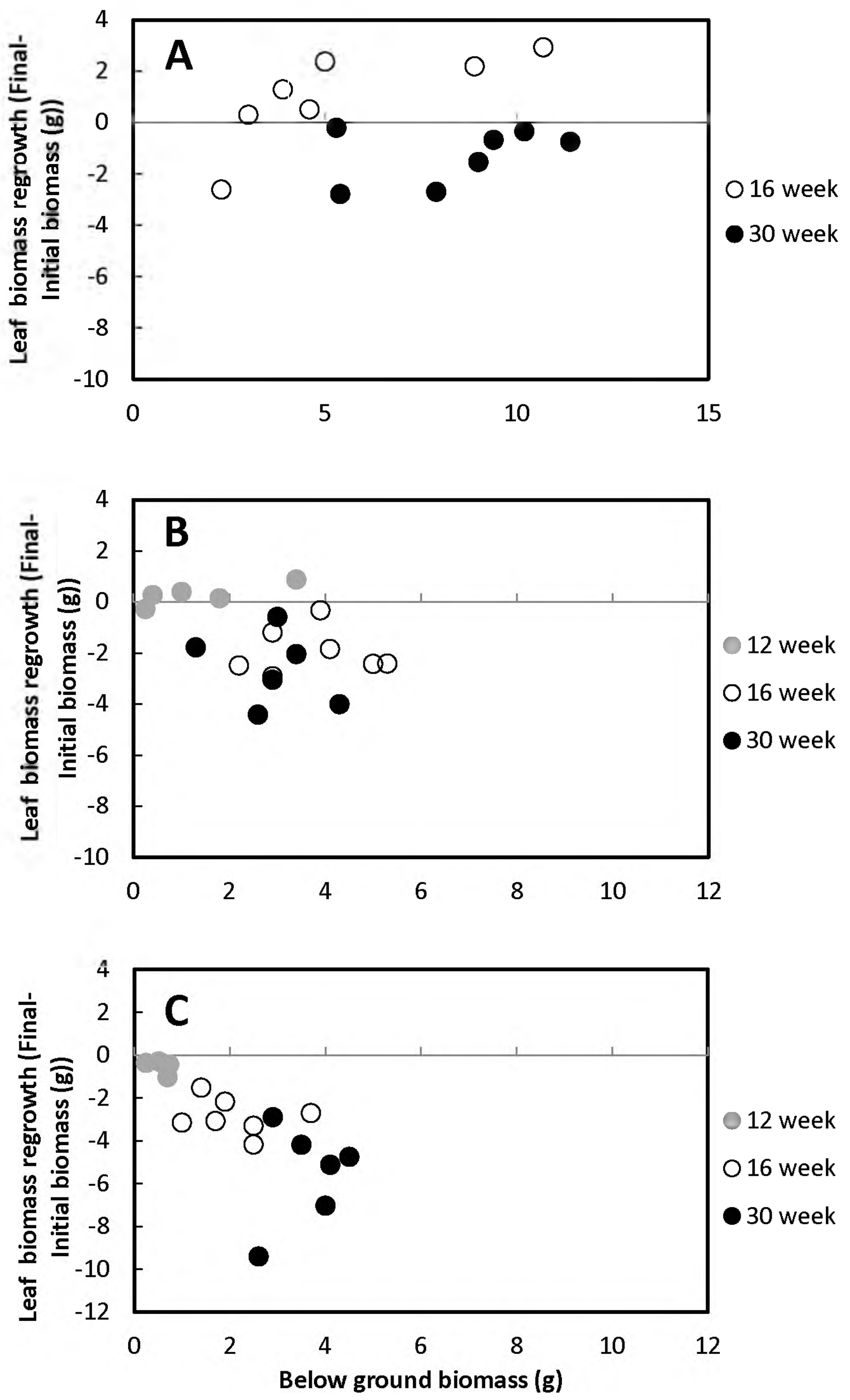


Figure 4.4: Leaf regrowth biomass in response to below-ground biomass for seedlings of different ages (12 week s- grey dots), 16 weeks - white dots, and 30 weeks - black dots) subjected to simulated browsing (100 % of leaves removed) and repeated browsing events (**A** - one defoliation - F1, **B** - two defoliations – F2 and **C** - three defoliations – F3). Due to mortality no data was available for 6W at F1, 2 or 3 and 12W old seedlings at F1 browsing frequencies.

Experiment 2 investigated the influence of defoliation intensity (50% and 100%) and frequency (once, twice or three times at 4-week intervals) on seedling responses.

Although overall, markedly less biomass was recovered by those seedlings subjected to 100% defoliation, there was no significant difference in the percentage of leaf biomass recovered between intensity treatments following a 1^{st} and 2^{nd} defoliation event (Fig 4.5A). Only a 3^{rd} 100% defoliation significantly lowered the recovery of leaf biomass, reaching a low of 23%. Seedlings undergoing less intense (50%) defoliation were able to overcompensate for photosynthetic loss following a 3^{rd} defoliation and had 52% higher biomass than control plants (Fig 4.5A, F3). Leaf biomass recovery responses of seedlings were directly calculated from final leaf biomasses, similarly regardless of intensity treatment total leaf biomass was significantly lower than the control. The magnitude of this effect was increased with repeated browsing events under intense defoliation (Fig 4.5B).

Like biomass responses, where the influence of 100 % was greater than that of the 50 % defoliations, SBD growth, above- and below-ground biomass showed similar responses (Fig 4.5C, D and E). SBD growth was only significantly lower than in control plants under intense (100%) defoliation, while final above- and below-ground biomass were all sensitive to both intensities of defoliation (See Appendix Table 2). Irrespective of browsing intensity, all growth variables except below ground biomass were similar following a 1^{st} and 2^{nd} defoliation. After a 1^{st} defoliation, seedlings subjected to 100% intensity had 46% lower below ground biomass than seedlings subjected to 50% defoliation. The magnitude of this effect was increased when plants were defoliated a second and third time.

Findings from the previous experiment (Fig 4.3F, G and H also See Appendix Table 2), where spine growth was not affected by age, indicated that higher browsing frequency reduced the number of spines per plant (Fig 4.3F). Results revealed a reduction in the number of spines produced with repeated defoliation events. Spine size, however, was not affected by the different browsing intensity treatments (Fig 4.5G and H), It is thus the number of spines, and not the diameter or length, that is affected by browsing, with only a significant influence on spine number following a 3^{rd} defoliation ($z = 3.552$, $p < 0.01$). Both above ($F = 66.95$; df= 1; $p < 0.001$) and below ($F = 46.53$; df = 1; $p < 0.001$) ground biomass positively influenced the number of spines produced by seedlings These findings suggest a strong dependence of spines on plant size.

