Seed fate and density of soil seed banks of four *Acacia* species in the Kruger National Park, South Africa

Samanta Adele Stelli

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DECLARATION

I declare that this thesis is my own, unaided work, unless specifically acknowledged in the text. It has not been submitted in any form for another degree or diploma award at any other university or institution of tertiary education. This thesis is being submitted for the Degree of Masters of Science at the University of the Witwatersrand, Johannesburg.

Supervisor:

Professor E.T.F. Witkowski

University of the Witwatersrand, Johannesburg, South Africa.

Signed:

Date:

07 October 2011

ABSTRACT

Observations of the changes in woody plant density in the Kruger National Park (KNP) over 58 years have shown an increase in large woody plant density on granite substrates, which is attributed to fire and herbivore density. Woody plants persist in areas with frequent fires, herbivory and drought by resprouting or protecting seeds in the ground. Soil seed banks, which are stores of seeds below ground or in leaf litter, provide 'insurance' for trees and allow populations to persist in unfavourable environments. No comprehensive studies have been conducted on soil seed bank ecology of *Acacia* species in the Kruger National Park, a research gap which this study aimed to fill.

The spatial distribution and density of *in situ* soil seed banks for four *Acacia* species, *A. grandicornuta, A. nilotica, A. senegal* and *A. tortilis* was assessed in the Skukuza land system of the KNP, South Africa. *In situ* soil seed banks were quantified for eight mature trees per species during 2005/2006. Greenhouse and field seed burial trials were carried out for one year and 16 months respectively, between 2005 and 2007, to investigate the persistence of *Acacia* seeds over an extended period of time. Post-dispersal seed predation of *Acacia* seeds was investigated during July 2006 in six demarcated grids within 15 km of Skukuza.

Overall soil seed bank density differed significantly among species, being highest for *A. tortilis* (19.5 ± 6.4 seeds m^{-2}), followed by *A. grandicornuta* (12.1 ± 6.9 seeds m^{-2}), *A. nilotica* (4.9 ±1.8 seeds m^{-2}) and lowest for *A. senegal* (0.6 seeds ± 0.4 seeds m^{-2}). Generally, seed bank density decreased with depth in the soil and distance from the centre of the tree canopy. Seed bank density increased significantly with a decrease in soil compaction for *A. senegal* only, while it was not related to over-storey canopy shading or herbaceous biomass for any of the species. No significant relationship was found between seed bank density and tree characteristics such as stem diameter, bark thickness or tree canopy area for any of the species. Viability of seeds from the seed bank decreased between species as follows: *A. tortilis* (77% of 142 seeds), *A. nilotica* (61% of 39 seeds), *A. grandicornuta* (58% of 87 seeds), and *A. senegal* (0% of 4 seeds). For all species with viable seeds, viability decreased with distance from the centre of the tree canopy. Bruchid beetle predation (assessed on 100 newly produced seeds) was low for all four species.

Fifty seeds each of *A. grandicornuta, A. senegal* and *A. tortilis* and 100 *A. nilotica* seeds were destroyed by fire during the field seed burial trial, of which four hundred seeds/species were used. Of the remaining seeds, 15% of *A. senegal,* 19% of *A. grandicornuta,* 34% of *A. nilotica* and 66% of *A. tortilis* remained intact after 16 months

in the field. Of these, 65% of *A. tortilis,* 27% of *A. nilotica,* 5% of *A. grandicornuta* and no *A. senegal* seeds were still viable. The percentage of remaining intact, viable seeds was highest under tree canopy cover and buried for *A. tortilis* (86%), *A. nilotica* (39%) and *A. grandicornuta* (6%), but the micro-site placement of seeds had a significant effect on viability for *A. nilotica* only (d.f. = l; $\chi^2 = 7.5$; $P = 0.006$).

In the greenhouse seed burial trial (150 seeds/species/treatment), one percent of the total seed lot germinated, which was 2.9% of *A. grandicornuta*, 0.7% of *A. senegal* and 0.2% of both *A. nilotica* and *A. tortilis*. *A. tortilis* had the highest percentage of remaining intact, viable seeds (92.2%), followed by *A. nilotica* (58.3%), *A. grandicornuta* (57.6%) and *A. senegal* (0%). The number of remaining intact, viable seeds was highest when watered with the average rainfall (327 seeds), followed by the highest (314 seeds) and lowest rainfall (296 seeds). There was no association between rainfall treatments and the number of remaining intact, viable seeds for any of the species, except for *A. grandicornuta* where the number of remaining intact, viable seeds increased significantly with the average rainfall.

Across six grids in the Skukuza land system, *A. grandicornuta* was the most dominant woody plant of six study species, followed by *Dichrostachys cinerea, A. tortilis, A. nilotica, A. senegal* and *A. nigrescens.* Woody plant density in grids varied between 226 plants ha^{-1} (Grid 3) to 1618 plants ha^{-1} (Grid 5), with a mean density of 862 ± 195 plants ha^{-1}. Overall, woody plant species diversity was low (Shannon Wiener Index, 1.8 ± 2.8; Evenness Index, 0.7 ± 0.02; Simpson's Reciprocal Index, 4.5 ± 0.6). The dung of nine species of large herbivore was recorded across all six grids. Large herbivores favoured seeds of indehiscent (55 *A. tortilis* seeds and 11 *A. nilotica* seeds) over dehiscent pods (1 *A. grandicornuta* seed). Only 9% (five *A. tortilis* seeds and one *A. grandicornuta* seed) of the 67 seeds extracted from dung germinated after a six-week germination trial. Less than half the remaining ungerminated *A. nilotica* seeds (46%) and *A. tortilis* seeds (40%) tested viable.

There was no correlation between the number of termitaria recorded and the number of *Acacia* trees growing on them ($r = 0.07$). Termite mounds occupied 0.0009 ± 0.0003 ha per grid matrix (0.8%).

Only four rodent species were recorded across all six grids, *Mastomys coucha* (multimammate mouse), *Rhabdomys pumilio* (striped mouse), *Aethomys chrysophilus* (red veld rat) and *Tatera leucogaster* (highveld gerbil). Rodent species diversity was low (Shannon Wiener Index, 0.6 ± 0.2; Evenness Index, 0.6 ± 0.2; Simpson's Reciprocal Index, 1.9 ± 0.3). In the field cafeteria trial there was a significant difference in the percentage of seeds removed between seed species ($P < 0.05$; $F = 2.8$; d.f. = 3, 236). There was a

significant difference in the percentage of seeds removed from trays placed under vegetation cover compared with trays placed in the open *(P* = 0.034).

This study suggests that *A. grandicornuta, A. nilotica* and *A. tortilis* seeds form short-term persistent seed banks, while *A. senegal* seeds are transient and do not form seed banks. Seeds of several woody plants were ingested by large herbivores and selected by rodents. The relevance of soil seed banks to regeneration of *Acacia* trees needs to be evaluated by investigating whether these species rely more on seed production or resprouting for individual recruitment into tree populations. Once this issue is clarified the effect of certain factors on seed fate and consequently, recruitment of individuals into plant populations, can be more clearly understood. This will assist in managing and understanding these potentially encroaching species in the Kruger National Park, South Africa.

KEYWORDS: *in situ* soil seed banks, persistence, germination, post-dispersal seed predation.

ABBREVIATIONS: KNP (Kruger National Park), NPNR (Nylsvley Provincial Nature Reserve), CSF (Corey Shape Factor), GI (Germination Index), PCQ (Point-Centred Quarter), GUD (Giving-Up Density), GPS (Global Positioning System), SE (Standard Error).

DEDICATION

I would like to dedicate this dissertation to my supervisor, my parents, my sister and my partner for all their patience, support and advice. Also to all my friends who assisted me in completing my field work.

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CHAPTER ONE

Introduction

1.1. Seed ecology

1.1.1. Plant regeneration

Plants may regenerate asexually through vegetative coppicing, or sexually through the production of seeds (Fenner and Thompson 2005). Seed production allows the shuffling of genetic material in a population (Fenner and Thompson 2005), species persistence and genetic variation (Bradbeer 1988; Grime and Hillier 1992), successful plant regeneration (defined by Grubb (1977) as the replacement of one mature individual by another of the next generation) and long-term plant population maintenance (Garner and Witkowski 1997).

Many savanna woody plant species will often produce shoots if above-ground parts are damaged or removed (Shackleton 1997; Bond and Midgley 2001; Neke *et al.* 2006). Most *Acacia* trees are able to resprout, especially following fire or damage by herbivores and there exists a growth continuum amongst these species, from tall single-stemmed reseeders to short multi-stemmed resprouters (Midgley and Bond 2001). Resprouting is defined, in terms of individual tree persistence, as the production of secondary trunks as a response to injury or changes in growing conditions (Bond and Midgley 2001). There are four types of sprouting, namely sprouting from the base of the trunk, from underground stems, from roots, and from layered branches (Del Tredici 2001). Resprouters often show minimal changes in population size over centuries. Seeders on the other hand, are subject to threats to seed-producing processes, such as pollination and dispersal failure (Bond and Midgley 2001). Bond and Midgley (2001) suggest that trees with an enhanced ability to resprout would thereby be able to persistently and successfully occupy a position in the ecosystem.

In the savanna environment, a trade-off occurs in resource allocation that favours resprouting over seed-based regeneration in trees (Bond and Midgley 2001). Resprouters tend to allocate more nutrients to underground storage systems when in competition with grasses and are then less affected by competition than obligate seeders (species that are dependent on seedling recruitment for population survival) (Clarke and Knox 2009). Obligate seeders are woody plants which may also not resprout from their trunks (Bond and van Wilgen 1996). Seedlings of obligate seeders are more efficient at capitalising on above-ground resources i.e. allocating nutrients to shoots and leaves to escape the light-limiting factor of grass clumps, however, fewer nutrients are allocated to roots, which weakens the root system and makes it more vulnerable to the fibrous root systems of competing established grasses (Clarke & Knox 2009). In the early stages of seedling growth, resprouters will not be suppressed by grass

competitors at the rate that obligate seeders will (Clarke & Knox 2009).

Many factors can affect the recruitment of a plant species, such as propagule production and transportation, competition, predation and herbivory (Clark *et al.* 2007). Seed limitation, which impacts recruitment, may be due to low seed numbers, but also to a failure to arrive at a suitable micro-site as a result of poor dispersal (Eriksson and Ehrlen 1992; Fenner and Thompson 2003; Clark *et al.* 2007). Factors affecting seed dispersal can include environmental dynamics such as wind direction and strength, the behaviour of animal dispersal agents and the patterns of rainfall and relative humidity, while characteristics of the parent plant such as genetics, fruit and seed size, and ease of dehiscence also affect the size and shape of the seed shadow (Willson and Traveset 2000). The seed shadow is the spatial distribution of dispersed seeds around their source (Janzen 1971).

Seeds disperse primarily to avoid natural enemies and sibling interactions and to find sites physically suitable for successful establishment, as plants species often have very specific physical requirements for germination and establishment; even with good dormancy mechanisms, dispersal will ensure that a seed will encounter the conditions required for successful establishment (Willson and Traveset 2000). Often seed dispersal does not result in successful seedling establishment, if seeds are exposed to threats such as rodent and insect predation (Wenny 2001). The availability of a safe site is necessary for the establishment of seedlings. Safe sites are described as places where the requirements for successful establishment including dormancy-breaking and germination occur, as well as a reduction in the effects of competition, predation and pathogen activity (Fenner and Thompson 2005).

For all plant species one of the most important functions of seed dispersal is to place seeds in safe sites (Green 1983). For example, Enright and Lamont (1989) described the litter layer under *Banksia* species as safe sites for seeds. However, in their analysis of the effect of leaf litter on seedling survival, Lamont *et al.* (1993) found that although litter provides a safe site for seeds, the survival of seedlings in litter was lower than that of seedlings on bare-soil patches. Indeed for many plant species only a small proportion of the seeds produced end up in locations suitable for establishment (Fenner and Thompson 2005). Thereby, safe site availability rather than absolute seed number may often determine future plant recruitment and hence population abundance (Maron and Gardner 2000). Eriksson and Ehrlen (1992), however, suggest that many species, if adequately studied, would turn out to be both micro-site and seed-limited. Species with large seeds, species in disturbed micro-sites and species with relatively short-lived seed banks often experience greater seed-limitation (Clark *et al.* 2007).

1.1.2. In situ soil seed banks

Soil seed banks are defined as all the viable seeds found in and on the soil or in associated leaf litter (Simpson *et al.* 1989) and consist of ungerminated seeds that have the potential to replace adult plants in a population (Baker 1989). Soil seed banks are ecologically and evolutionarily significant to plant population dynamics (Kalisz 1991; Auld 1995) as they allow a species to survive unfavourable environmental conditions (Mbalo and Witkowski 1997) and affect resilience and resistance of ecosystems to environmental disturbances (Pugnaire and Lazaro 2000). The formation of persistent soil seed banks is especially important for regeneration in obligate seeders (Bond *et al.* 1990). Seed bank ecology has become a subject of increasing interest to scientists, mainly because of the role seed banks play in conservation (Fenner 1995). They act as gene reservoirs, maintain genetic variation, buffer both populations and genotypes from extinction (Holmes and Newton 2004) and preserve the representation of a plant population within a community (Kalisz 1991). Seed banks are therefore regarded as natural 'storage facilities' (Fenner and Thompson 2005).

Seeds that have the potential to remain in the soil for long periods of time are often small, compact and smooth with specific requirements for germination (Thompson 1987). Seeds with hard seed coats, such as *Acacia* species from the sub-family Mimosaceae (van Wyk and Malan 1998) have the characteristics to potentially form soil seed banks (Witkowski and Garner 2000). When seeds are dispersed from the parent plant they can be in a state of dormancy (Hartmann *et al.* 2002). A dormant seed is one that even when viable, experiences a state of desiccation and does not germinate even when placed in an environment normally conducive to germination (Roberts 1972; Mayer and Poljakoff-Mayber 1975; Baskin and Baskin 1989). Dormancy, which is also defined as the long-term persistence of viable seeds (Taylorson and Hendricks 1977), is common in seeds with hard seed coats that act as mechanical barriers to germination and allow seeds to potentially remain viable for several decades (Figure 1) (Mayer and Poljakoff-Mayber 1975; Taylorson and Hendricks 1977; Coe and Coe 1987; Bradbeer 1988; Murdoch and Ellis 1992). This function is known as coat-imposed or exogenous physical dormancy (Hartmann *et al.* 2002). An impermeable testa or seed coat will prevent water from being imbibed until surrounding conditions are favourable for seedling growth (Van Staden *et al.* 1989). Consequently *Acacia* seeds that are kept dry are able to remain dormant for many years (Tybirk *et al.* 1992).

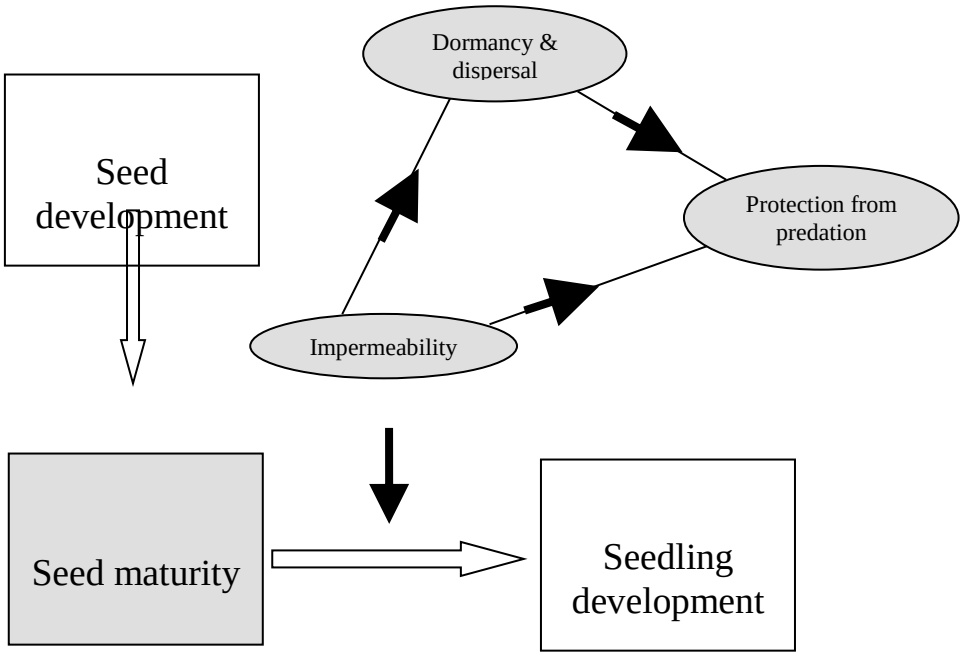

Figure 1. The functional aspect of the legume testa or seed coat (adapted from Van Staden *et al*. 1989).