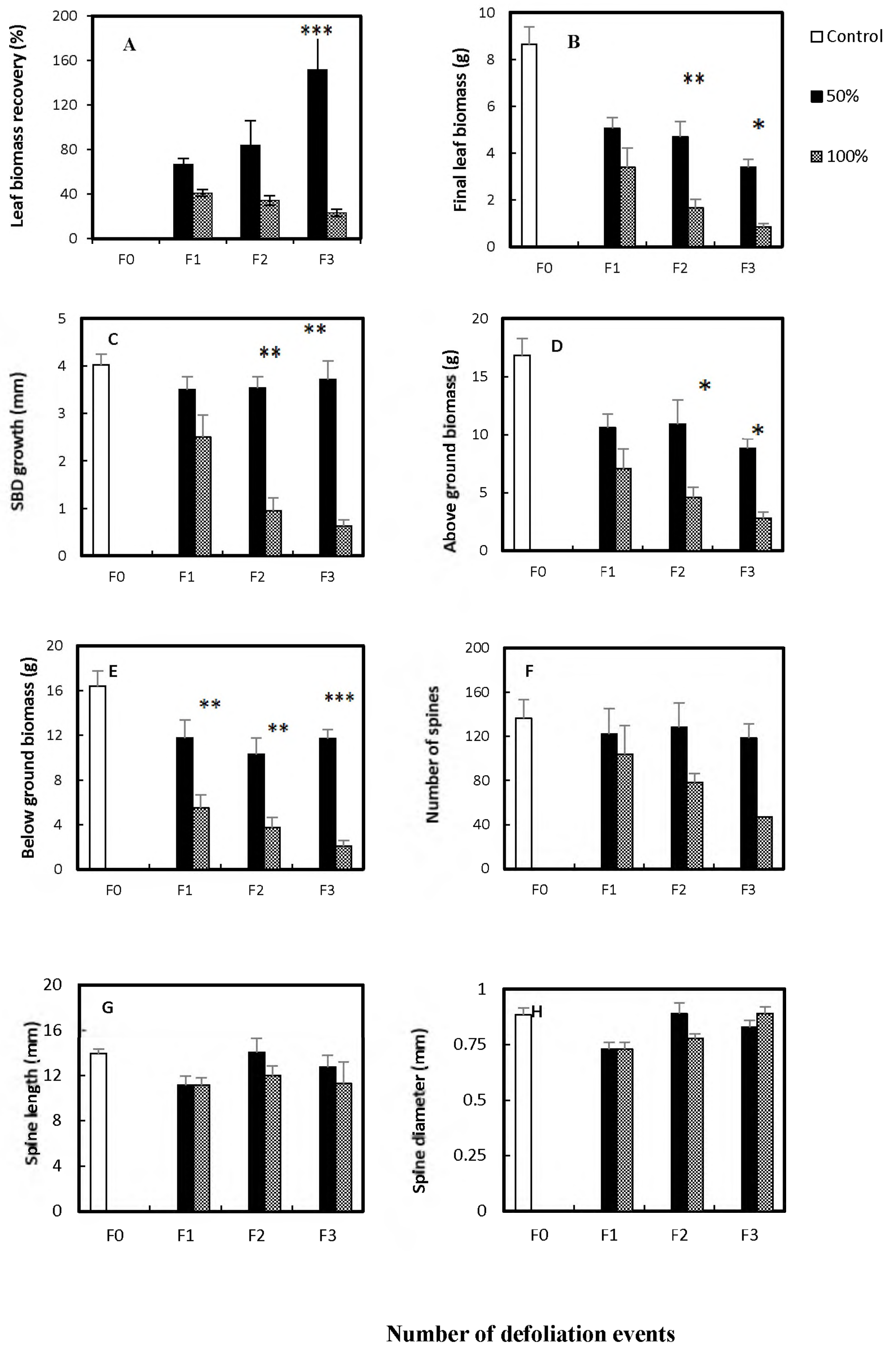


Figure 4.5 : Mean (±SE) of % Leaf biomass recovered (**A**), final leaf biomass (**B**) Stem Basal Diameter growth (mm) (**C**), above ground biomass (g) (**D**), below ground biomass (g) (**E**), number of spines (**F**), length of spines (mm) (**G**) and spine diameter (mm) (**H**) of *V karroo* seedlings subjected to two intensities (50 (solid black bars) and 100% (patterned grey bars) defoliation) and frequencies (F0 (control), F1, F2, F3) of herbivory . Significance symbols represent differences in seedling response within each frequency *P < 0.05, **P < 0.01, ***P < 0.001.

There was a significant difference in the effect of 50% (Fig 4.6A) and 100% (Fig 4.6B) defoliation on the regrowth of leaves ($z = 5.511$; $p < 0.001$). The effect of below ground biomass on regrowth appeared to be less pronounced for seedlings under a 50% defoliation treatment (Fig 4.6A). Contrasting effects were seen for seedlings subjected to 100% defoliation (Fig 4.4 B), where below ground biomass had a significant influence on leaf biomass regrowth ($t = 3.195$; $p < 0.01$). As the frequency of herbivory increased (F1 to F3) regrowth decreased. The magnitude of this effect was increased with below ground biomass. Additionally there is a difference in the effect of a single defoliation and a 2nd defoliation ($z = -4.241$; $p < 0.001$) and 3rd defoliation event ($z = -5.588$; < 0.001) but no difference is seen between a 2nd and 3rd defoliation event ($z = -1.347$; $p > 0.05$).

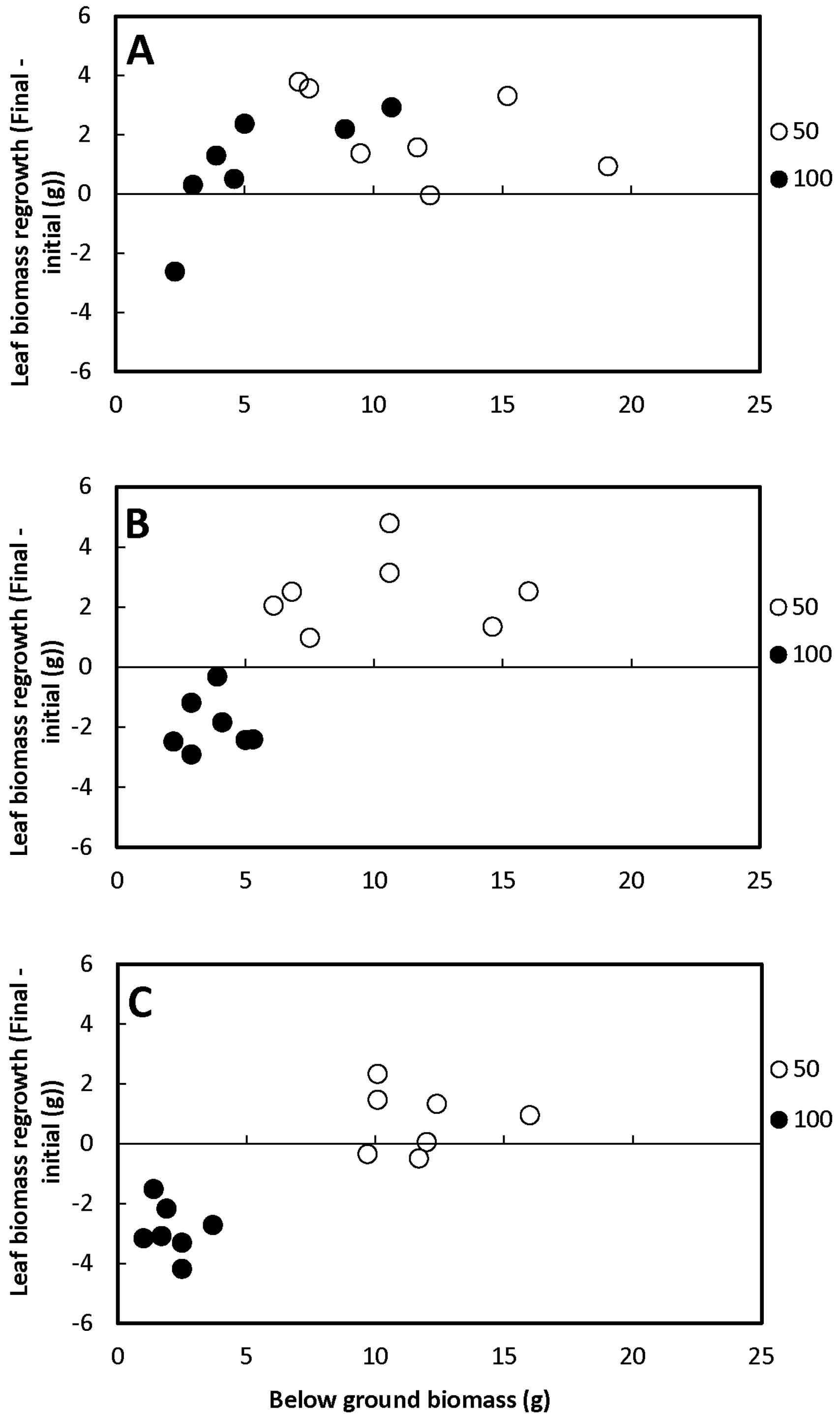


Figure 4.6: The effect of below ground biomass (g) on a seedling's ability to regrow following different browsing frequencies (**A** (one defoliation event), **B** (two defoliation events) and **C** (three defoliation events)). Leaf regrowth biomass (g) is shown in response to two defoliation intensities, 50% (white dots) 100% (black dots).