The distribution of seeds in the soil is affected by the interaction between factors like seed production, seed dispersal, habitat quality, environmental fluctuations and germination (Parker *et al.* 1989). Seeds in a seed bank may be introduced from the current year's seed rain or from previous years' and are removed from the system through germination, predation, senescence (the loss of viability with age) and pathogens (Simpson *et al.* 1989; Fenner 1995; Solomon *et al.* 2006). Seeds that become buried or cached too deeply in the soil by rodents, birds and ants become ineffective as seed banks (Baker 1989), unless there is a mechanism which allows these seeds to be brought up to more shallow surface layers, such as through the activity of burrowing animals. There are two main factors that may limit the density of seed banks, namely, the amount of seed arriving at the site and the persistence of viable and ungerminated seeds in the soil (Solomon *et al.* 2006). In order for seeds to contribute to regeneration, they need to survive disturbances and environmental fluctuations as well as long-term exposure to predation while retaining viability in the seed bank (Parker *et al.* 1989). Seed banks are composed of seeds of different ages, which means the probability of seed germination will vary temporally as germination stimulants, like scarification, are affected by age (Valleriani and Tielborger 2006). Older seeds appear to respond more readily to germination cues such as water than do recently produced seeds, which creates more opportunities for plant recruitment (Ribas-Fernandez *et al.* 2009) if there are more seeds from past seasons stored in the seed bank.

Generally, the number of seeds in a seed bank does not accurately reflect the number of seedlings that will emerge after germination as the number of seedlings that emerge is only a small portion of the seeds initially present in the soil (Fenner 1995; Witkowski and Wilson 2001). However, knowing the size, species richness and composition of seed banks is important in reflecting the past, current and future state of ecosystems (Solomon *et al.* 2006). Johannesmeier and Van Rooyen (2007) suggest that when fully describing a plant community, buried viable seeds in the seed bank should be included as they represent the community as much as the aboveground components.

1.1.3. Seed dispersal and predation

Seeds form a large portion of the diet of a range of vertebrates including baboons, vervet monkeys, squirrels, dormice, rats and mice (Willson and Traveset 2000; Stuart and Stuart 2001; Vander Wall *et al.* 2005). Research has also documented the widespread removal of seeds by caching and food-storing rodents (Mittelbach and Gross 1984; Vaughton 1998; Ostfeld *et al.* 1997; Lortie *et al.* 2000; Blaney and Kotanen 2001; Jansen *et al.* 2004). Invertebrate-dispersed seeds often offer a reward in the form of a nutritious food body containing sugars, proteins and fats called elaiosomes (Fenner and Thompson 2005). In certain cases, removal of seeds by rodents facilitates the establishment of seedlings, while seeds that are not removed and buried by scatter-hoarding rodents do not establish seedlings (Jansen *et al.* 2004). Consequently, buried seed caches of granivorous rodents can represent a means of seed dispersal and plant propagation for certain species (Geluso 2005). Clarke *et al.* (2007) suggest that the effect of seed predation on plant population density may be influenced by whether a population is seed- or seedling establishment-limited.

The consumption of seeds by ungulates may facilitate seed scarification and lessen the effect of pre-dispersal insect predators such as bruchid beetle predation, as infested pods consumed by ungulates have been shown to germinate better than infested pods not consumed (Miller 1994). The lower incidence of bruchid beetle infestation of seeds found in ungulate dung may be a result of the break-down and mastication of infested seeds (Coe and Coe 1987). If *Acacia* pods are consumed by ungulates before seeds and pods are dispersed, there is a possibility that a significant proportion of the seeds will evade infestation by bruchid beetles (Kriticos *et al.* 1999).

The dispersal of seeds by ants is a form of mutualism called myrmecochory whereby the ant is rewarded with food and the seed is potentially dispersed to a site more favourable to seedling establishment (Wenny 2001; Fenner and Thompson 2005). There are gaps in the

literature regarding seed dispersal by termites, as opposed to ants. A study in a Sudanian woodland savanna shows that termite mounds provide safe sites for the recruitment of woody plants; most abundant on mounds are seedlings regenerated by seeds, while sprouting is more abundant in areas surrounding mounds (Traore *et. al.* 2008). A study on post-dispersal seed predation of *Acacia karroo* Hayne. and *Acacia nilotica* (L.) Willd. Ex in an African savanna showed that ants selected *A. nilotica* over *A. karroo* seeds, possibly due to the sweet and sticky substance secreted by *A. nilotica* pods (Walters *et al.* 2005). Additionally evidence of termite and ant activity in the Nylsvley Provincial Nature Reserve has been ascribed to the burial of *A. nilotica* and *Acacia tortilis* Hayne pods beneath the soil surface by these invertebrates (Miller 1994).

Seeds may be dispersed further from the parent tree by termites, ants (Dean and Yeaton 1992) or caching rodents (Vander Wall *et al.* 2005) and dispersal of large tree seeds by rodents and birds can be very effective (Fenner and Thompson 2005). Seed fate can also be affected by pre-dispersal predation by insects such as bruchid beetles, as well as post-dispersal predation by rodents and other granivores (Coe and Coe 1987; Stuart and Stuart 2001; Vander Wall *et al.* 2005). Wind and vertebrates often disperse seeds further from the parent plant, while invertebrates such as ants typically disperse within shorter distances (Willson and Traveset 2000).

1.1.4. Savanna Acacia tree species

Savanna ecosystems have a net water-deficit for most of the year and are closely associated with frequent burning (Scholes and Walker 2004). Savannas in southern Africa experience highly variable rainfall patterns and intra-seasonal droughts are common (Smith and Shackleton 1988). *Acacia* trees are widespread and play significant roles in savanna ecosystems (Goheen *et al.* 2004), where almost all *Acacia* species provide a valuable source of browsing material for a range of herbivores (Coe and Coe 1987), particularly during the dry season when grassland pasture is deficient in these nutrients (Savadogo and Elfving 2007). *Acacia* tree bark is also frequently sought after by elephants during droughts or dry periods, the cause of which is the death of large numbers of mature trees as elephants also tend to push trees over to reach foliage and pods (Coe and Coe 1987).

Midgley and Bond (2001) discuss the demographics of *Acacias* (Figure 2) and ask pertinent questions such as whether *Acacia* recruitment is dependent on resprouting or inputs from seeds. Most *Acacias* can resprout after fire and browsing (Bond 2008) and the ability to resprout in savanna tree species is essential to persistence in environments where seedling recruitment may be limited (Bond and Midgley 2001). The Nylsvley Provincial Nature

Reserve (a savanna region) has experienced summer drought conditions for more than half its recorded history. These conditions are described by Frost (1987) as one or more months between December and February where the area receives less than 50 mm of rain. It is likely that conditions for germination and successful seedling establishment are met during above average rainfall periods, where cohorts of seedlings are established, with long periods of time where no or very few seedlings establish (Wilson and Witkowski 1998). Seed production is an important regeneration mechanism in South African *Acacia* tree species such as *A. karroo* and *A. nilotica* (Munkert 2009).

There have been a number of studies conducted on soil seed banks of African savanna *Acacia* species (see Argaw *et al.* 1999; Witkowski and Garner 2000; Loth *et al.* 2005). Witkowski and Garner (2000) found that the savanna shrubs/trees, *Dichrostachys cinerea, A. nilotica* and *A. tortilis* form at least short-term persistent seed banks. In a study of seed demography in an Ethiopian *Acacia* woodland, Argaw *et al.* (1999) found associated soil seed banks for both *A. senegal* Brenan and *A. tortilis*, although *A. senegal* seed banks were relatively small, which was attributed to high pre-dispersal seed predation and seed abortion. However, there are definite gaps in the literature with regards to soil seed banks in the Kruger National Park (KNP). Botha (2006) conducted a preliminary investigation of the seed banks of two savanna tree species, *Acacia nigrescens* Oliv. and *Philenoptera violacea*,(Klotzsch) Schrire in the KNP but found no evidence of seed banks for either species. It is possible that this is a result of a poor seed crop subsequent to poor rains at the start of the rainy season (Botha 2006).

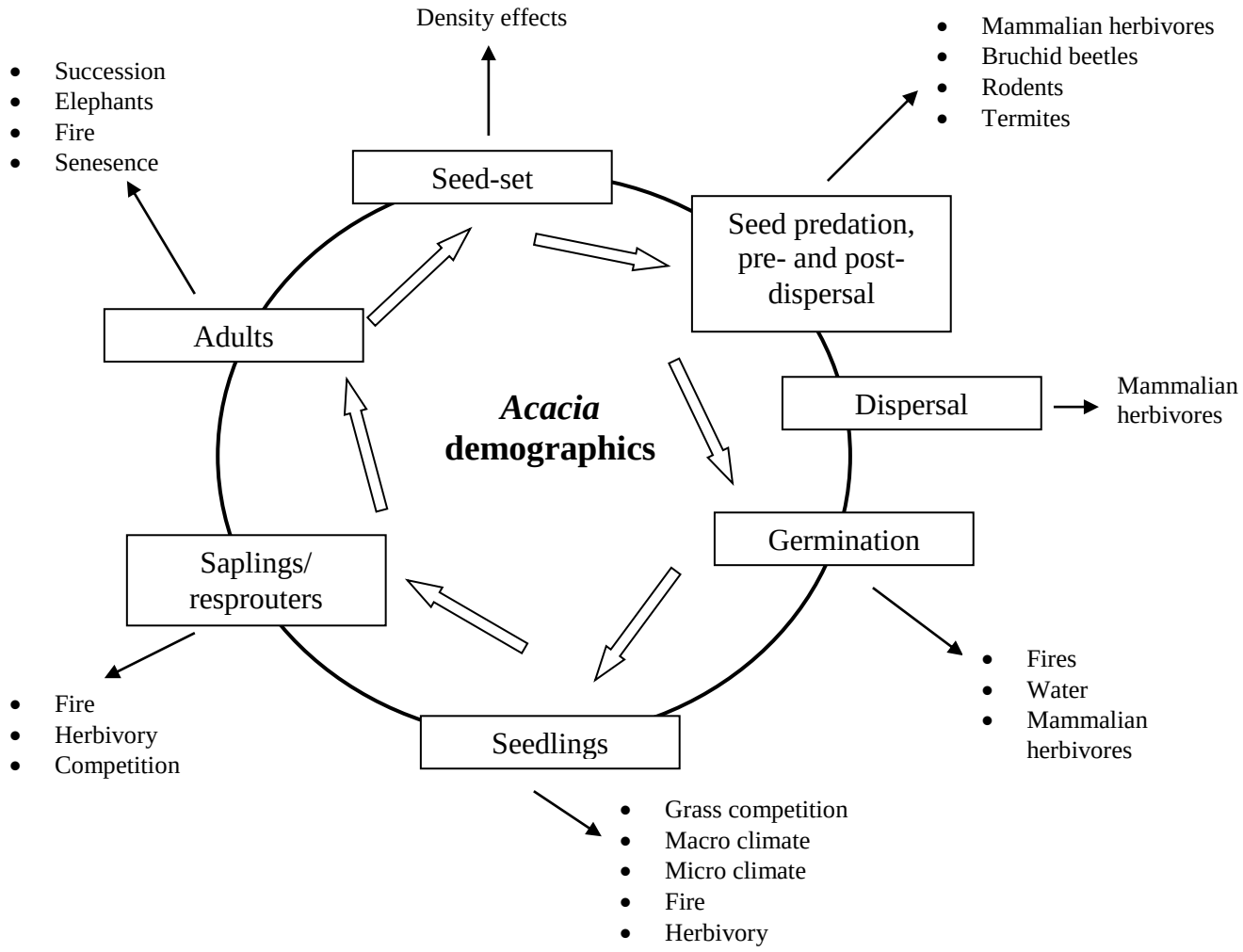

Figure 2. Demographic barriers occurring in African *Acacia* species (adapted from Midgley and Bond 2001).

1.2. Rationale

The KNP is situated in the low-lying savannas of South Africa and is one of the largest and most intensely managed savanna areas in Africa (Eckhardt *et al.* 2000). A recent assessment of woody plant cover and density showed an overall increase in woody plant density and cover on the granite substrates of the KNP over 58 years, which is attributed to a decrease in competition from grasses. This, in turn, is thought to be a result of overgrazing by herbivores, the numbers of which are kept high possibly as a result of the provision of surface water (Eckhardt *et al.* 2000). The process of an increase in woody plant density is known as bush encroachment (Wiegand *et al.* 2006). Generally, tree and shrub densities increase in savanna ecosystems due to overgrazing, fire suppression and CO_2 fertilization (Bond 2008). An increase in tree density at the landscape scale can have a negative effect by depleting the nutrients available to grasses, which compromises the nutrition available to wild ungulates (Riginos *et al.* 2009).

Grass production can also decline dramatically with an increase in woody plant

density (Scholes and Archer 1997). While grasses on basalt substrates can recover more effectively from increased grazing pressure because of the abundance of nutrients in basalt-derived soils, granite substrates are nutrient-poor (Eckhardt *et al.* 2000). Although bush encroachment is an obviously serious problem in African savannas, it is still poorly understood (Midgley and Bond 2001). *Acacia* species such as *A. nilotica* and *A. karroo* are often responsible for bush encroachment and thicket formation in African savannas (Garner and Witkowski 1997; Kriticos *et al.* 1999; Radford *et al.* 2001; Munkert 2009), as are other savanna woody plant species such as *Dichrostachys cinerea* (L.) Wight & Arn. (Stuart-Hill and Tainton 1999).

The persistence of tree populations is dependent on the storage of successful recruitment events between generations, which will allow strong recruitment opportunities when conditions are favourable (Bond and Midgley 2001). For example, plants in fire-prone environments will protect their seeds from fire by storing them underground or in fire-proof seed 'heads' (Fornara 2005). Long-term persistent seed banks (seeds persist in the soil for longer than 5 years) are generally formed in ecosystems that only irregularly allow successful seedling establishment (Wagner and Mitschunas 2008). Soil seed banks enable seeds to be stored in the soil for extended periods of time until conditions are most suitable for seed germination as well as seedling establishment (Crawley 1990). Burial in a seed bank offers protection from disturbances which may kill seeds or reduce their viability such as extreme temperature fluctuations, granivory or seed consumption (Thompson *et al.* 1993). Soil seed banks of *Acacias* are a highly complex subject (Tybirk *et al.* 1992). There are gaps in the understanding of seed bank dynamics in the savanna biome, and filling those gaps may provide an explanation in part to the recruitment process in bush-encroaching tree and shrub species (Garner and Witkowski 1997).

Understanding the consequences of changes in tree density on grasses, and other ecosystem services such as maintenance of wild ungulate populations, is critical to management decisions in conserved areas (Smit 2004; Riginos *et al.* 2009). Thereby, sexual reproduction in species with the potential to be encroachers is an important consideration in the management of bush encroachment in southern African savannas (Munkert 2009). The KNP management has set 'thresholds of potential concern' in savanna management, which if reached, activate an assessment of the causes of concern in the ecosystem and initiate corrective action (Eckhardt *et al.* 2000). Thresholds of potential concern represent hypotheses of the limits in acceptable change in the structure, function and composition of an ecosystem (Rogers 2003). Analysing the density and distribution of *in situ* soil seed banks and assessing *Acacia* seed fate will assist KNP management in further understanding the

regeneration of savanna trees and the general increase in tree and shrub density and cover in certain areas of the KNP.