4.3.2 Field experiment

Figure 4.7 illustrates scaling relationships between *V. karroo* plant traits following a single defoliation event of either 50 % or 100 % in relation to control plants. While there was no significant effect of size ($z = 1.356$; $p > 0.05$) or defoliation intensity ($z = 0.012$; $p > 0.05$) on survival, there was a significant combined influence ($z = -2.147$; $p < 0.05$), where bigger plants subjected to 50% defoliation had higher survival. There was some difference in survival following a single 100% defoliation, larger seedlings had at least 32% increase in survival. High survival rates allowed me to test the relationships between plant traits. The allometric nature of the relationships between plant traits suggested that there was a strong effect of plant size, and responses often changed at a size threshold of 9 mm SBD (Fig 4.8). Additionally, seedlings and saplings grown under natural conditions had much lower growth rates (SBD 0.08 mm $month^{-1}$) than those seedlings subjected to controlled greenhouse conditions (SBD 1.36 mm $month^{-1}$).

SMA slopes were fitted separately for each defoliation treatment (Fig 4.7). These slopes were then compared between small (< 9 mm SBD) and large (> 9 mm) size classes (See appendix Table 3). SMA fits to the relationships varied with plant size, but in intercept rather than in slope. There was some evidence for an effect of defoliation intensity on intercept between size classes among several allometric lines of best fit. However this difference in intercept was restricted to large seedlings. Only the following allometric lines differed: a slight difference in the relationships between final leaf biomass and SBD (Wald statistic = 5.69, df = 2, $p = 0.05$), initial leaf biomass and final leaf biomass (Wald statistic = 7.002, df = 1, $p < 0.01$), and finally leaf biomass recovered and SBD (Wald statistic = 8.359, df = 1, $p < 0.01$).

Despite the similar slopes between defoliation intensities within small and large size classes, there were substantial slope and intercept differences by age class within each defoliation intensity treatment (Fig 4.7, also See Appendix Table 3). Slopes and intercepts of allometric lines were significantly different for most plant trait regressions (slopes are not equal and differences in intercept; $p < 0.05$ (See Appendix)). However SBD regrowth and SBD and final leaf biomass revealed similar slopes which can be referred to as an isometric relationship ($p > 0.05$) for small and large plants subjected to all defoliation intensities. I also found, despite size, final leaf biomass was strongly correlated to SBD at all intensities (R^2 ranges from 0.81-0.94; $p < 0.001$) suggesting that plant leaf recovery following defoliation is strongly dependent on plant size and presumably size of lignotuber.

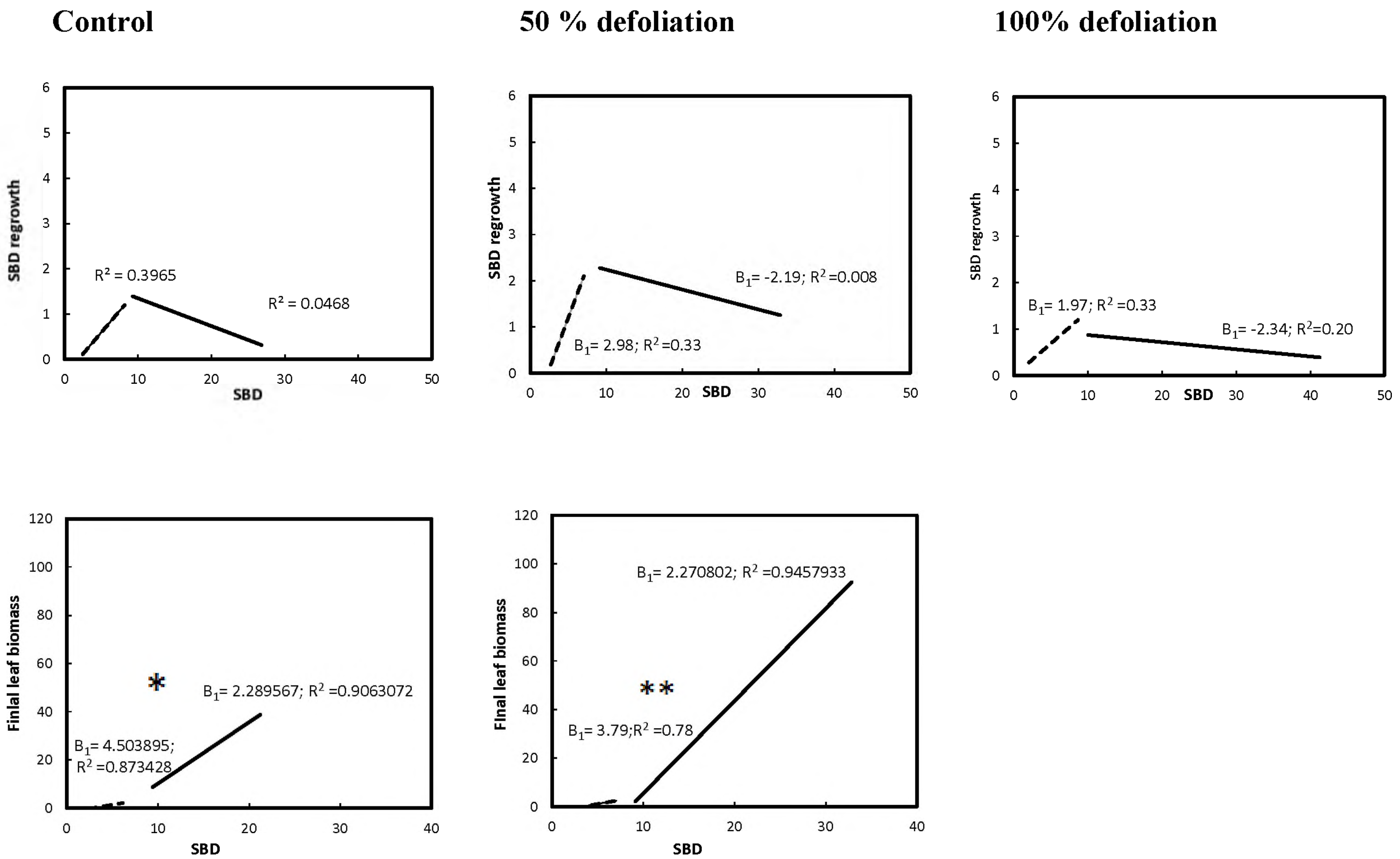


Figure 4.7a: The scaling relationships between *V. karroo* plant traits (grey regression lines represent seedlings with SBD < 9 mm, while black lines represent saplings with SBD > 9 mm) following defoliation. Controls (n=30) were only available for plant traits: SBD growth, final leaf biomass and change in height. Plants (n=30) were subjected to a single simulated defoliation event (50% defoliation= removal of half leaf biomass and 100% defoliation= removal of all leaf biomass). Significant slope (B_1 = slope) differences between large and small seedling and R^2 values for the relationships between growth traits were calculated using SMA analysis. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ represent significant levels for differences in relationships between small and large plants. There was no significant difference in slopes of relationships between 50 or 100% defoliation intensities for the measured plant traits. Continued (Fig4.8b) on page 64.