1.3. Research aim and objectives

The main aim of this study was to assess the density and spatial distribution of *in situ* soil seed banks of four *Acacia* species in the Kruger National Park and analyse processes which may affect soil seed bank characteristics in each species. To understand the processes which affect seed fate and the formation of soil seed banks, the following objectives were developed:

1. To quantify the spatial distribution of seeds in *in situ* soil seed banks of four *Acacia* species and analyse various seed, pod, tree and micro-site characteristics of each species and determine how these characteristics may affect seed bank density.
2. To compare the results of field and greenhouse seed burial trials to further understand the dormancy, persistence, germination and viability of seeds of four *Acacia* species in soil seed banks over various periods of time and environmental conditions.
3. To investigate the role that large herbivores, termites and rodents play as seed predators and dispersers of various savanna woody plant species, as well as their impact on seed fate.

1.4. Dissertation structure

The study has been written up as five chapters, with the intent to publish Chapters 2 - 4 as separate journal papers (Figure 3). Consequently, certain details may be referred to more than once throughout the dissertation, although this is kept to a minimum. The first chapter provides an overall introduction to the subject of the study and topics discussed include general seed ecology such as soil seed bank dynamics, seed dispersal and seed limitation, as well as a brief description of the environmental characteristics of the KNP. The introduction also includes the rationale for the study and lists the major aims and objectives. The second chapter investigates the spatial and temporal distribution and density of *in situ* soil seed banks for four *Acacia* species in the KNP, as well as characteristics of seeds, pods, parent-trees and micro-sites which may affect density and distribution. The third chapter looks more closely at processes that affect seeds such as dormancy, persistence and viability in the soil of seeds of the same four *Acacia* species. Also investigated are the effects of exposure to natural environmental conditions and simulated rainfall patterns over extended periods of

time. The fourth chapter analyses the role of potential seed predators and dispersers such as termites as well as small and large mammalian herbivores on seed germinability and viability, at the same time investigating the associated effects of woody plant density and diversity. Also included in this chapter is a study of rodent seed preference and selection of four savanna woody plant seeds, as well as the effect of vegetation cover on rodent seed selection. The growth of *Acacia* trees on termite mounds is briefly discussed. The dissertation is concluded by a brief chapter (Chapter 5) that summarises and discusses the main results obtained, as well as providing recommendations and suggestions for KNP management regarding future studies and management.

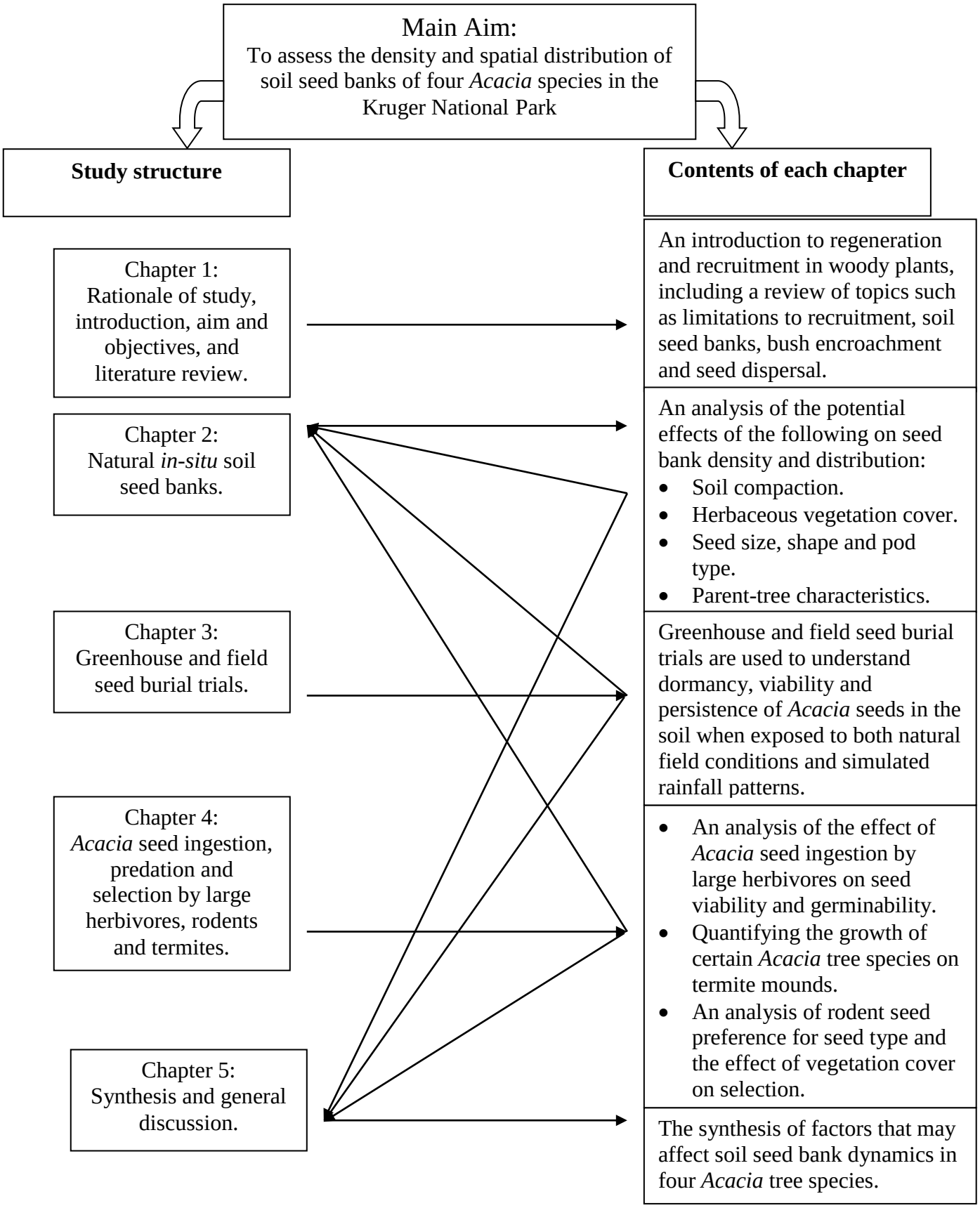

Figure 3. The overall structure of the MSc dissertation.

1.5. Study region and regional context

The KNP (Figure 4) spans the low-lying savannas of the Northern and Mpumalanga Provinces in South Africa and is situated in the Lowveld, bordering Mozambique in the east and Zimbabwe in the north (Eckhardt *et al.* 2000; Mabunda *et al.* 2003). High-density communal areas and private and provincial game reserves form the western border of the KNP (Mabunda *et al.* 2003). Natural boundaries to the south, north and east are formed by the Crocodile River, the Luvuvhu and Limpopo Rivers and the Lebombo Hills, respectively (Mabunda *et al.* 2003).

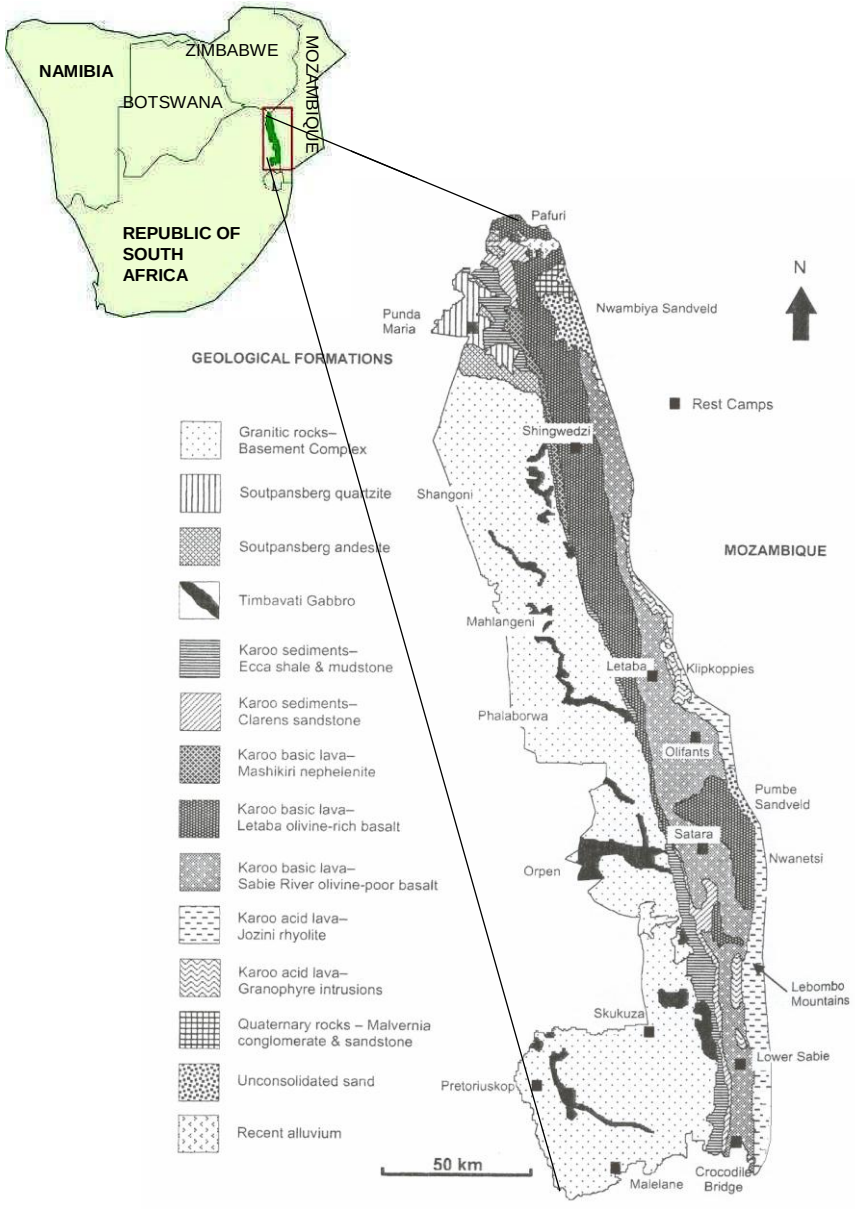

Figure 4. The geographical location of the Kruger National Park in southern Africa, as well as the geology of the area (adapted from Botha 2006).

1.5.1. Topography and drainage patterns

The KNP is comprised of a range of landform types, from the low mountains and hills in the north-west and south-west, and the Lebombo Hills bordering Mozambique, to the low-lying plains covering the western half of the park (Venter *et al.* 2003). The eastern half of the KNP has slight to moderately undulating slopes, separated at the mid-point by extremely irregular areas at the Olifants River Rugged Veld (Venter *et al.* 2003). The geology of the KNP is made up of a combination of granite gneiss, schists, amphibolites, sediments, basalts, rhyolites and gabbros (Schutte 1986). The KNP can loosely be divided down its middle based on basic geology (Figure 4 and 5). The geological sequence changes from west to east (Venter *et al.* 2003): the western parts of the Park are dominated by underlying granitic features from the Basement Complex, while the east comprises level plains underlain by basalt (Karoo basic lava) (Mabunda *et al.* 2003; Venter *et al.* 2003). The granite and basalt bands are separated through the middle by a thin north-south strip of sedimentary rock (Venter *et al.* 2003) (Figure 5). With a decrease in rainfall towards the north of the KNP, soils become shallower and soil type diversity decreases. Granitic soils in the south of the Park can be up to five times deeper than granitic soils in the north (Venter *et al.* 2003).

The KNP is drained from the west to east and forms a pediplain towards the sea (Venter and Bristow 1986), which follows the east-west altitude gradient (Mabunda *et al.* 2003). Six large perennial rivers form the main drainage systems, namely the Limpopo, Luvuvhu, Letaba and Olifants Rivers in the north and the Sabie and Crocodile Rivers in the south (Venter and Bristow 1986; Eckhardt *et al.* 2000). The majority of smaller drainage channels in the KNP are ephemeral and only run during the rainy season (Venter and Bristow 1986), although larger seasonal rivers form pools that provide a constant supply of water through most years (Mabunda *et al.* 2003). The density of streams in the eastern basaltic areas is up to four times less than the density of streams in the western granitic areas (Venter and Bristow 1986).

1.5.2. Climate and weather patterns

The southern half of the KNP falls into a temperate climatic zone, while the north is sub-tropical to tropical (Mabunda *et al.* 2003). As a result of this, temperatures rarely reach freezing point in winter and often exceed 35°C in the summer months (Venter and Gertenbach 1986). Frost rarely occurs (Mabunda *et al.* 2003).

The KNP experiences summer rainfall, which occurs from November to March, with an average rainfall of 440 mm annum^{-1} in the north-east (Pafuri) and 740 mm annum^{-1} in the

south-west (Pretoriuskop); there is a decrease in mean annual rainfall from south to north and east to west (Venter *et al.* 2003). There is a rainfall peak in January and February and little rain between the winter months of June to August (Venter and Gertenbach 1986). Humidity varies from 50-53% in the summer months to 37-42% in winter (Venter *et al.* 2003).

1.5.3. Fauna and flora

The KNP falls into the savanna biome and has a wide range of different mammals, birds, reptiles, fish, amphibians, insects and plants (Venter *et al.* 2003). Approximately 1 968 plant species occur in the KNP, comprising 457 shrubs and trees, 235 grasses, 27 ferns, 16 woody lianas, 20 aloes, and 1213 forbs (Venter and Gertenbach 1986). There is a strong correlation between vegetation and soil type in the KNP, with a number of plants considered as indicator species of specific soil conditions (Venter and Gertenbach 1986; Mabunda *et al.* 2003). None of the four main study species to be analysed in this study (*Acacia grandicornuta* Gerstner, *A. nilotica*, *A. senegal* and *A. tortilis*) are mentioned as indicator species of soil conditions by Venter *et al.* (2003). The KNP is divided into land systems defined by the association between geology, terrain, morphology, soils and woody vegetation, and the Skukuza land system, which is where the study site was situated, is defined by *Combretum* spp., *Terminalia* spp., *Acacia gerrardii* Benth and *Acacia nigrescens* trees (Venter *et al.* 2003). The savannas of the KNP are loosely divided into infertile and fertile areas based on soil properties, where broad-leaved savannas (trees with no thorns and broad leaves) occupy up to 75% of the KNP and are generally found on granite or sandstone derived soils (Venter *et al.* 2003). The remainder of the KNP is occupied mainly by fine-leaved savannas, which are usually associated with fertile clay soils and have deciduous trees with thorns and fine, compound leaves (Venter *et al.* 2003).

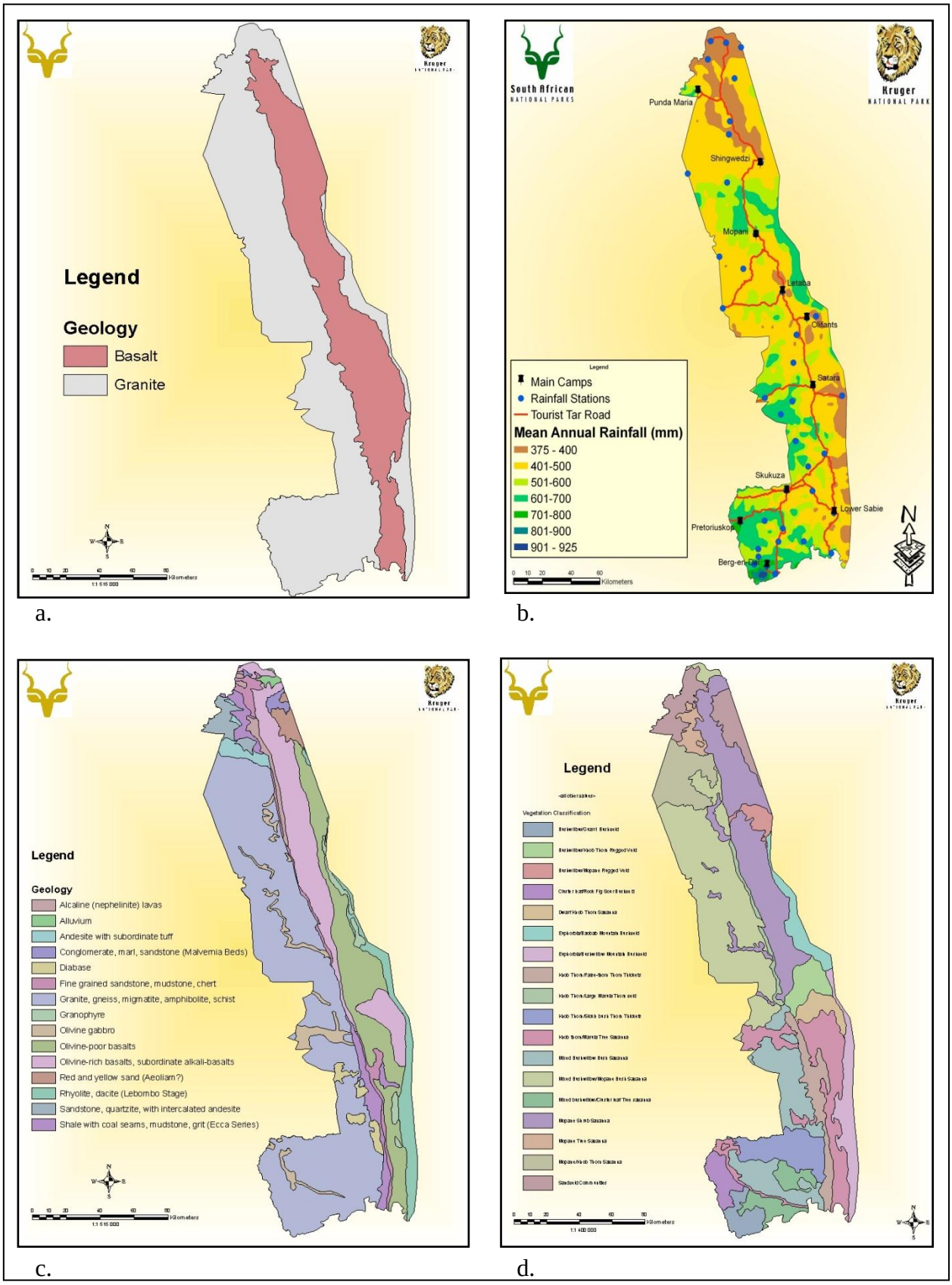

Figure 5 a-d) Environmental systems of the Kruger National Park: a) basic geology, b) annual rainfall, c) geology, and d) land systems.