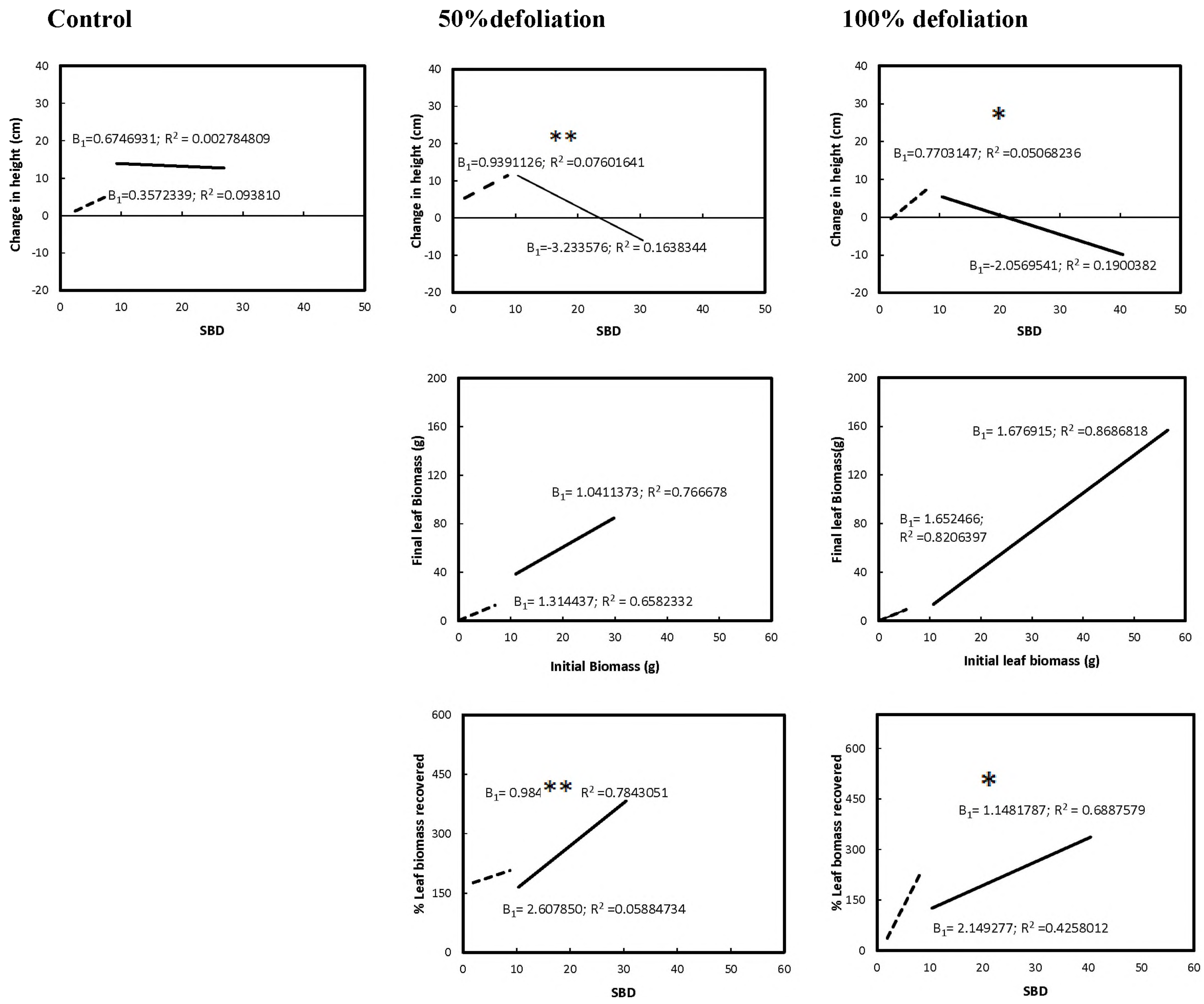


Figure4.7b: Continued

4.4 Discussion

The aim of this study was to investigate how survival and growth of the common African savanna species, *V. karroo*, are influenced by its age and size. I was especially interested in the sensitivity of seedlings and saplings to varying intensities of repeated browsing in the form of simulated defoliation. As expected, I found large differences in mortality between the different seedling age groups. Furthermore, age of seedlings as well as repeated browsing had an effect on seedling survival and the ability for the seedlings to re-grow. Older seedlings had greater survival and higher browsing frequencies resulted in greater mortality and reduced growth. Results from pot experiments were complemented by field trials indicating that plant response varied with size which can be directly correlated to plant age. Among field plants there were two distinct demographic stages (< 9 mm and > 9 mm SBD) which differed markedly in their rates of regrowth in response to defoliation – regardless of defoliation intensity. As predicted, younger seedlings subjected to frequent browsing were found to have increased mortality and reduced regrowth. For the first time we show that when plants reach a certain age (28 weeks under controlled conditions) and size (9 mm basal diameter under natural conditions), their tolerance to browsing disturbance increased significantly. Additionally results indicated that both age and size and browsing intensity have significant individual and combined effects on the response of seedlings and saplings.

4.4.1 Survival

Statistical survival analysis suggests that survival and growth were strongly age-dependent and younger seedlings had lower rates of recovery and survival following disturbance. In the absence of browsing, seedling survival was 100 percent at all ages. As expected, seedling mortality was greatest for the youngest age (6 week old) exposed to only a single defoliation. In the field experiment, the highest mortality occurred among small (less than 9mm SBD) seedlings that were also subjected to an intense defoliation event. Higher mortality among smaller seedlings could be a result of smaller and less developed root systems and therefore being reliant on their reduced nutrient content and carbon reserves for regrowth (Schutz *et al.* 2009; Hanley *et al.* 2004). According to the resource limitation hypothesis this is expected where smaller reserves contained within younger plants negatively influence secondary growth (Bryant *et al.* 1991). Hence they are less able to recover following browsing and therefore even the slightest disturbance can supress plant growth (Hodar *et al.* 2008). These survival results imply that frequent intense browsing events cause greatest mortality in young plants below 12 weeks in age or seedlings in the field.

4.4.2 Growth responses

It has been shown that after disturbance plants react positively and compensate for any tissue loss, but following repeated defoliation under compensation occurs during which plants are unable to recover the leaf biomass that existed prior to defoliation (Moyo *et al.* 2015). By quantifying biomass production and recovery I was able to determine whether *V. karroo* seedlings were able to fully compensate for biomass loss and how this was affected by plant age and defoliation frequency and intensity. Greenhouse results revealed that a seedlings ability to recover lost biomass was strongly influenced by both its age and the number of times it was defoliated. After an initial disturbance seedlings generally recovered more than their initial biomass (overcompensation) but thereafter this proportion of recovery gradually decreased for all ages. Those seedlings undergoing repeated browsing also appeared to invest very little in photosynthetic biomass production but this reduced investment in photosynthetic resources may be attributed to short recovery intervals between frequency events. Although plants within the field were not subjected to repeat browsing I expect a decrease in regrowth following additional browsing. In terms of defoliation intensity while there was markedly less biomass recovered by those seedlings subjected to 100% defoliation there was no difference in the effect of a 1st and 2nd defoliation. Seedlings that were moderately defoliated were able to overcompensate for leaf biomass loss, even after a 3rd defoliation. These results suggest that a moderate browsing intensity from smaller herbivores or arthropods may increase the rate of transition to adulthood. . Saplings in the field were also able to overcompensate for biomass loss while smaller seedlings produced lower leaf biomass. Although both demographic plant sizes were able to recover, saplings were better at recovering from browsing. This strategy of producing low-cost photosynthetic biomass, adopted by many acacia species, is known to promote seedling survival. However adopting this strategy will probably result in a browse trap escape cost where a plants rate of transition to adulthood is reduced (Hean and Ward 2012; Choeni and Sebata 2014).

Browsing, regardless of age, significantly reduced SBD growth. This implies that browsing is effective at reducing secondary growth of seedlings even at an age of 42 weeks and with repeated browsing events resulting in a further reduction. Plant growth rates are important for the development of models and understanding savanna dynamics (Scogings 2014). Despite the importance of growth rates, several studies have looked at the influence of browsing on plant height and not stem sizes within African savannas (Birkett and Stevens-Wood 2005; Fornara and du Toit 2008; Levick and Rogers 2008). Interestingly, in both natural and controlled greenhouse conditions, SBD only seemed to be strongly influenced by an intense 100% defoliation while above and below ground biomasses were sensitive to even half defoliation. It is important to consider the difference in growth under natural versus controlled greenhouse conditions. Seedlings and saplings grown under natural conditions had much lower growth rates but when browsing, following a single intense defoliation was introduced SBD growth doubled under both greenhouse and natural conditions. Although faster growth rates were observed under controlled conditions, I found that seedlings subjected to intense repeated defoliation had an average monthly growth rate of 0.18 mm (across ages).This drastic drop (1.36 to 0.18mm month^{-1}) in SBD growth implies that seedlings subjected to intense repeated defoliation may not persist and dominate a natural landscape while seedlings growing at average rates may find it difficult to escape to reproductive adult size (Higgins *et al.* 2000). Similar effects were observed for above and below ground biomass as well as final leaf biomass where there was a negative effect of intense simulated browsing on growth. The magnitude of this effect increased with repeated herbivory events. Additionally a moderate (50%) defoliation also reduced growth. This negative influence on growth, despite the level of browsing intensity suggests that further frequent browsing (> 3 defoliation events) may result in mortality.

Another aspect of the study investigated the influence of below ground biomass on a seedlings ability to recover following disturbance. Frequent disturbance in the form of biomass removal should select for increased storage of carbohydrate reserves within below ground organs. This should support compensatory regrowth (Schutz *et al.* 2011; Choeni and Sebata 2014). Overall, following initial browsing plants were able to recover and invest in leaf biomass. The effect of age was very pronounced, I found that older seedlings had higher below ground biomass but this decreased with repeated browsing, as did the ability to regrow above-ground biomass. This may be because older plants relative to their initial biomass had more to recover over a shorter period of time as opposed to smaller seedlings with less initial leaf biomass. Depletion in carbon reserves generally associated with repeated disturbance events may be another possible explanation for lack of leaf biomass regrowth seen in older plants (Schutz *et al.* 2011). Although an intense defoliation appeared to supress recovery, a moderate 50% defoliation resulted in increased below ground storage which may explain the overcompensation for lost leaf biomass. The reduced below ground biomass and lack of recovery associated with intense repeated browsing may be due to the short recovery intervals and thus strong effect of time on exponential growth. If plants respond quickly by altering biomass allocation patterns they may have to deal with suboptimal growth (Poorter *et al.* 2012). Therefore frequent browsing, at an intense severity, which results in shorter regrowth periods, may have a major influence on a plants ability to tolerate and persist in a highly browsed environment. Consequently, further browsing may result in mortality and not just growth suppression due to the decrease in availability of stored carbohydrate reserves. Disturbance adapted plants like *V. karroo* are expected have increased resistance due to their increased growth rates and below ground storage. However, their ability to participate in compensatory regrowth is influenced by more than just browsing intensity and frequency (Wigley *et al.* 2015). The magnitude of regrowth will also depend on climate, plant competition and resource availability (Wigley *et al.* 2015). In the field small plants had a positive response to a single defoliation and increased growth (SBD height and leaf biomass) while large plants appeared to be investing in leaf biomass as opposed to stem and height growth in the field.

4.4.3 Spinescence

Due to restricted photosynthesis and poorly developed root systems I expected smaller seedlings to have a low investment in defences (Boege and Marquis 2005). However, as a plant ages their allocation to defence could increase or decrease dependent on a plants resource allocation patterns (Boege and Marquis 2005 and Barton and Hanley 2013). Previous research supports these ideas and shows that browsing, particularly repeated browsing can influence structural defences with clear increases in spine lengths and diameters observed (Young *et al.* 2003; Staver *et al.* 2009; Wigley *et al.* 2015). However, results from my greenhouse study reveal the effect of herbivory on spinescence is less pronounced. In response to disturbance *Acacia* species are often found to develop branched, cage like structures following herbivory, as this offers protection to the plant and particularly the growing shoots (Archibald and Bond 2003). Many other studies have indicated a strong correlation between increased defence structures and decrease in herbivory (Cooper and Owen-Smith 1986; Gowda 1997; Young and Okello 1998). There was a slight effect of repeated herbivory and plant age on spine size. Older seedlings produced larger spines but were not able to increase the number of spines per plant. Plants tend to avoid herbivory through the production of structural defences and in a semi-arid savanna, these deterrents represent the first line of defence (Scogings *et al*. 2004). If plants do not respond to herbivory by investing in defence they may be prone to further herbivory. It is however noteworthy that *V. karroo* is known to alter its chemical defences in response to disturbance (Teague 1989; Wigley *et al*. 2015), a variable not measured in this study.

4.4.4 Conclusion

This chapter revealed that age and plant size was important in determining *V. karroo*'s growth and survival responses to browsing. Research findings indicate that seedlings at all ages were sensitive to browsing. I suggest that a threshold size at which seedlings become resistant to herbivory induced mortality occurs at as little as 28 weeks (under controlled conditions). Within natural conditions larger seedlings (> 9mm SBD) were more resistant to browsing following a single defoliation. In terms of a particular intensity of herbivory that may contribute to mortality and represent a meaningful release bottleneck, an intense repeated defoliation is most effective. The magnitude of the effect also depends on plant recovery periods. Decreased recovery periods between browsing events were directly related to a decrease in the ability to recover leaf biomass. Therefore *V. karroo*, at lower stocking densities < 4ha LSU^{-1}, may be able to compensate for lost biomass. With intense repeated herbivory seedlings are less inclined to recover significant proportions of lost biomass leading to reduced rates of transition to sapling and less commonly mortality. Hence herbivory in the form of browsing, through decreasing seedling survival can impose a 'demographic bottleneck' to establishment and thus reduce further encroachment by woody plants.

Critical to understanding woody thickening in rangelands of the Eastern Cape was to understand the factors that limit seedling establishment and the mechanisms responsible for seedling mortality or suppression (decrease in transition to adulthood). Here I confirm that herbivory is a critical management tool to dealing with woody thickening and maintaining the openness of semi-arid savannas. Although there is a general understanding of how individual factors may facilitate bush encroachment, research on how underlying mechanisms interact is necessary. Especially since it is now widely accepted that drought, disturbance and atmospheric CO_2 play a role and interact to influence an outcome. Hence by investigating how these underlying mechanisms interact and facilitate encroachment we will be able to identify accurate thresholds behind shifts towards more thicket like biomes.

4.5 Appendix

Table 1: TukeyHSD post hoc results indicating effect of simulated herbivory and repeated herbivory (**F0** (control), **F1** (one defoliation), **F2** (two defoliations) and **F3** (three defoliations)) within each age (12, 16 and 30 week) on various plant traits. Significant differences between herbivory frequency treatments are shown in bold.