(http://www.sanparks.org/parks/kruger/conservation/scientific/gis/default.php**).**

1.5.4. Study species

Four *Acacia* study species are analysed in Chapters 2 and 3, namely *Acacia grandicornuta* Gerstner (horned thorn), *A. nilotica* (L.) Willd. ex Delile subsp. *kraussianna* (Benth.) Brenan (scented-pod thorn), *A. senegal* (L.) Willd. var. *rostrata* Brenan (bushy three-hook thorn) and *A. tortilis* (Forssk.) Hayne subsp. *heterocantha* (Burch.) Brenan (umbrella thorn) (see Schmidt *et al.* 2002). In Chapter 4, the seeds of *Acacia nigrescens* Oliv. (knob thorn) and *Dichrostachys cinerea* (L.) Wight & Arn. subsp. *africana* Brenan & Brummitt (small-leaved sickle-bush) are used in the rodent seed preference trial to complement a PhD study running simultaneously on these species.

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CHAPTER 2

Soil seed bank ecology of four selected savanna *Acacia* tree species in the Kruger National Park, South Africa

Abstract

There has been an increase in the density of large woody plants on granite substrates over 58 years in the Kruger National Park (KNP), South Africa. Seed production by trees in the KNP varies between years and sites, and tree recruitment into a population is often episodic. The contribution of soil seed banks to *Acacia* tree regeneration in this southern African savanna is poorly understood. An investigation was done on soil seed bank density of four *Acacia* species in the KNP during 2005/2006.

There were no significant differences in soil seed bank density between micro-sites for *A. grandicornuta*, *A. nilotica* or *A. senegal*. Seed bank density of micro-sites under canopies of associated *A. tortilis* trees was significantly higher than any of the other micro-sites ($P < 0.05$). There were no significant differences in seed density with depth in the soil for any of the species. Generally, however, seed bank density decreased with depth in soil and distance from the centre of the tree canopy. There was a significant difference in overall seed bank density between species ($P < 0.05$), where *A. tortilis* had the most dense seed banks (19.5 ± 6.4 seeds m-^{2}), followed by *A. grandicornuta* (12.1 ± 6.9 seeds m^{-2}), *A. nilotica* (4.9 ± 1.8 seeds m^{-2}) and *A. senegal* (0.6 ± 0.4 seeds m^{-2}).

Seed bank density increased significantly with a decrease in soil compaction for *A. senegal* ($P = 0.01$) but not for the other species. Seed bank density was not related to herbaceous biomass or over-storey canopy shading for any of the species. Seed bank density increased significantly with tree height for *A. senegal* ($P = 0.001$) and *A. tortilis* ($P = 0.02$) and with tree canopy volume for *A. nilotica* ($P = 0.02$). No other significant relationships were found between seed bank density and other associated tree characteristics such as stem diameter, bark thickness and tree canopy area for any of the species.

A. tortilis seeds had the smallest mass (0.03 ± 0.001 g), followed by *A. nilotica* (0.04 ± 0.007 g), *A. grandicornuta* (0.06 ± 0.003 g) and *A. senegal* (0.11 ± 0.036 g). The most spherical seeds were *A. nilotica* (0.04 ± 0.006), followed by *A. tortilis* (0.07 ± 0.002), *A. grandicornuta* (0.12 ± 0.004) and *A. senegal* (0.21 ± 0.015). Viability of seed bank seeds decreased between species as follows: *A. tortilis (*11% of 142 seeds), *A. nilotica* (61% of 36 seeds), *A. grandicornuta* (58% of 87 seeds) and *A. senegal* (0% of 4 seeds). A larger percentage of viable *A. nilotica* and *A. grandicornuta* seeds were found in the leaf litter (68 and 62%, respectively) than in the other two soil depth categories, while more viable

A. tortilis seeds were found in the 0-2 cm soil depth category (59%) than in the leaf litter (32%) and 2-5 cm soil depth category (9%). For all three species, seed viability decreased with distance from the centre of the tree canopy.

Bruchid beetle predation, assessed on 100 newly produced seeds per species, was low, ranging between species as follows: *A. nilotica* (15.8%), *A. senegal* (5.5%), *A. grandicornuta* (2.5%) and *A. tortilis* (2.4%).

This study showed that the seeds of three species, namely *A. grandicornuta, A. nilotica* and *A. tortilis* are able to persist in the soil for at least 8 months (and possibly longer). Therefore seeds of these species can initially be classified as short-term persistent (seeds persist in the soil for between one and five years), which is consistent with seed banks studies undertaken in the NPNR. A field-based longevity study must be undertaken to substantiate these results. Seed density varied between species, as did seed size and shape and viability. No viable *A. senegal* seeds were found in the soil, where seeds were at least 8 months old, so this species does not form persistent seed banks. This is the first study of *in situ* soil seed banks of *Acacia* trees in the KNP. As seed banks play an important role in *Acacia* population recruitment, understanding seed bank dynamics can assist managers in determining conservation strategies in the KNP, especially as *Acacia* trees are often responsible for an increase in thicket formation and bush encroachment.

Keywords: *in situ* soil seed banks, Kruger National Park, *Acacia,* persistence, seed fate.

2.2 Introduction

Over thirty years ago Thompson and Grime (1979) stated that although studies had been conducted on soil seed banks, little was known about the processes affecting both seed entry into and removal from the soil. Further, Garwood (1989) noted that the spatial and temporal distribution of buried seeds was poorly understood. Witkowski and Garner (2000) mentioned the gaps in knowledge of the role of soil seed banks in bush encroachment in southern African savannas, while through the years a number of other authors have noted the lack of understanding of general soil seed bank processes in various plant species (see Kalisz and McPeek 1992; Auld 1995; Garner and Witkowski 1997; Radford *et al.* 2001). A soil seed bank is all the viable seeds found in and on the soil or in associated leaf litter (Simpson *et al.* 1989) and consists of ungerminated seeds that have the potential to replace adult plants in a population (Baker 1989). The presence of a seed bank is often an essential stage in a plant's life history and can have an effect on both the resilience and resistance of vegetation to environmental disturbances (Pugnaire and Lazaro 2000). The accumulation of viable seeds

in the soil allows recruitment of plants into a population even in the absence of a productive seed year, thereby decreasing any effects of seed limitation in that species. Seed limitation indicates that plant recruitment is limited by the availability of seeds (Clark *et al.* 2007). Maron and Gardner (2000) suggest that recruitment into plant populations that have abundant seed banks is limited by safe-site availability rather than absolute seed abundance. A safe site is a site suitable for successful seedling establishment (Green 1983). A study on *Acacia tortilis* recruitment in Kenya showed a poor correlation between seed banks and seedling recruitment, suggesting that recruitment in this species is not seed-limited but is rather limited by successful seedling establishment (Stave *et al.* 2006).

A number of authors have explored the impacts, both negative and positive, of soil seed banks on plant populations. Seed banks provide a species with the opportunity to delay germination until conditions are favourable for successful seedling establishment. If all seeds germinated together under unfavourable conditions there would be a greater chance of the species becoming locally extinct (Valleriani and Tielborger 2006). The impression of 'pulsed' or cohorted recruitment in *Acacia* species in arid environments may be a result of environmental conditions such as good rainfall (Pucheta *et al.* 2006). A mass release of seeds may result in a cohort of seedling establishment (Seymour 2008). In an analysis of seed limitation and a critique on seed addition experiments, Clark *et al.* (2007) found that seed bank longevity, seed size, seed origin and disturbance affect seed limitation in plant populations. Kalisz and McPeek (1992) suggest that a seed bank may destabilize a population if the rate of seeds entering the seed bank exceeds the rate of germination from the seed bank (in species relying on seed banks as their main source of regeneration). On the other hand, Crawley (1990) noted that long-term persistent seed banks can provide stabilized protection for plant populations.

The burial rate of seeds in the soil and their horizontal and vertical distribution can be affected by factors at different spatial and temporal scales. These factors include soil texture, structure, compaction, water content and deposition, seed size and shape, seed longevity, animal and insect activity, and other local environmental conditions (Parker *et al.* 1989; Guo *et al.* 1998). Accordingly, both temporal and spatial variation in micro-site characteristics can considerably influence the probability of seeds entering, remaining in, and emerging from a seed bank (Kalisz 1991). If the soil beneath the parent-tree canopy is hard and compact, seeds may not be able to easily penetrate the soil surface. Soil compactness can be affected by temperature, rainfall, light and soil origin, i.e. the parent rock. If micro-site characteristics do indeed affect seed distribution in the soil, then seeds from plants growing in semi-arid regions will be clustered, as noted by Page *et al.* (2006), in small patches according to specific environmental conditions. In arid environments, wind-dispersed seeds, nutrients, litter and soil often accumulate under widely-spaced patches of vegetation, which creates heterogeneous environments (Lamont *et al.* 1993; Pucheta *et al.* 2006) and affects the spatial distribution of seeds in a seed bank.

Besides local environmental influences, seed characteristics of a species, such as size and shape as well as pod type may influence seed bank dynamics. There are two main types of *Acacia* pods, classified according to the method by which the seeds are dispersed (see Coe and Coe 1987). In dehiscent pods the fruit's pericarp splits and seeds are dispersed by wind or gravity, while indehiscent seeds are not released from the pericarp: the whole fruit along with the seeds remains intact and on the tree (or on the ground) until browsed or removed (Bradbeer 1988; Miller 1993). Seeds from indehiscent pods such as *Acacia tortilis* and *A. nilotica* are typically ungulate-dispersed, while seeds from dehiscent pods such as *A. senegal* are mostly wind-dispersed (Tybirk *et al.* 1992). *A. grandicornuta* produces dehiscent pods and consequently seeds should also be wind-dispersed (*pers. obs*). African *Acacia* pods are an important source of food to ungulates during the dry seasons and intense browsing pressure by elephants has been observed on *A. nigrescens* trees in the KNP (Coe and Coe 1987). The pods of *A. nilotica* give off a strong sweet scent believed to attract herbivores to facilitate seed dispersal (E.T.F. Witkowski, *pers. comm.* 2005).

Bruchid beetles also use *Acacia* seeds as a food source (Ernst *et al.* 1990). The female beetle lays an egg on a ripening pod, whereby on hatching, the larvae will bore through the pod surface and into a developing seed. Here it will enlarge a chamber in the seed and eat away at the cotyledon and epidermal layer as it grows. Once the larvae has pupated and emerged as an adult, it will leave the seed through a window in the testa

(Southgate 1978). The seed is then exposed to bacteria and fungi, as well as loss of moisture through the hole. However, it has been suggested that so long as the beetles do not destroy the embryo of the seed there is a chance the seed will remain intact and the hole created by the beetle may even facilitate germination by allowing the seed to imbibe water (Lamprey *et al.* 1974).

Seed germination does not take place within a fruit or pod. A seed will only germinate once it has been dispersed and the pod has been decomposed, broken open, damaged or eaten by animals (Mayer and Poljakoff-Mayber 1975). Species producing indehiscent pods benefit from ungulate dispersal, as the seed is often removed from the vicinity of the parent plant and the pod is 'opened' to release the seeds (Mayer and Poljakoff-Mayber 1975). Some legumes produce indehiscent fruit as a dispersal unit and to postpone germination (Kigel 1995). Seeds also require scarification before they germinate. Scarification, or breakdown of the seed coat, naturally occurs when seed coverings become 'damaged' and germination is initiated (Bradbeer 1988). Natural scarification of seeds in the soil is a slow process that prevents the detrimental effect of mass germination in arid and semi-arid areas and allows, instead, germination at irregular intervals and in small batches (Coe and Coe 1987; Wilson and Witkowski 1998). Scarification is facilitated by wind, rain, trampling by animals and bacterial action, with fire playing an important role in making seeds of certain savanna *Acacia* species, such as *A. tortilis*, more permeable to water (Coe and Coe 1987).

Unlike the indehiscent pods of *A. nilotica* and *A. tortilis*, species with dehiscent pods such as *A. senegal* (Coe and Coe 1987) and *A. grandicornuta* (*pers. obs.*) often release seeds directly onto the ground. The seeds are usually initially deposited below and around the parent tree and if not further dispersed (secondary dispersal), seed banks are likely to be strongly linked to parent trees in terms of genetics and spatial distribution (Witkowski and Garner 2000).

Is it more beneficial to a species to produce many smaller seeds, or fewer larger seeds? Haig and Westoby (1988) discuss the theory of seed size to seed number trade-offs. Producing a larger number of smaller seeds increases the chance of a single seed's survival. Producing fewer, larger seeds means that each seed is at a higher risk of seed attrition. However, larger seeds have a larger energy store and it has been shown by Saverimuttu and Westoby (1996) that cotyledon-stage seedlings of larger-seeded species persist for longer in deep shade. Bond *et al.* (1999) developed a model to predict maximum seedling emergence depth and found that seed size was allometrically related to

maximum emergence depth. In other words, a significantly larger number of bigger seeds emerged from deeper in the soil than smaller seeds. Nonetheless, larger seeds may not percolate into the soil as easily as smaller, more compact seeds (Thompson *et al.* 1993). With reference to seed bank formation, this means that producing larger numbers of smaller seeds may be more beneficial than producing fewer, larger seeds.

Seed shape may also affect the chance of a seed entering the seed bank and there are a number of ways to quantify seed shape. The Corey Shape Factor (CSF) (Corey 1949) reflects the flatness of a seed. A CSF value of 0 represents a perfectly flat seed, while a value of 1 represents a perfect sphere (Chambert 2006). Thompson *et al.* (1993) describe a measure of sphericity that is expressed by the variance of the transformation of seed length, width and depth so that length is unity. Here a spherical seed will have a variance of 0 while needle or disc-shaped seeds have a variance of 0.3. Smaller, more spherical seeds may be able to penetrate cracks, tunnels and holes in the soil more easily than larger and flatter seeds (Thompson *et al.* 1993) and as a result may theoretically form more dense soil seed banks.

As mentioned by Hill and Vander Kloet (2005), seed bank density in general, is a function of seed longevity, seed rain and soil conditions. What effects then, if any, do the characteristics of parent-trees or trees associated with a soil seed bank have on seed bank density? While various studies have focused on the factors affecting seed bank density mentioned by Parker *et al.* (1989) above, not many studies have looked at the effect of parent-tree characteristics on seed bank density. However, Witkowski and Garner (2000) showed that the size of soil seed banks of three savanna woody species, specifically *A. tortilis*, *A. nilotica* and *Dichrostachys cinerea*, was positively related to 'parent' tree size. In contrast, in a study of desert shrubs, Guo *et al.* (1998) found that seed bank densities under shrubs did not relate to the size of the shrub canopy. There is a large gap in the literature regarding soil seed banks of dehiscent species such as *A. senegal* and *A. grandicornuta.*

The main aim of this study was to investigate the soil seed bank density of four *Acacia* species in the KNP and the influence and relationship of various ecological factors, such as tree, micro-site and seed characteristics on and to seed bank density. The following objectives were developed to fulfill the aim of this study:

1. To investigate whether pre-dispersal bruchid beetle predation, seed size and shape as well as pod characteristics (dehiscent vs. indehiscent) of each *Acacia* species relate to soil seed bank density.

2. To investigate whether there is a relationship between tree characteristics such as stem diameter, canopy area and volume, bark thickness and tree height and soil seed bank density.
3. To investigate whether micro-site characteristics such as soil compaction, over-storey canopy shading and herbaceous biomass influence soil seed bank density.

2.3. Materials and Methods

2.3.1. Study region and site selection

Soil seed bank samples were taken in various sites within a ± 15 km radius of Skukuza in the Kruger National Park (KNP). The study region falls under the Skukuza land system, which makes up 19.6% of the KNP's total area and covers 382 045 ha (Venter *et al.* 2003). The Skukuza land system is slightly undulating plains underlain by granitic rock, with sandy soils in the uplands and duplex sodic clay soils in the bottomlands. It receives 500-750 mm of rainfall annually. The vegetation of this land system is broad-leaved bushveld in the uplands or catenal crests, dominated by moderately dense *Combretum apiculatum* Sond. subsp. *apiculatum* (red bushwillow) and *Combretum zeyheri* Sond. (large-fruited bushwillow) bushveld. Bottomlands or valleys are fine-leaved savannas with open *Acacia gerrardii* Benth. var. gerrardii (red thorn) and *Euclea divinorum* Hiern (magic guarri) shrubveld with prominent *Combretum hereoense* Schinz (russet bushwillow) and *Acacia nigrescens* Oliv. (knob thorn) vegetation (Venter *et al.* 2003).

Sites within the region were selected based on the presence of dominant stands/areas of various 'fruit-producing' *Acacia* species, as potential study species. Each study site was defined as an area where a particular *Acacia* species is prevalent and where a cluster of trees was at least 250 metres from the next cluster, in order to ensure that tree clusters were separate from one another. It was assumed for the purposes of the study, that areas with an abundance of one type of *Acacia* species were indicator sites in terms of soil type, climate, vegetation and other environmental characteristics, biotic and abiotic, of these species.

Within these selected sites, six different *Acacia* species were chosen as potential study species, namely *A. exuvialis* Verdoorn (flaky thorn), *A. grandicornuta* Gerstn. (horned thorn), *A. nilotica* (L.) Willd. ex Del. subsp. *kraussianna* (Benth.) Brenan

(scented-pod thorn), *A. senegal* (L.) Willd. var. *rostrata* Brenan (bushy three-hook thorn), *A. tortilis* (Forssk.) Hayne subsp. *heterocantha* (Burch.) Brenan (umbrella thorn) and *A. welwitschii* Oliv. subsp. *delagoensis* (Harms) J. H. Ross & Brenan (delagoa thorn). Although prevalent in this region, *A. nigrescens* and *Dichrostachys cinerea* (Mimosoideae) were already under study (B. Wilson, PhD study) and hence excluded.

During July 2005 mature pods were sampled from the six *Acacia* species for a pilot study on seed size, shape and pod characteristics. From a basic analysis of these characteristics of the six species, four were selected as study species, namely *A. grandicornuta, A. nilotica, A. senegal* and *A. tortilis.* Generally *A. nilotica, A. tortilis* and *A. senegal* trees co-occur naturally, while *A. grandicornuta* trees occur in large, dense stands and is the dominant tree species in the stands (*pers. obs.*). The species were selected because two of them produce indehiscent pods with different seed size and shape from each other (*A. nilotica* and *A. tortilis*) and two produce dehiscent pods (*A. grandicornuta* and *A. senegal*), again differing in seed size and shape from one another. It was also noticed on closer investigation during the pilot study that *A. welwitschii* trees only occurred in sparse isolated patches, while the seeds of *A. exuvialis* were similar in size and shape to the seeds of the more abundant *A. tortilis.*

2.3.2. Pre-dispersal bruchid beetle predation and seed characteristics

Pods from the four study species were collected from an average of three trees per species in the pilot study of July 2005. Between 200 and 1000 pods were collected per species, depending on the number of accessible pods in the canopy and once pods were de-seeded over 1000 seeds were collected per species. From this, one hundred seeds of each species were randomly selected and categorised as intact (large and swollen with no indications of bruchid beetle predation), predated (seeds with bruchid beetle exit holes and with the inside partially or completely eaten) or aborted (seeds that are flatter, less plump, small and shrivelled in comparison to intact seeds) (see Wilson and Witkowski 2003). Twenty intact seeds per species were further selected. Mass, volume, density, sphericity (see Thompson *et al.* 1993; Fenner and Thompson 2005) and Corey Shape Factor (CSF) (Corey 1949) were determined for all species from the intact air-dried seeds. Seeds were weighted on a Precisa Instruments AG 92SM-202A balance (Dietikon, Switzerland) to a precision of 0.0001 g, and measured with a set of steel calipers i.e. length (mm), width (mm) and depth (mm).Volume (V) was calculated using the formula $V = 4/3\ \Pi r^3$ and density (D) with the formula D = mass/volume. The seeds were also tested for viability using a 1% tetrazolium salt staining solution (2,3,5-triphenyl-tetrazolium chloride; Moore

1985; Mbalo and Witkowski 1997). Seeds only partially stained were assumed to have low viability and were recorded as non-viable (Mbalo and Witkowski 1997).

2.3.2.1. Data and statistical analyses

One-way ANOVAs and post-hoc Tukey Studentized Range tests were used to compare seed characteristics between the study species (α, $P = 0.05$). One-way ANOVAs denote overall significant differences in characteristics between species and the post-hoc Tukey test (denoted by superscript letters) specifies which species are significantly different. Seed characteristics were also regressed against one another using simple linear regressions. Data are mostly presented as means ± standard errors (SE).

2.3.3. Soil sampling for soil seed bank analyses

From the study region, eight trees per species were selected for soil seed bank sampling based on tree size, maturity and pod production. Only trees considered reproductively mature (*A. grandicornuta, A. nilotica* and *A. tortilis* trees that were greater than 2 metres high (Zambatis *et al.* 1997) and *A. senegal* trees that were greater than 1.5 metres high) were selected.

Micro-sites are small-scale sites that may be suitable for germination and survival of seedlings (Eriksson and Ehrlen 1992). Soil seed bank density was assessed at eight micro-sites along the North-South axis of each tree to provide the horizontal spatial distribution of soil seed banks. At each micro-site the vertical distribution of seeds (with depth in litter and soil) was also assessed (see Witkowski and Garner 2000). The following horizontal micro-sites were assessed (Figure 1):

a. 'Under' samples: taken under the tree canopy at the base of the trunk.
b. 'Half-under' samples: taken under the tree canopy midway between the trunk and the edge of the canopy.
c. 'Edge' samples: taken at the edge of the tree canopy.
d. 'Beyond' samples: taken mid-point between the canopy edge of the sample tree and the canopy edge of the nearest neighbouring tree, providing the distance to the neighbouring trees was greater than 10 m (i.e. at least 5 m away from the canopy edge of the sample tree).

Three vertical sub-samples were taken from (a) the litter layer, (b) 0-2 cm and (c) 2-5 cm soil depths, at each horizontal micro-site.

Samples were taken with a robust steel quadrat, 30 cm x 30 cm wide and 5 cm deep, with lines etched on the steel for easy depth determination (Plate 1). The quadrat was

pushed into the soil to the required depth and all the soil amassed to that depth was removed as sub-samples. Samples were only taken to a depth of 5 cm, as previous studies have shown that the majority (> 95%) of seeds were found in the top 5 cm of the soil (Garner and Witkowski 1997; Witkowski and Garner 2000; Wilson and Witkowski 2003). Given the granitic soils of the KNP, seed banks are likely to be generally quite shallow as the soil is generally more compacted than the sandy soils of Nylsvley Nature Reserve (see Garner and Witkowski 1997).

The samples were then sieved with a standard 2 mm soil sieve. This pore size is small enough to allow retention of any *Acacia* seeds but large enough to allow the majority of the soil to pass through (Witkowski and Garner 2000). Seeds retrieved from the samples were categorized as intact, predated or aborted. Seeds were classified as 'old' if they were brown in colour and not still in pods (see Witkowski and Garner 2000; Wilson and Witkowski 2003). The general species composition of each soil sample was recorded i.e. the number of *Acacia* study species seeds found versus other types of seeds large enough to be retained by the sieve. Other seeds were classified as seeds not from the four *Acacia* study species.

The following measurements (following Wilson and Witkowski 2003) were taken for each tree associated with seed bank samples (Plate 1):

- Stem diameter at a point on the stem 20 cm above ground level;
- Over-storey canopy shading at the four main cardinal points using a spherical densiometer Model A with a convex mirror of 2.5 inches in diameter and 24 quarter inch squares (Lemmon 1957);
- Herbaceous biomass using a disc pasture meter (following Barnsby and Tainton 1977) for a total of 32 measurements per tree. Within each horizontal micro-site (Figure 1) four measurements were taken;
- Bark thickness using a bark gauge at the same point on the stem measured for stem diameter;
- Soil compaction (soil hardness) for a total of 32 measurements per tree. Four measurements were taken within each micro-site (Figure 1) using a soil penetrometer (model CL-700, Soil test Inc. Evanston, Illinois);
- Height (H) of the associated parent tree;

- Lowest height of the canopy (LH) of the tree;
- Maximum canopy diameter (D_2) perpendicular to the maximum diameter (D_1) of the tree;

The canopy area and volume of each tree were calculated using the following equations:

- Canopy Area (CA) = II r^2 = II (D_1/2)(D_2/2)
- Canopy Volume ($CV_{cylinder}$) = (H-LH)(CA)

Seed banks were sampled over two periods, namely the 13th to the 24th of February and the 4th to the 11th of April 2006, before the current fruiting season had begun. Seeds were measured for various seed characteristics (sphericity, volume, density, CSF and mass) and intact seeds were tested for viability using tetrazolium staining. Throughout the thesis, these soil seed banks are referred to as *in situ* as sampling was done on site.

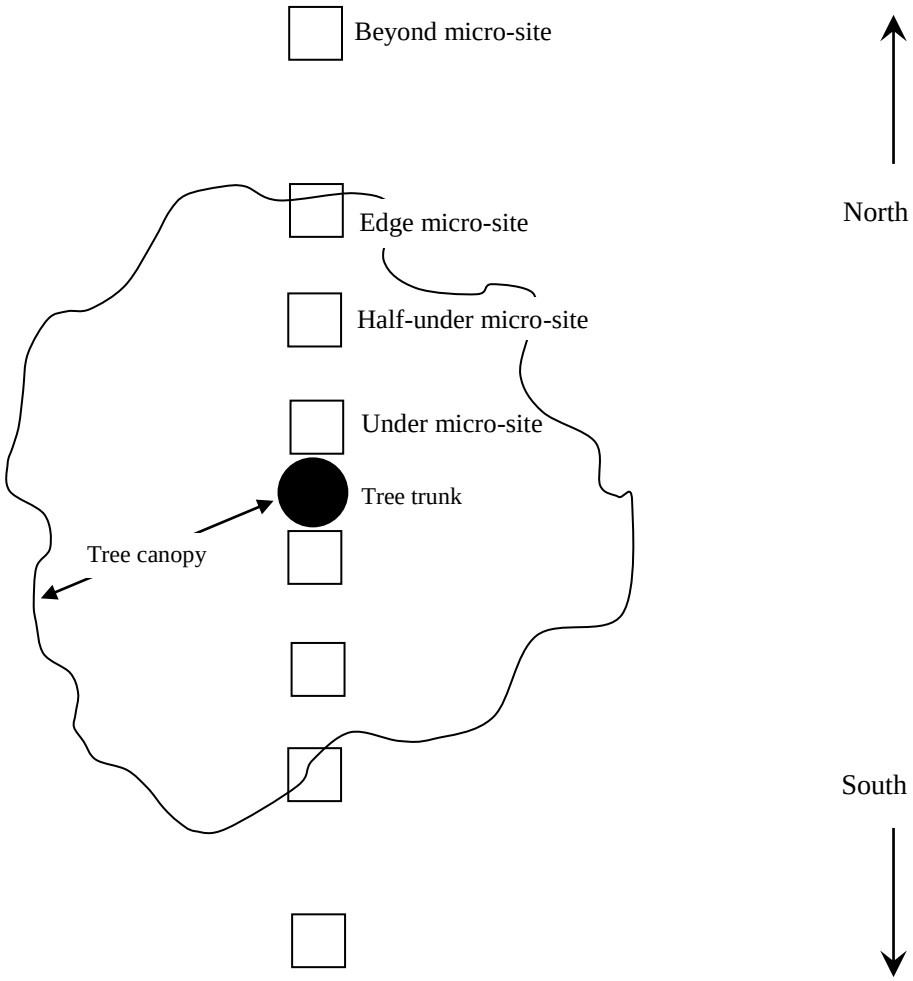

Figure 1. Top-down view and position of micro-sites used for soil seed bank sampling (adapted from Witkowski & Garner 2000).

Plate 1. Images of sampling *in situ* soil seed banks near Skukuza, Kruger National Park, South Africa in February and April 2006. Clockwise from top left: sampling the 'under canopy' micro-site; the steel quadrat used to sample soil seed banks; a disc pasture meter used to sample herbaceous biomass; a caliper used to record stem diameter; much-needed assistance in the field; and soil penetrometers (left and middle) used to record soil compaction and a spherical densiometer (right) used to record over-storey canopy shading.

2.3.3.1. Data and statistical analyses

One-way ANOVAs and post-hoc Tukey Studentized Range tests (α, $P = 0.05$) were used to compare associated tree characteristics (height, canopy area and volume, bark thickness and stem diameter) among species; seed characteristics (sphericity, volume, density, CSF and mass) of a sample of seeds between species; soil compaction and herbaceous biomass between micro-sites of associated trees within a species; soil compaction, herbaceous biomass and over-storey canopy shading among species; differences in seed density between micro-sites and different soil depth categories per species; differences in seed density at different micro-sites and soil depth categories among species; overall seed bank density among species; and seed characteristics (sphericity, volume, density, CSF and mass) of seed bank seeds among species.

The relationship between various seed characteristics of a sample of seeds per species, and the relationship between associated tree characteristics within species was determined using simple linear regressions, as was the relationship between soil compaction and herbaceous biomass associated with trees, per species. Regressions were also used to determine the relationship between both associated tree characteristics and micro-site characteristics (herbaceous biomass, soil compaction and over-storey canopy shading) with seed bank density per species.

Paired student *t*-tests were used to compare over-storey canopy shading, herbaceous biomass, soil compaction and seed density between the North and South aspects of associated trees per species.

Where necessary percentage data was transformed using arcsin-transformations prior to statistical analysis. Data are presented as means ± standard errors (SE) unless otherwise stated. For all statistical analyses, Microsoft Office Excel 2007, STATISTICA Release 9 Statsoft, Inc., USA (2010) and SAS ® Institute Inc., USA were used.

2.4. Results

2.4.1. Pre-dispersal seed predation of the pilot seed set

Of a batch of one hundred randomly selected seeds per species (seeds were removed from pods that had been collected from tree canopies from the 2005 fruiting season), 15.8% of *A. nilotica,* 5.5% of *A. senegal,* 2.5% of *A. grandicornuta* and 2.4% of *A. tortilis* seeds had evidence of bruchid beetle infestation (Table 1). A greater proportion of each seed set was aborted than predated (Table 1).

Table 1. Pilot study of percentage of intact, predated and aborted seeds extracted from pods sampled from the tree canopies of four *Acacia* species in the Kruger National Park, South Africa (*n* = 100 throughout).