		12 week		**16 week**		**30 week**	
Plant trait	**Treatment**	z	p	z	p	z	p
Leaf Biomass Recovery%	F0-F1						
	F0-F2						
	F0-F3						
	F1-F2			-1.424	0.8459	-2.906	0.0710
	F1-F3			-2.550	0.1745	-4.118	**<0.01**
	F2-F3	-1.535	0.7882	-1.125	0.9513	-1.167	0.9409
SBD Regrowth	F0-F1			-3.525	**0.00266**	-4.037	**<0.001**
	F0-F2	-8.486	**<1e-04**	-7.156	**<0.001**	-8.687	**<0.001**
	F0-F3	-9.265	**<1e-04**	-7.910	**< 0.001**	-8.084	**<0.001**
	F1-F2			-3.631	**0.00173**	-4.809	**<0.001**
	F1-F3			-4.385	**< 0.001**	-4.205	**<0.001**
	F2-F3	-0.751	0.733	-0.753	0.87521	0.582	0.938
Above ground biomass (g)	F0-F1			-5.554	**<0.001**	-7.530	**< 1e-04**
	F0-F2	-10.059	**<1e-05**	-6.960	**<0.001**	-11.485	**< 1e-04**
	F0-F3	-10.497	**<1e-05**	-7.990	**<0.001**	-11.069	**< 1e-04**
	F1-F2			-1.405	0.4959	-4.250	**0.000113**
	F1-F3			-2.435	0.0707	-4.250	**0.000113**
	F2-F3	0.422	0.906	-1.030	0.7317	0.401	0.978165
Below ground biomass (g)	F0-F1			-8.135	**<0.001**	-5.902	**< 0.001**
	F0-F2	-3.952	**0.000257**	-9.426	**<0.001**	-9.279	**< 0.001**
	F0-F3	-4.504	**<1e-04**	-10.665	**<0.001**	-8.450	**< 0.001**
	F1-F2			-1.292	0.5682	-3.609	**0.00164**
	F1-F3			-2.530	**0.0553**	-3.062	**0.01192**
	F2-F3	-0.511	0.865853	-1.238	0.6024	0.355	0.98469
Final leaf biomass (g)	F0-F1			-6.139	**< 0.001**	-7.683	**<0.001**
	F0-F2	-8.597	**<1e-04**	-8.319	**< 0.001**	-10.382	**<0.001**
	F0-F3	-10.104	**<1e-04**	-9.287	**< 0.001**	-11.108	**<0.001**
	F1-F2			-2.180	0.12873	-3.000	**0.0144**
	F1-F3			-3.148	**0.00936**	-3.726	**0.0011**
	F2-F3	-0.970	0.596	-0.969	0.76734	-0.700	0.8972
Thorn number	F0-F1			-1.422	0.4854	-1.079	0.7025
	F0-F2	-3.716	**<0.001**	-2.533	**0.0552**	-3.021	**0.0134**
	F0-F3	-6.366	**<0.001**	-3.892	**<0.001**	-3.064	**0.0120**
	F1-F2			-1.110	0.6832	-1.985	0.1935
	F1-F3			-2.470	**0.0646**	-2.028	0.1775
	F2-F3	-2.256	0.0622	-1.360	0.5247	-0.042	1.0000
Thorn length (mm)	F0-F1			-1.806	0.270	-0.148	0.9989
	F0-F2	-1.604	0.2437	-1.259	0.589	-2.565	**0.0503**
	F0-F3	-2.339	**0.0505**	-1.713	0.317	-0.755	0.8747
	F1-F2			0.547	0.948	-2.423	0.0727
	F1-F3			0.092	1.000	-0.613	0.9280
	F2-F3	-0.598	0.8211	-0.454	0.969	1.744	0.3005
Thorn diameter (mm)	F0-F1			-3.009	0.01386	0.251	0.9945
	F0-F2	-1.780	0.1760	-2.074	0.16175	2.345	0.0880
	F0-F3	-2.561	0.0281	0.263	0.99364	-0.509	0.9570
	F1-F2			0.935	0.78617	-2.586	0.0477
	F1-F3			3.272	0.00588	-0.750	0.8768
	F2-F3	-0.631	0.8026	2.337	0.08963	1.770	0.2879

Table 2: TukeyHSD post hoc results showing the effect of simulated herbivory intensity treatments (50% and 100% defoliation) between defoliation events (**F0** (control), **F1** (one defoliation), **F2** (two defoliations) and **F3** (three defoliations)) on various measured plant traits. Significant differences between herbivory frequency treatments are shown in bold.

		50% Defoliation		**100% Defoliation**	
Plant Trait	**Treatment**	z	p	z	p
SBD Regrowth	F0-F1	1.234	0.9219	3.561	**<0.01**
	F0-F2	1.137	0.9488	7.230	**<0.01**
	F0-F3	0.734	0.9960	7.991	**<0.01**
Above ground biomass (g)	F0-F1	3.165	**0.03344**	5.013	**<0.001**
	F0-F2	3.026	**0.05113**	6.282	**<0.001**
	F0-F3	4.108	**<0.001**	7.211	**<0.001**
Below ground biomass (g)	F0-F1	2.797	0.09567	6.598	**<0.001**
	F0-F2	3.671	**0.00594**	7.645	**<0.001**
	F0-F3	2.823	0.08952	8.650	**<0.001**
Final leaf biomass (g)	F0-F1	4.353	**<0.01**	6.244	**<0.01**
	F0-F2	4.789	**<0.01**	8.461	**<0.01**
	F0-F3	6.354	**<0.01**	9.446	**<0.01**
Thorn number	F0-F1	0.569	0.9921	1.298	0.90009
	F0-F2	0.319	0.99998	2.311	0.28691
	F0-F3	0.717	0.99653	3.552	**0.00873**
Thorn length (mm)	F0-F1	1.957	0.5111	1.950	0.516
	F0-F2	-0.070	1.000	1.360	0.875
	F0-F3	0.837	0.991	1.851	0.585
Thorn diameter (mm)	F0-F1	2.845	0.0843	2.713	0.1189
	F0-F2	-0.237	1.0000	1.870	0.5717
	F0-F3	0.948	0.9812	-0.237	1.0000

Table 3: The bivariate relationships between several plant traits were analysed by defoliation intensity treatment (control, 50 and 100% defoliation and small and large size classes). Relationships were analysed using Standardized Major Axes (SMA) techniques. heterogeneity of regression slopes and differences in intercept of regression slopes are indicated in bold.

Plant trait regression	**Treatment(control Vs 50% defoliation Vs 100% defoliation**	**H0: slopes are equal**			**H0: no difference in intercept**			**Treatment (small Vs large seedlings)**	**H0: slopes are equal**			**H0: no difference in intercept**		
		Likelihood ratio statistic	p value	df	Wald statistic	p value	df		Likelihood ratio statistic	p value	df	Wald statistic	p value	df
SBD- SBD growth	All	1.264	0.53	2	2.763	0.25	2	Control	0.1543	0.69442	1	6.287	**0.012165**	1
	Small	1.469	0.479	2	3.301	0.19	2	50% defoliation	0.5574	0.45532	1	12.38	**0.00043343**	1
	Large	0.5971	0.74	2	3.042	0.21	2	100% defoliation	0.1666	0.68311	1	10.02	**0.0015443**	1
SBD- Final Biomass	All	3.672	0.15	2	2.054	0.35812	2	Control	5.357	**0.02**	1	5.939	**0.014809**	1
	Small	4.27	0.11	2	1.069	0.586	2	50% defoliation	7.964	**0.0047**	1	3.835	**0.050186**	1
	Large	1.569	0.45629	2	5.697	**0.057923**	2	100% defoliation	5.201	**0.022**	1	1.047	0.306	1
Initial Biomass-Final Biomass	All	0.3654	0.54554	1	3.743	**0.053039**	1	Control						
	Small	0.8261	0.36341	1	0.954	0.32871	1	50% defoliation	0.6234	0.42979	1	0.1402	0.70804	1
	Large	0.6226	0.43007	1	7.002	**0.0081428**	1	100% defoliation	0.005693	0.93985	1	5.874	**0.015364**	1
SBD- Biomass recovery	All	0.039	0.84344	1	3.147	**0.076075**	1	Control						
	Small	0.3248	0.56872	1	1.611	0.2043	1	50% defoliation	8.621	**0.0033222**	1	11.07	**0.0008787**	1
	Large	0.2881	0.59146	1	8.359	**0.0038373**	1	100% defoliation	3.72	**0.053762**	1	11.87	**0.00056946**	1
SBD- Height growth	All							Control	0.9491	0.32995	1	4.509	**0.033725**	1
	small	0.6746	0.71368	2	2.847	0.24082	2	50% defoliation	6.951	**0.0083754**	1	14.77	**0.00012148**	1
	large	4.378	0.11201	2	0.2763	0.87096	2	100% defoliation	4.5	**0.033898**	1	6.246	**0.012445**	1

Table 4: The bivariate relationships between several plant traits were analysed by defoliation intensity treatment (control, 50 and 100% defoliation) for each small and large size class. Relationships were analysed using Standardized Major Axes (SMA) techniques. heterogeneity of regression slopes and differences in intercept of regression slopes are indicated in bold. Each estimate is associated with a confidence interval (CI) limit and R^2 (correlation with p value).