Acacia species	Intact (%)	Predated (%)	Aborted (%)
A. grandicornuta	81.2	2.5	16.3
A. nilotica	55.2	15.8	29.0
A. senegal	71.1	5.5	23.4
A. tortilis	87.0	2.4	10.6

2.4.2. Seed characteristics

While *A. tortilis* and *A. nilotica* produce indehiscent pods, *A. senegal* and *A. grandicornuta* pods are dehiscent (Figure 2). Each *Acacia* species showed obvious visual differences in seed shape and size (Figure 3). Both *A. nilotica* and *A. senegal* seeds had significantly larger mass and volume than *A. tortilis* and *A. grandicornuta* seeds, while there was no significant difference in mass ($F_{3,57} = 50.7$; $P = 0.67$) or volume ($F_{3,57} = 43.3$; $P = 0.88$) between *A. nilotica* and *A. senegal* seeds (Table 2). Seed density comparisons between species showed that *A. grandicornuta* and *A. tortilis* seeds were not significantly different from one another ($F_{3,57} = 32.8$; $P = 0.99$), nor were *A. nilotica* and *A. senegal* seeds ($F_{3,57} = 32.8$; $P = 0.88$). However, *A. grandicornuta* and *A. tortilis* seeds were significantly denser than both *A. nilotica and A. senegal* seeds (Table 2). Seed sphericity and CSF values of all species were significantly different from one another (Table 2). The most spherical seeds were *A. nilotica*, followed by *A. tortilis*, *A. grandicornuta* and *A. senegal* (Table 2).

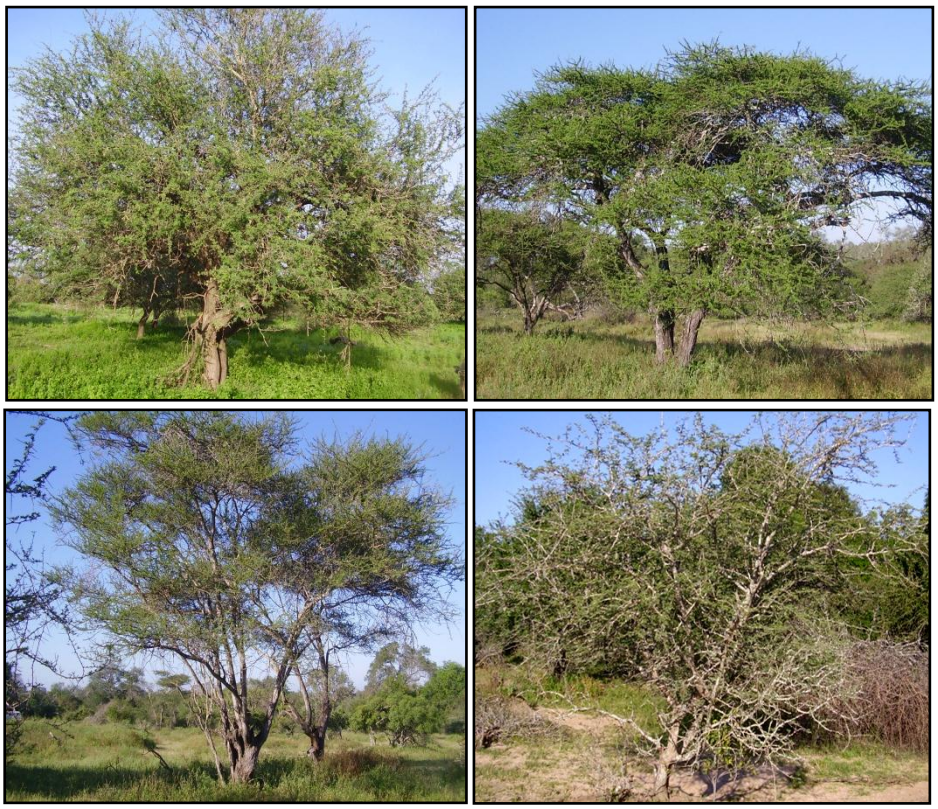

Figure 2. Representative trees of each *Acacia* study species (clockwise from top left): *A. grandicornuta, A. nilotica, A. senegal and A. tortilis.*

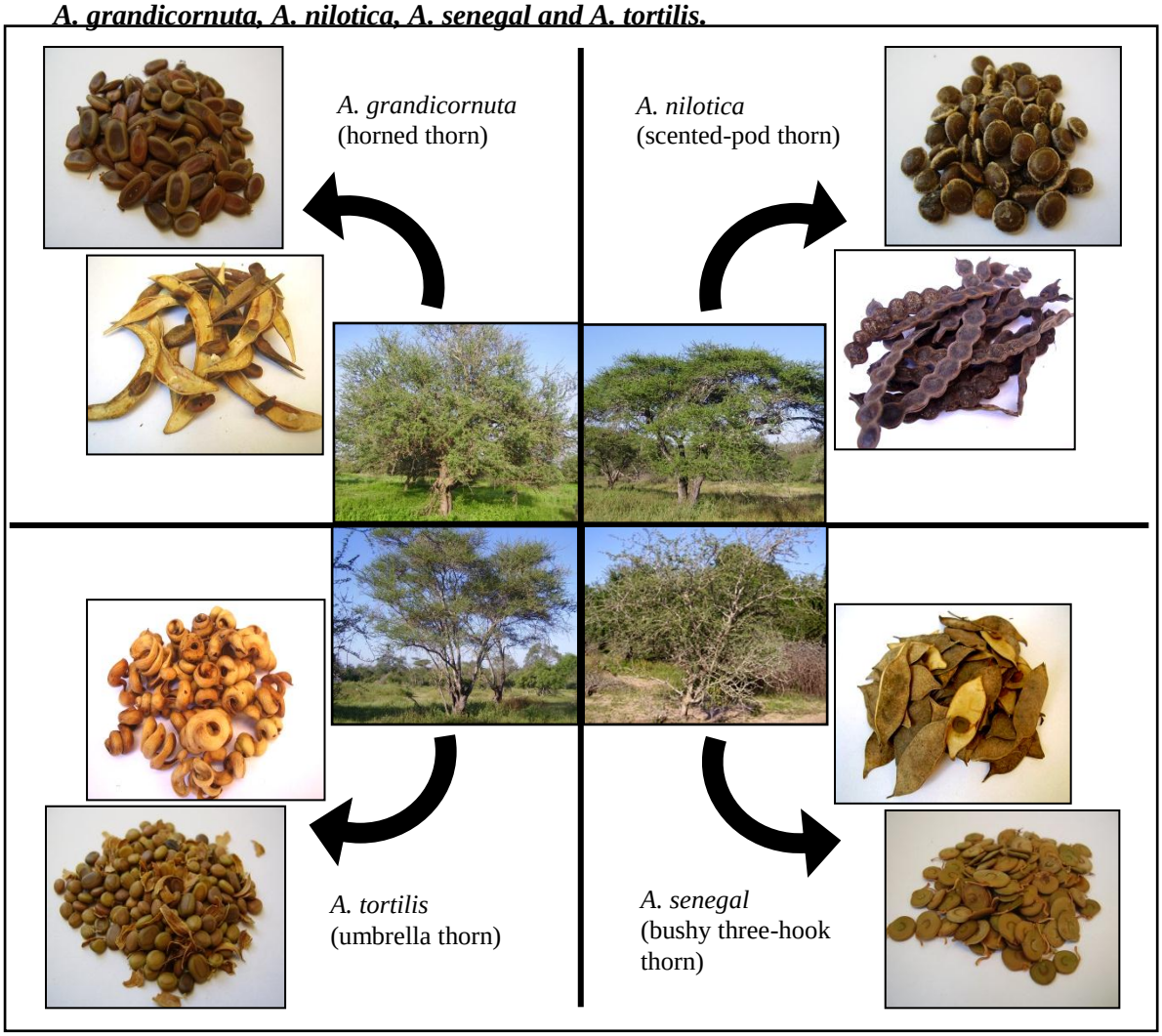

Figure 3. Pictorial comparison of pods and seeds of the four *Acacia* study species.

Table 2 a) Comparisons of seed mass, volume, density, sphericity and Corey Shape Factor (CSF) (mean ± SE) of seeds sampled from pods, between four *Acacia* species in the Kruger National Park, South Africa. Means with different superscript letters are significantly different at the *P* = 0.05 level; *n* = 20 throughout.

Species	Mass (g)	Volume (mm³)	Density (g mm⁻³)	Sphericity	CSF
A. grandicornuta	0.09 ± 0.004^{B}	55.15 ± 4.38^{B}	0.0016 ± 0.0007^{A}	0.14 ± 0.002^{B}	0.34 ± 0.006^{C}
A. nilotica	0.11 ± 0.004^{A}	90.32 ± 3.62^{A}	0.0012 ± 0.00001^{B}	0.06 ± 0.003^{D}	0.57 ± 0.011^{A}
A. senegal	0.10 ± 0.006^{A}	85.59 ± 7.00^{A}	0.0013 ± 0.00004^{B}	0.21 ± 0.002^{A}	0.19 ± 0.003^{D}
A. tortilis	0.04 ± 0.002^{C}	26.03 ± 1.11^{C}	0.0017 ± 0.00002^{A}	0.11 ± 0.002^{C}	0.41 ± 0.007^{B}

b) One-way ANOVA results for comparisons of seed mass, volume, density, sphericity and CSF values between four *Acacia* species (d.f. = 3, 57).

	Mass (g)	Volume (mm³)	Density (g mm⁻³)	Sphericity	CSF
P	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
F	50.65	43.25	32.78	792.70	483.76

Seed viability of a sample of seeds removed from pods varied among species, namely 81.2 % of *A. grandicornuta*, 55.2% of *A. nilotica*, 71.1% of *A. senegal* and 87.1% of *A. tortilis* seeds (Figure 4).

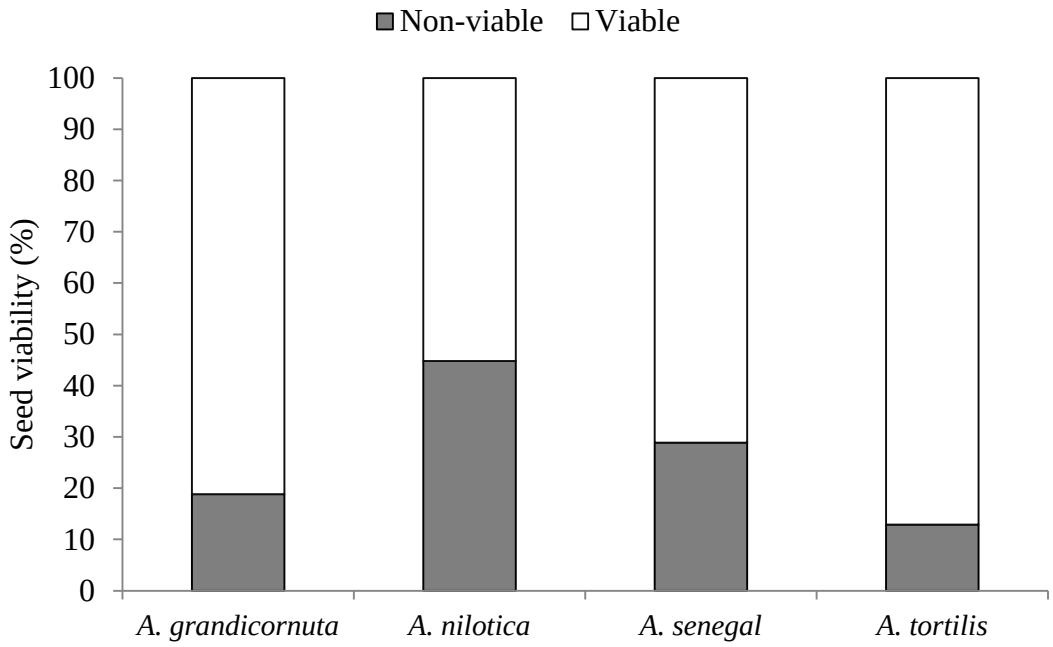

Figure 4. Percentage of viable and non-viable seeds from samples of pods of four *Acacia* species in the Kruger National Park, South Africa (*n* = 20).

Simple linear regressions showed a significantly negative relationship of both volume and mass with CSF, while a significantly positive relationship was found for both volume and mass with sphericity for *A. tortilis* seeds (Table 3). Generally, the smaller *A. tortilis* seeds are more spherical. There were poor fits between seed sphericity and mass for *A. grandicornuta* (r^2 = 0.20; P = 0.06), *A. nilotica* (r^2 = 0.07; P = 0.29) and *A. senegal* (r^2 = 0.01; P = 0.78), as well as between seed sphericity and volume for *A. grandicornuta* (r^2 = 0.40; P = 0.003), *A. nilotica* (r^2 = 0.07; P = 0.004) and *A. senegal* (r^2 = 0.004; P = 0.79) (Figure 5). Poor fits also occurred between CSF and seed mass for *A. grandicornuta* (r^2 = 0.07; P = 0.27), *A. nilotica* (r^2 = 0.06; P = 0.33) and *A. senegal* (r^2 = 0.02; P = 0.55) as well as between CSF and seed volume for *A. grandicornuta* (r^2 = 0.16; P = 0.10), *A. nilotica* (r^2 = 0.05; P = 0.36) and *A. senegal* (r^2 = 0.02; P = 0.53) (Figure 5).

Table 3. Simple linear regressions (r^2- and P - values) between seed characteristics for *A. tortilis* ($P < 0.05$; $n = 20$). Values for the three other study species have not been included in this table as the results were not significant.

	Slope of relationship	r^2	P
CSF vs. seed mass (g)	-	0.954	< 0.0001
CSF vs. seed volume (mm^3)	-	0.759	< 0.0001
Sphericity vs. seed mass (g)	+	0.859	< 0.0001
Sphericity vs. seed volume (mm^3)	+	0.776	< 0.0001

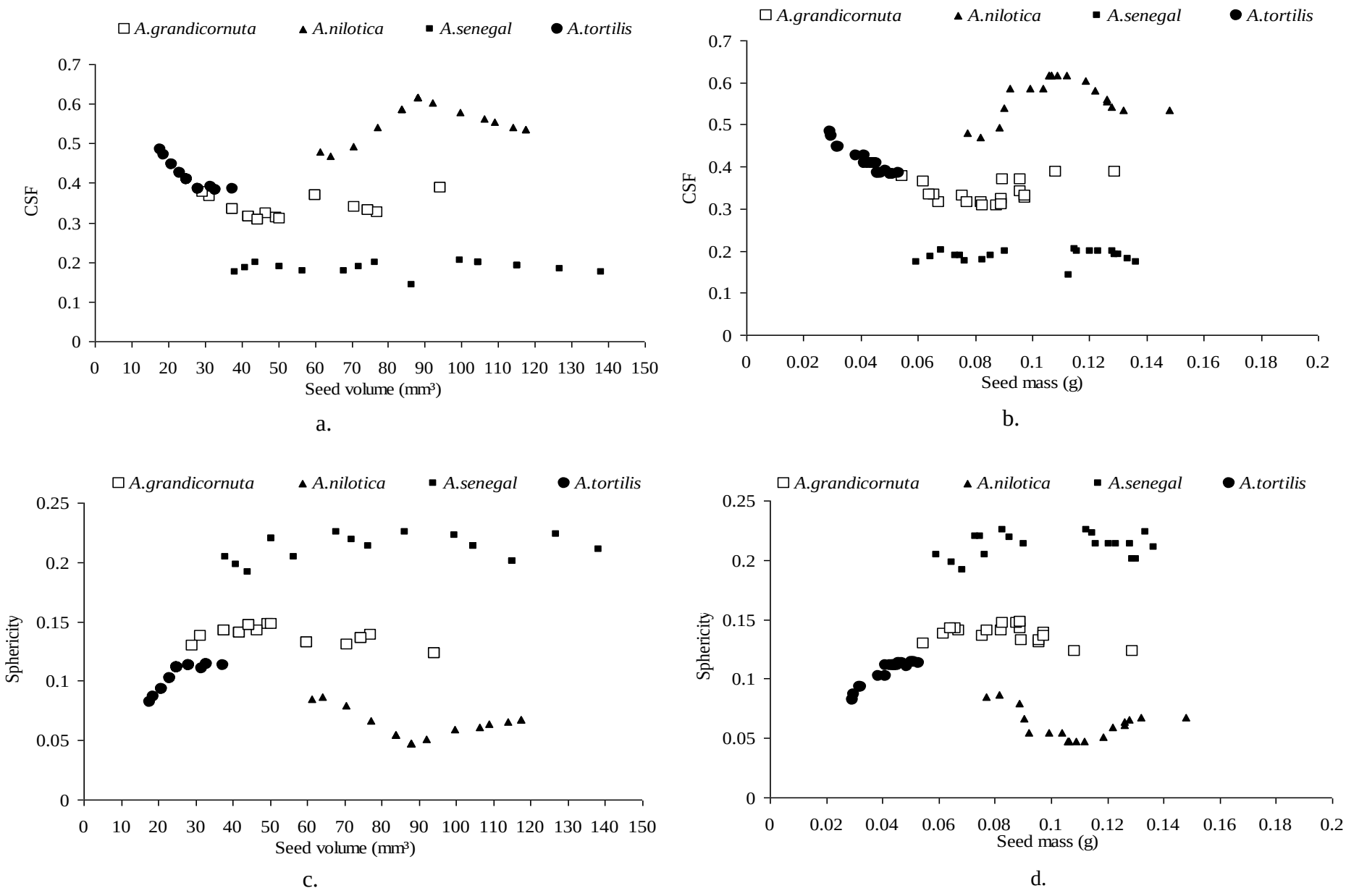

Figure 5. Relationship between a) Corey Shape Factor (CSF) and volume, b) CSF and mass, c) sphericity and volume, and d) sphericity and mass of seeds of four *Acacia* species in the Kruger National Park, South Africa (*n* = 20).

2.4.3. Associated tree characteristics

There was a significant difference in tree height and canopy volume between *A. senegal* trees and the other three species. The tree canopy area of *A. tortilis* was significantly larger than the other three species and while there was no significant difference between the canopy area of *A. grandicornuta* and *A. nilotica* trees, canopy area of *A. senegal* trees was significantly smaller than the other three species (Table 4).

Table 4 a) Comparisons of tree height, canopy area and canopy volume (mean ± SE) between four *Acacia* species in the Kruger National Park, South Africa. Means with different superscript letters are significantly different at the *P* = 0.05 level; *n* = 8 throughout.

Species	Height (m)	Canopy area (m^2 $tree^{-1}$)	Canopy volume (m^3 $tree^{-1}$)
A. grandicornuta	2.61 ± 0.11^{A}	59.98 ± 6.63^{B}	103.61 ± 11.36^{A}
A. nilotica	2.30 ± 0.09^{A}	52.83 ± 7.73^{B}	79.67 ± 11.03^{A}
A. senegal	1.75 ± 0.10^{B}	16.73 ± 2.50^{C}	20.07 ± 3.27^{B}
A. tortilis	2.46 ± 0.08^{A}	90.76 ± 13.04^{A}	148.10 ± 20.35^{A}

b) One-way ANOVA results for comparisons of tree height, canopy area and canopy volume between four *Acacia* species in the Kruger National Park, South Africa (d.f. = 3,21).

	Height (m)	Canopy area (m^2 $tree^{-1}$)	Canopy volume (m^3 $tree^{-1}$)
p	< 0.0001	< 0.0001	< 0.0001
F	17.02	22.72	30.53

The relationship between stem diameter and bark thickness was positive for *A. tortilis*, as was the relationship between tree height and canopy volume for *A. senegal* (Table 5). Linear regressions indicated no significant relationships between the various other combinations of tree characteristics of all four species; however, the relationship of both tree height and canopy area with stem diameter of *A. nilotica* tended to be significant, as did the relationship of both tree canopy area and bark thickness with stem diameter of *A. grandicornuta* (Table 5).

Table 5. Relationships between five tree characteristics of four *Acacia* species in the Kruger National Park, South Africa (significant values, where $P \leq 0.05$, in bold).

		A. grandicornuta (n = 8)	*A. nilotica* (n = 8)	*A. senegal* (n = 8)	*A. tortilis* (n = 8)
Tree height (m) and stem diameter (cm)	r^2	0.08	0.42	0.11	0.09
	P	0.49	0.08	0.42	0.46
Bark thickness (mm) and tree canopy volume (m^3 $tree^{-1}$)	r^2	0.20	0.004	0.001	0.25
	P	0.26	0.88	0.95	0.21
Bark thickness (mm) and tree canopy area (m^2 $tree^{-1}$)	r^2	0.17	0.003	0.02	0.26
	P	0.31	0.91	0.74	0.19
Tree canopy volume (m^3 $tree^{-1}$) and stem diameter (cm)	r^2	0.30	0.18	0.01	0.11
	P	0.16	0.30	0.81	0.43
Tree canopy area (m^2 $tree^{-1}$) and stem diameter (cm)	r^2	0.44	0.39	0.003	0.05
	P	0.08	0.09	0.89	0.58
Tree height (m) and tree canopy area (m^2 $tree^{-1}$)	r^2	0.05	0.18	0.19	0.03
	P	0.57	0.29	0.29	0.69
Tree height (m) and tree canopy volume (m^3 $tree^{-1}$)	r^2	0.01	0.01	0.49	0.0001
	P	0.82	0.81	**0.05**	0.99
Bark thickness (mm) and tree height (m)	r^2	0.01	0.0003	0.04	0.31
	P	0.81	0.97	0.67	0.15
Bark thickness (mm) and stem diameter (cm)	r^2	0.45	0.03	0.04	0.51
	P	0.07	0.67	0.66	**0.05**

2.4.4. Micro-site characteristics

Generally there was no relationship between soil compaction and herbaceous biomass in the *Acacia* study species (r^2 = 0.07). Simple linear regressions did however, show a negative relationship between herbaceous biomass and soil compaction for both *A. grandicornuta* and *A. tortilis* trees (Table 6) but no relationships for *A. nilotica* or *A. senegal* trees (Figure 6).

Table 6. Simple linear regressions (*P* & r^2-values) between soil compaction (kg cm^{-2}) and herbaceous biomass (kg ha^{-1}) associated with four *Acacia* species in the Kruger National Park, South Africa (*P* < 0.05; *n* = 32 throughout).

	A. grandicornuta	*A. nilotica*	*A. senegal*	*A. tortilis*
r^2	0.62	0.03	0.01	0.39
P	0.001	0.21	0.58	0.02

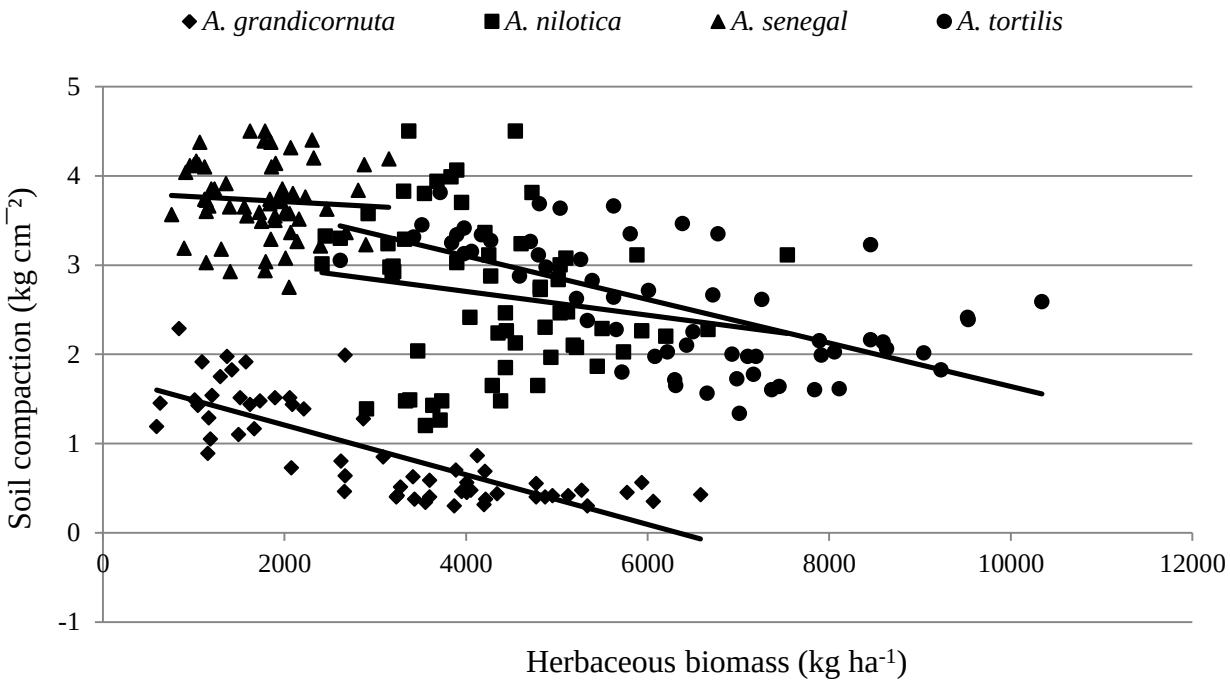

Figure 6. Soil compaction in relation to herbaceous biomass associated with trees of four *Acacia* species in the Kruger National Park, South Africa (*n* = 32).

There was no significant difference in herbaceous biomass between micro-sites for *A. senegal* (*P* = 0.44). For *A. grandicornuta* and *A. tortilis*, micro-sites under the tree canopy (under canopy micro-sites) had significantly more herbaceous biomass than micro-sites in the

open (beyond micro-sites). There was no significant difference in herbaceous biomass between micro-sites under *A. nilotica* tree canopies (under canopy micro-sites) and micro-sites in the open (beyond micro-sites) (Table 7). Generally herbaceous biomass was higher below canopy (under and half-under micro-sites) and at the edge micro-sites than in the open (beyond micro-sites) for all species.

Table 7 a) Comparisons of herbaceous biomass (kg ha^{-1}) (mean ± SE) between micro-sites under canopy, half-under, edge and beyond canopy of four *Acacia* species in the Kruger National Park, South Africa. Means (in columns) with different superscript letters are significantly different at the *P* = 0.05 level; *n* = 32 throughout.

Micro-sites	*A. grandicornuta*	*A. nilotica*	*A. senegal*	*A. tortilis*
Under canopy	4414 ± 793^{A}	3638 ± 138^{B}	1744 ± 92	7294 ± 310^{A}
Half-under	4609 ± 371^{A}	4927 ± 244^{A}	1535 ± 191	7251 ± 482^{A}
Edge	2411 ± 238^{B}	5003 ± 337^{A}	1814 ± 203	6274 ± 342^{A}
Beyond canopy	1366 ± 216^{B}	2956 ± 108^{B}	1468 ± 187	4432 ± 251^{B}

b) One-way ANOVA results for comparisons of herbaceous biomass between micro-sites under canopy, half-under, edge and beyond canopy of four *Acacia* species (d.f. = 3,27).

	A. grandicornuta	*A. nilotica*	*A. senegal*	*A. tortilis*
P	0.002	0.0003	0.44	0.001
F	11.42	19.66	0.92	14.11

There was a significant difference in soil compaction between under canopy micro-sites and beyond canopy micro-sites for all four species. Soil compaction of beyond canopy micro-sites and edge canopy micro-sites did not differ significantly from one another for any of the species. For *A. grandicornuta* and *A. senegal* there was no significant difference in soil compaction between micro-sites under tree canopy (under and half-under). Soil compaction of under canopy micro-sites was significantly lower than that of half-under micro-sites for both *A. nilotica* and *A. tortilis* (Table 8). Generally, soil compaction increased for all species with distance from the centre of the tree canopy.

Table 8 a) Comparisons of soil compaction (kg cm^{-2}) (mean ± SE) between micro-sites under canopy, half-under, edge and beyond canopy of four *Acacia* species in the Kruger National Park, South Africa. Means (in columns) with different superscript letters are significantly different at the *P* = 0.05 level; *n* = 32 throughout.

Micro-sites	*A. grandicornuta*	*A. nilotica*	*A. senegal*	*A. tortilis*
Under canopy	0.45 ± 0.02^{B}	1.47 ± 0.07^{C}	3.32 ± 0.11^{B}	1.68 ± 0.03^{C}
Half-under	0.53 ± 0.04^{B}	2.25 ± 0.08^{B}	3.46 ± 0.09^{B}	2.11 ± 0.07^{B}
Edge	1.11 ± 0.11^{A}	3.02 ± 0.06^{A}	4.04 ± 0.09^{A}	2.89 ± 0.12^{A}
Beyond canopy	1.33 ± 0.08^{A}	3.21 ± 0.09^{A}	4.11 ± 0.08^{A}	3.21 ± 0.09^{A}

b) One-way ANOVA results for comparisons of soil compaction between micro-sites under canopy, half-under, edge and beyond canopy of four *Acacia* species (d.f. = 3,27).

	A. grandicornuta	*A. nilotica*	*A. senegal*	*A. tortilis*
P	< 0.0001	< 0.0001	< 0.0001	< 0.0001
F	45.33	110.99	13.97	74.02

There was a significant difference in herbaceous biomass associated with trees between all species, with values decreasing between species as follows:

A. tortilis > *A. nilotica* > *A. grandicornuta* > *A. senegal* (Table 9). Soil compaction was significantly higher for *A. senegal* trees than for *A. grandicornuta*, *A. nilotica* and *A. tortilis* trees and there was no significant difference in soil compaction between *A. nilotica* and *A. tortilis* trees. Soil compaction for *A. grandicornuta* trees was significantly lower than the other three species (Table 9).

Table 9 a) Comparison of herbaceous biomass and soil compaction (mean ± SE) associated with trees between four *Acacia* species in the Kruger National Park, South Africa. Means (in columns) with different superscript letters are significantly different at the *P* = 0.05 level; *n* = 32 throughout.

	A. grandicornuta	*A. nilotica*	*A. senegal*	*A. tortilis*
Herbaceous biomass (kg ha^{-1})	3035 ± 204^{C}	$4285 \pm {}^{135B}$	1739 ± 73^{D}	6272 ± 230^{A}
Soil compaction (kg cm^{-2})	0.9 ± 0.04^{C}	2.7 ± 0.07^{B}	3.7 ± 0.05^{A}	2.5 ± 0.06^{B}

b) One-way ANOVA results for comparisons of herbaceous biomass and soil compaction associated with trees between four *Acacia* species in the Kruger National Park, South Africa (d.f. = 3,177).

	Herbaceous biomass (kg ha^{-1})	Soil compaction (kg cm^{-2})
P	< 0.0001	< 0.0001
F	199.3	654.9

Paired student *t*-tests showed no significant difference in herbaceous biomass between the North and South aspects of trees for *A. nilotica* (*t* = 0.44; *P* = 0.33), *A. senegal* (*t* = 0.50; *P* = 0.31) or *A. tortilis* (*t* = 1.13; *P* = 0.13). Herbaceous biomass on the South aspect of *A. grandicornuta* trees was significantly higher than that on the North aspect (*t* = 3.1; *P* < 0.05) (Figure 7).

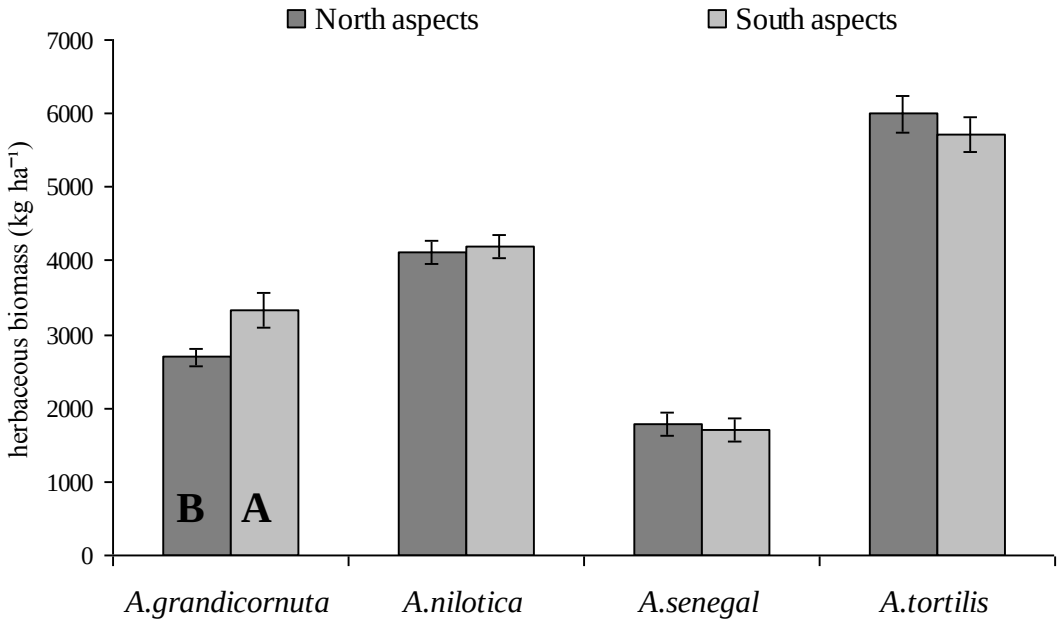

Figure 7. Comparisons of herbaceous biomass (mean ± SE) between the North and South aspects of trees of four *Acacia* species in the Kruger National Park, South Africa (*n* = 32). Bars with highlighted letters are significantly different at the *P* = 0.05 level.