Plant trait regression	Defoliation treatment	Size class (sma)	Intercept estimate	CI lower limit	CI upper limit	Slope estimate	CI lower limit	CI upper limit	R-squared	P value
SBD-SBD regrowth	Control	small	-2.1286687	-3.3209604	-0.9363771	2.670692	1.441254	4.948882	0.02078516	0.63842
		large	-3.8519152	-7.5893703	-0.1144601	3.277076	1.365865	7.862583	0.01537816	0.76986
	50	small	-2.1060530	-3.2398156	-0.9722903	2.981205	1.777141	5.001059	0.3361306	**0.037815**
		large	2.8518493	0.9082933	4.7954052	-2.194111	-4.219919	-1.140810	0.008281745	0.7785
	100	small	-1.6610112	-2.4050723	-0.9169501	1.973571	1.205341	3.231438	0.3339661	**0.030427**
		large	2.7493575	0.3416125	5.1571024	-2.347220	-4.875326	-1.130066	0.2031616	0.22337
Final biomass-SBD	Control	small	-3.202073	-3.894186	-2.509959	4.503895	3.644996	5.565183	0.873428	**3.3659e-07**
		large	-1.363448	-2.117883	-0.609012	2.289567	1.747343	3.000051	0.9063072	**7.61e-05**
	50	small	-2.784542	-3.606526	-1.962558	3.799283	2.803353	5.149031	0.7836128	**5.7447e-05**
		large	-1.3830097	-1.8494987	-0.9165207	2.270802	1.928635	2.673673	0.9457933	**1.1787e-07**
	100	small	-2.979908	-3.791001	-2.168816	4.071551	3.107887	5.334018	0.8110866	**1.1204e-05**
		large	-1.8718358	-2.7846631	-0.9590086	2.645280	2.018226	3.467159	0.88716	**4.6486e-05**
Final- Initial biomass	Control	small								
		large								
	50	small	0.11084167	-0.09081892	0.31250226	1.314437	0.987887	1.748928	0.6582332	**1.419e-05**
		large	0.4310341	-0.4565272	1.3185954	1.0411373	0.5553998	1.9516875	0.766678	0.02225
	100	small	-0.05370981	-0.27300495	0.16558533	1.652466	1.285513	2.124165	0.8206397	**3.3314e-06**
		large	-0.6370140	-1.4507037	0.1766756	1.676915	1.176368	2.390445	0.8686818	**0.00074553**
Biomass recovered- SBD	Control	small								
		large								
	50	small	0.4914585	-0.4176423	1.4005593	2.607850	1.563929	4.348588	0.05884734	0.34817
		large	1.1481145	0.6199347	1.6762944	0.9847627	0.6574702	1.4749831	0.7843051	**0.0014879**
	100	small	0.5657963	-0.1329683	1.2645608	2.149277	1.356544	3.405265	0.4258012	0.01142
		large	0.79238558	0.06526054	1.51951061	1.1481787	0.7104955	1.8554857	0.6887579	**0.0056336**
Height change-SBD	Control	small	1.359082	1.217254	1.500910	0.3572339	0.2024810	0.6302621	0.0938102	0.28686
		large	0.9119551	0.2743678	1.5495423	0.6746931	0.3207208	1.4193365	0.002784809	0.88488
	50	small	0.8009603	0.4627277	1.1391929	0.9391126	0.5547836	1.5896872	0.07601641	0.30133
		large	5.345869	1.924109	8.767628	-3.233576	-6.818754	-1.533420	0.1638344	0.27986
	100	small	0.8022137	0.4851461	1.1192814	0.7703147	0.4422687	1.3416838	0.05068236	0.41983
		large	3.742375	1.634538	5.850213	-2.0569541	-4.2942985	-0.9852739	0.1900382	0.24081

5. Thesis Summary

The thesis comprises two studies that aim to improve our knowledge of tree-grass coexistence within savannas. The study specifically focused on the role of browsing as a demographic bottleneck for bush encroachment, using *V.karroo* as a test case. This final chapter attempts to summarise the main findings from both chapters 3 and 4 as well as discuss its implications for future research.

Staver and Bond (2014) proposed a “browse trap”, similar to the more common “fire trap” discussed in Chapter 1, as a way to think about the effect of browsing in savannas. To determine whether *V.karroo* experiences a release bottleneck in this semi-arid savanna I examined the relationship between plant size and age and browsing. I then assessed whether natural browsing, like fire, can limit tree cover by preventing the transition of smaller plants to adults (chapter 3). If browsing did impact survival and growth how did this effect change with plant size and age? This allowed me to determine a cut-off size and age after which browsing had less impact (Chapter 4).

Research findings as shown in Fig 5.1 illustrate the effects of browsing from herbivores, which is largely influenced by herbivore biomass in a region. Browsing, which in this study was examined in terms of defoliation intensity and frequency (A) and vertebrate exclusion (B), was largely impacted by plant size. Plant age was also directly related to plant size which strongly affected mortality and plant regrowth following browsing. Shade (B), another aspect of the study, proved to have a strong limiting effect on plant regrowth. Herbivore browsing can ultimately, through its direct effect on size and regrowth impact a plant’s rate of transition into adulthood.

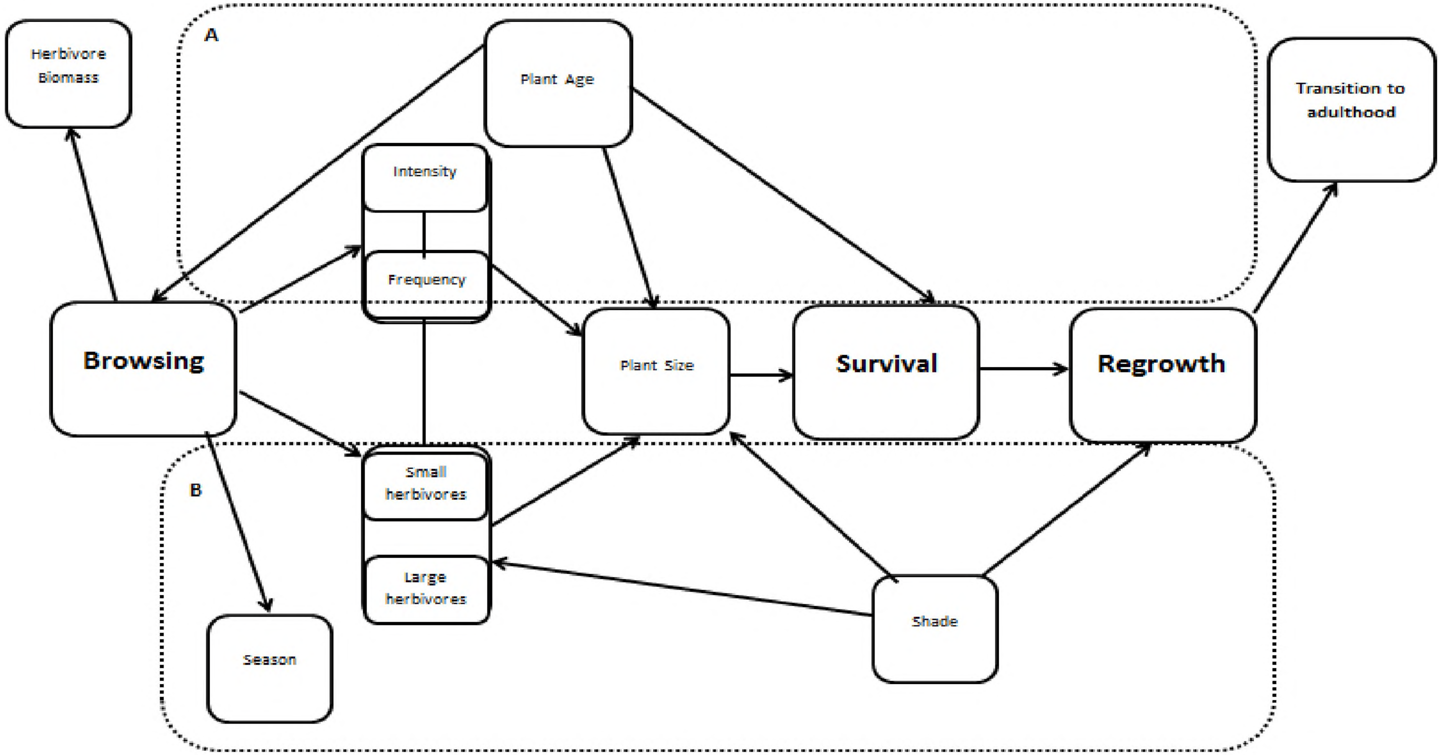


Figure 5.1: Flow diagram illustrating main relationships observed between browsing and survival and regrowth of *V.karroo* in a semi-arid savanna. Directions of arrows indicate an effect. Two main research ideas are examined to understand browsing effects: **A-** describes the effect of natural browsing from different herbivore guilds, and **B-** shows the effects of specific browsing intensities and frequencies of different plant ages on survival and growth.

5.1 Main findings

5.1.1 Does browsing limit tree cover by preventing release of smaller plants into adults?

Chapter 3 determined the probability of a *V. karroo* seedling being defoliated. I assessed the role different herbivore guilds under varying microhabitat conditions, in causing a seedling release bottleneck in a semi-arid savanna (Fig 5.1A). Results revealed that herbivory was prominent in this semi-arid savanna and can be suggested as an important release bottleneck for bush encroachment here. Browsing was severe and frequent from both small and larger herbivores. However the effects of browsing from both small and larger herbivores were far greater on mortality than on compensatory growth responses. The exclusion of larger vertebrates such as kudu and impala resulted in drastic increases in seedling survival. Additionally seedling growth was largely impacted by shade from established trees. Growth was limited in subcanopy while full exposure to sunlight enhanced growth suggesting *V.karroo* may be shade intolerant. Within this semi-arid savanna browsing can impact woody tree cover through imposing a release bottleneck for tree seedlings and saplings that can result in suppression and mortality.

5.1.2 How did browsing impact survival and growth and how did this effect change with plant size and age?

In chapter 4 I learned that age and size were important in determining growth and survival responses to browsing of *V. karroo*' seedlings and saplings. Results indicate that there is a distinct age threshold, associated with a specific intensity and frequency of browsing, at which seedlings become less susceptible to browsing. Older seedlings, specifically older than 28 weeks were better at recovering and surviving browsing. Seedlings at 6 weeks old or less have very little chance of survival after just a 1st defoliation. Generally there was very little or no difference between a 1st and 2nd defoliation but a 3rd defoliation frequency was very effective at suppressing plant growth. Seedlings responded positively to moderate browsing intensity (50% leaf removal), in some cases overcompensating for leaf loss but intense defoliation (100% leaf removal) had a strong negative effect on plant growth for both young and old seedlings. Leaf regrowth following intense defoliation comes at the expense of root resources but with frequent repeated defoliation the root reserves are not able to sustain repeated regrowth requirements. Following moderate defoliation, on the other hand, a frequent 50 % defoliation is sustainable without reducing the amount of regrowth. Under field conditions, similar response patterns were observed. Seedlings differed markedly in their response to a single browsing event as opposed to saplings. Saplings (> 9mm SBD) appeared to have a higher chance of survival and were better at recovering following browsing. These findings have important implications for tree-grass coexistence dynamics, suggesting that specific size and browsing thresholds should be considered when attempting to understand savanna biome shifts, since it may increase our predictive power.

5.2 Future research

This study demonstrated that herbivory and specifically browsing is an important component of tree-grass coexistence in semi-arid savanna systems. Woody plant response is however size and age dependent. Small seedlings appeared to be most vulnerable to browsing, but when plants reached ~ 48 weeks or 9mm in SBD, the impact of browsing on their development and transition to adulthood was very low. Investigating plant responses, at various demographic stages, to top-down disturbances proves useful and is important when determining disturbance thresholds and biome tipping points. Additionally woody seedling growth and survival in the field is impacted by more than just herbivory. Other well-known disturbances include fire, drought and competition with grasses (Sankaran *et al.* 2004; Bond 2008). Some future research initiatives should consider the findings of this study and incorporate them into research areas discussed below.

1. My current research reveals that there is a size and age threshold to which seedlings become more resistant to browsing. I predict that this threshold may change along environmental gradients. However this has not been contextualised in terms of a rainfall gradient. Moreover relationships between disturbance and seedling establishment are likely to be affected by atmospheric CO_2 levels. While there is considerable research indicating expected increases in CO_2 and land cover change, there are few studies quantifying the influence of elevated CO_2 on savanna trees and demography (Bond 2008; Kgope *et al.* 2010; Ripley *et al.* (in submission)). A recent study by Ripley *et al.* (in submission) found that CO_2 fertilisation has large effects on *V.karroo* growth. By modelling the probability of being eaten and the effect of CO_2 fertilisation they found seedlings would be less susceptible to damage from browsing and mortality through increased plant growth rates in the future. These findings are important for research development but more direct measurements of woody growth rates under elevated CO_2 and herbivory are critical. Previously effective disturbance

mechanisms that control tree distributions and abundance in grassy ecosystems may change in effectiveness with changing CO_2 concentrations. Hence there is a need for more field trials and experiments to validate the importance of these factors (Midgley *et al*. 2007) and how they interact with each other across environmental gradients.

2. In arid systems fire played a minor ecological role in rangeland management when compared to in mesic regions of South Africa. However with current increases in bush encroachment fire is now being introduced in many regions of the Eastern Cape, where previously more natural mechanisms were utilised. Therefore determining the intensity and frequency at which a fire is most effective is important, but for fire to be useful it should be used in combination with browsing initiatives. For instance intense browsing following fire may significantly reduce tree cover as well as reduce the chances of encroachment at a later stage.

3. Our knowledge about woody plant resource allocation is increasing but specific allocation patterns associated with disturbance and climate is poor. Understanding *V.karroo* allocation strategies, although not explicitly covered in this study are important for predicting accurate plant responses to climate change. This study revealed that resource allocation varies with size, especially between seedling and saplings in the field. A better understanding of plant investment trade-offs and resource partitioning will allow us to determine economically important plant organs. This will make a significant contribution to our modelling predictive power and understanding of tree-grass coexistence.

4. Lastly, research on the effect of shade on plant growth and survival is varied. There is evidence for positive, negative and no effect of shade. Here I found shade had a strong limiting effect on growth. These findings support evidence suggesting *V. karroo* may be intolerant to shade. More experimental work on how the effect of shade may directly influence specific plant size classes is necessary. Further research into this may provide valuable insight into the role of shade in limiting growth and its interactive effect with disturbance and CO_2 fertilisation in preventing seedling release in a semi-arid savanna.

5.3 Conclusion

An increase in woody trees in grassy ecosystems has serious implications for savannas. The success of woody plants is largely determined by seedling and sapling dynamics, establishment and survival in the face of disturbance. This represents the most important period for implementing management initiatives to prevent bush encroachment since *V. karroo* seedlings and saplings are prone to disturbance and competition. Within mesic regions there has been extensive research on drivers influencing encroachment, this is not as common in semi-arid regions and while there is a general understanding of all potential drivers the effects of individual drivers in absence of other disturbances is lacking (Bond 2008; Staver and Bond 2014). Woody increase patterns are not simply a function of a single factor such as CO_2, but are rather a function of how CO_2 alters the balance within savannas favouring trees. Therefore determining critical environmental thresholds such as a specific CO_2 concentration, fire intensity or in my case maximum browsing associated with plant size and how it interacts with the changing climate to lead to a biome transition is necessary.

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