Paired student *t-tests* showed a significant difference in soil compaction between the North and South aspects *of A. grandicornuta* (*t* = 3.5; *P* < 0.05), *A. senegal* (*t* = 1.94; *P* < 0.05) and *A. tortilis* (*t* = 1.99; *P* < 0.05) trees (Figure 8). There was no significant difference in soil compaction between the North and South aspects of trees for *A. nilotica* (*t* = 0.62; *P* = 0.27).

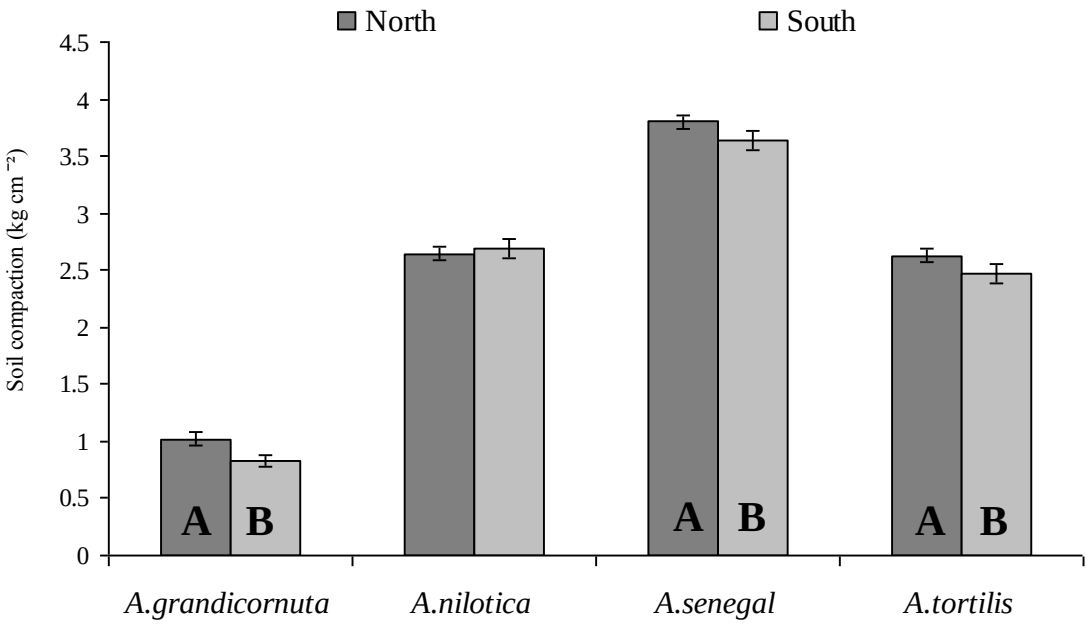

Figure 8. Comparisons of soil compaction (mean ± SE) between the North and South aspects of trees of four *Acacia* species in the Kruger National Park, South Africa (*n* = 32). Bars with highlighted letters are significantly different at the *P* = 0.05 level.

One-way ANOVAs showed that over-storey canopy shading by *A. senegal* trees was significantly lower than that of the other three species. There were no significant differences in over-storey canopy shading between any of the other species (Table 10).

Table 10 a) Comparisons of tree over-storey canopy shading (mean ± SE) between four *Acacia* species in the Kruger National Park, South Africa. Means with different superscript letters are significantly different at the *P* = 0.05 level; *n* = 32 throughout. Percentage data was transformed using arcsin-transformations prior to statistical analysis.

	A. grandicornuta	*A. nilotica*	*A. senegal*	*A. tortilis*
Over-storey canopy shading (%)	81.1 ± 3.9^{A}	80.6 ± 5.8^{A}	68.2 ± 2.1^{B}	87.1 ± 1.9^{A}

b) One-way ANOVA results for comparisons of tree over-storey canopy shading between four *Acacia* species (d.f. = 3, 93).

	Over-storey canopy shading
P	0.0004
F	12.96

Paired student *t*-tests showed no significant difference in tree over-storey canopy shading between the North and South aspects of any of the species (Figure 9).

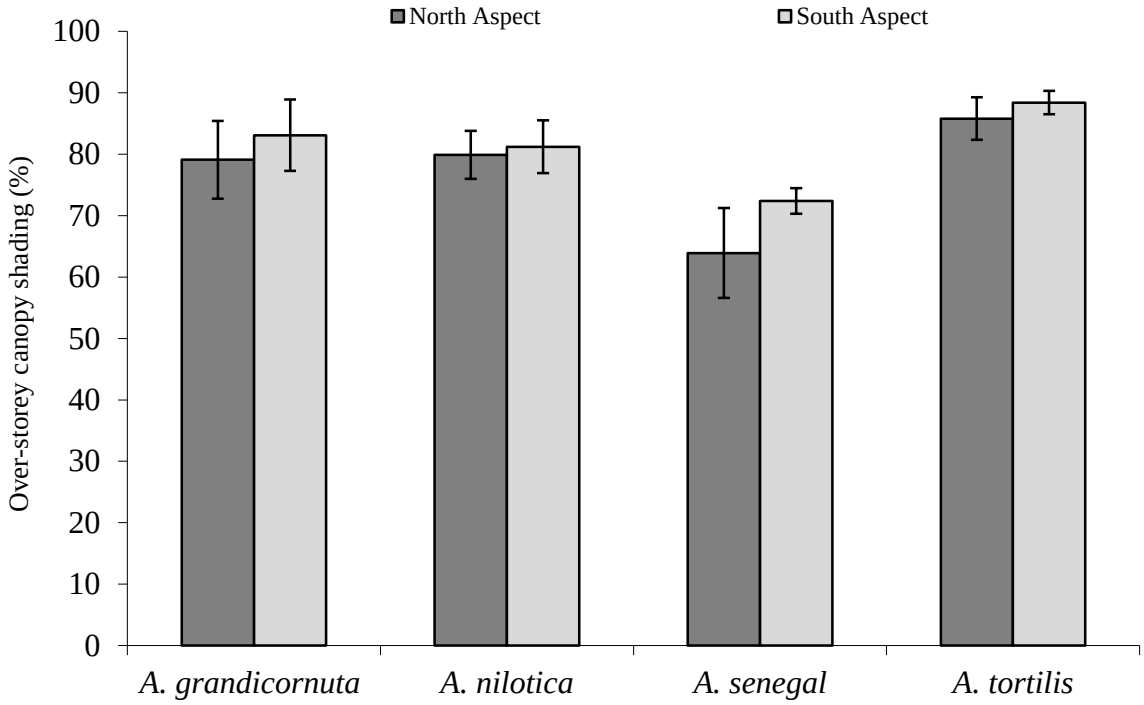

Figure 9. Comparisons of over-storey canopy shading (mean ± SE) between the North and South aspects of trees of four *Acacia* species in the Kruger National Park, South Africa (*n* = 32).

2.4.5. Species composition and densities of soil seed banks.

An analysis of the species composition of soil seed banks of large seeds (seeds retained by the 2 mm sieve) showed that seed banks were composed primarily of seeds of species other than the four study species (Table 11). No 'current' seeds were found (seeds that are either green or still in their pods) as sampling was done before the new fruiting season (June/July 2006).

Table 11. Species composition and densities of soil seed banks of four *Acacia* species in the Kruger National Park, South Africa. Any seeds sampled from soil seed banks other than the four study species were categorized as 'other seeds'. The percentage value in brackets is the proportion of the total sample.

Species	*Acacia* seeds	'Other seeds'
A. grandicornuta	87 (27 %)	233 (73%)
A. nilotica	36 (4%)	864 (96%)
A. senegal	4 (1%)	396 (99 %)
A. tortilis	142 (23%)	458 (76%)

One-way ANOVAs showed no significant difference in seed densities between micro-sites for *A. grandicornuta, A. nilotica* or *A. senegal* . There was a significant difference in seed densities between micro-sites for *A. tortilis* (Table 12). There was no significant difference in seeds densities between soil depth categories for *A. grandicornuta, A. nilotica, A. senegal* or *A. tortilis.* Seed density generally decreased with depth in the soil and with distance from the centre of the canopy (Figure 10).

One-way ANOVAs showed a significant difference in seed densities of the under canopy *(P* < 0.05) and half-under canopy micro-sites *(P* < 0.05) among species but no significant difference in seed densities of the edge micro-sites *(P* = 0.4) and beyond canopy micro-sites *(P* = 0.2) among species. There were significant differences in seed densities of all three depth categories (litter, 0-2 cm and 2-5 cm deep) among species *(P* < 0.05) (Table 12).

Table 12 a) Comparisons of seed density (seeds m^{-2}) (mean ± SE) between four micro-sites (under canopy, half-under, edge and beyond canopy), and three different soil depth categories (litter, 0-2 cm and 2-5 cm) for four *Acacia* species in the Kruger National Park, South Africa (*n* = 8). Means with different superscript letters are significantly different at the *P* = 0.05 level. Superscript letters in bold indicate differences between species, while letters in italics represent differences within species.

	Soil depth categories			Micro-site categories			
	Litter	0-2 cm	2-5 cm	Under	Half-under	Edge	Beyond
A. grandicornuta	6.5 ± 3.7^{A}	3.9 ± 2.9^{A}	1.7 ± 0.7^{A}	6.9 ± 4.4^{B}	2.5 ± 1.3^{B}	1.5 ± 0.8	1.2 ± 1.1
A. nilotica	2.3 ± 1.1^{A}	1.8 ± 0.7^{B}	0.8 ± 0.4^{B}	1.8 ± 0.7^{B}	1.9 ± 1.2^{B}	0.8 ± 0.8	0.4 ± 0.4
A. senegal	0.3 ± 0.3^{B}	0.3 ± 0.2^{B}	0 ± 0^{B}	0.1 ± 0.1^{B}	0.1 ± 0.1^{C}	0.4 ± 0.3	0 ± 0
A. tortilis	5.7 ± 1.4^{A}	9.8 ± 3.7^{A}	4.2 ± 2.2^{A}	$12.2 \pm 3.8^{A, A}$	$4.2 \pm 2.1^{\mathbf{A},\ B}$	2.6 ± 1.9^{B}	0.7 ± 0.3^{B}

b) One-way ANOVA results for comparisons of seed density (seeds m^{-2}) between four micro-sites: under canopy, half-under, edge and beyond canopy, for four *Acacia* species (d.f. = 3,124).

	A. grandicornuta	*A. nilotica*	*A. senegal*	*A. tortilis*
P	0.5	0.4	0.3	0.00002
F	0.8	1.1	2.7	8.8

c) One-way ANOVA results for comparisons of seed density (seeds m^{-2}) between three different soil depth categories: litter, 0-2 cm and 2-5 cm, for four *Acacia* species (d.f. = 3,93).

	A. grandicornuta	*A. nilotica*	*A. senegal*	*A. tortilis*
P	0.3	0.4	0.8	0.4
F	1.3	1.0	0.3	0.9

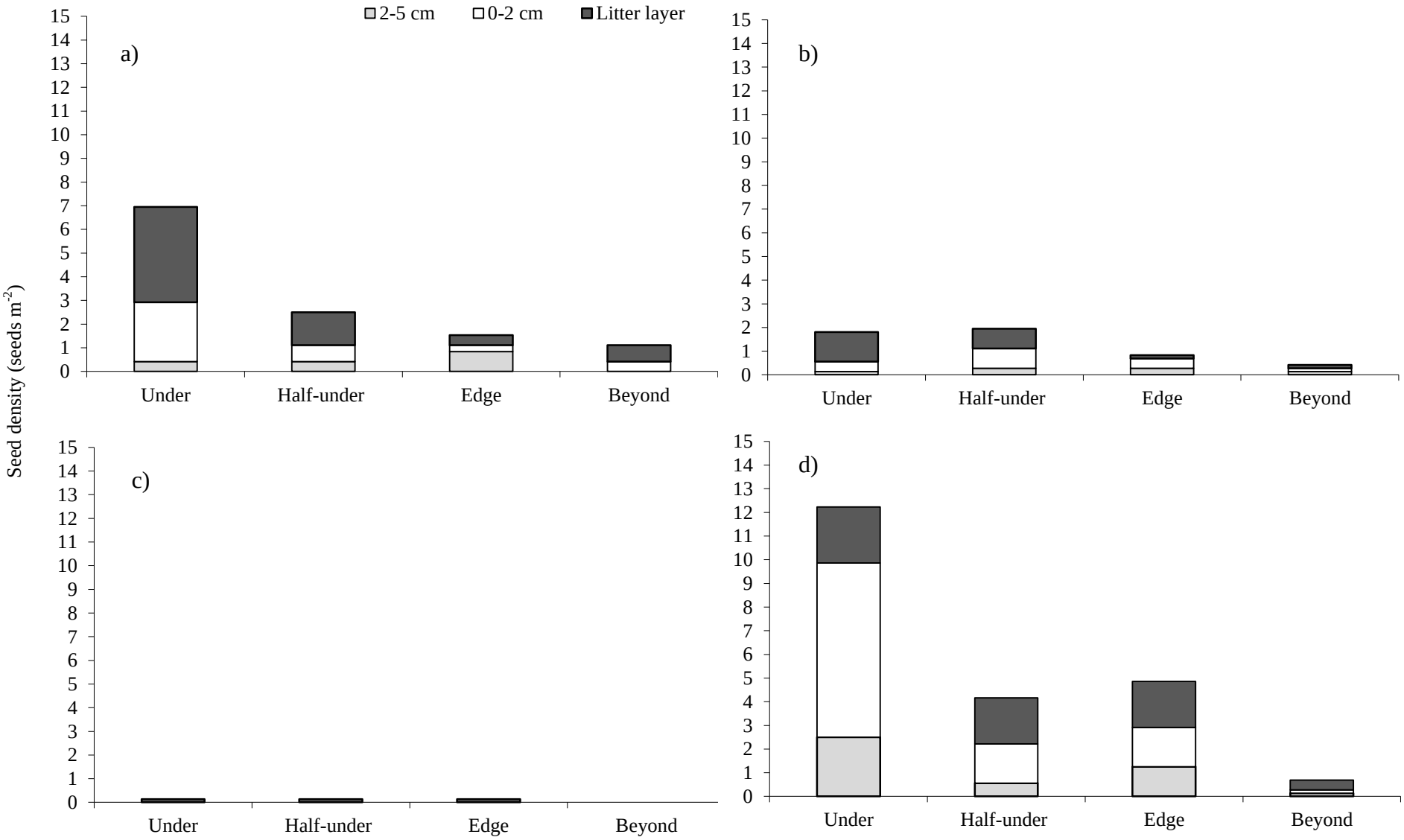

Figure 10 a-d) Seed density in relation to presence in the litter and with soil depth of a) *A. grandicornuta*, b) *A. nilotica*, c) *A. senegal* and d) *A. tortilis* within the micro-sites under canopy, half-under, edge and beyond canopy (see text for definitions).

Paired student *t*-tests showed no significant difference in seed densities between the North and South aspects of trees for any of the four *Acacia* species (Table 13). The South aspect of both *A. nilotica* and *A. tortilis* trees had a slightly higher seed density than the North aspect, while the North aspect of *A. grandicornuta* trees had a higher seed density than the South aspect. Seed densities of both aspects were similar for *A. senegal* trees (Figure 11).

Table 13. Paired student f-test results for a comparison of seed density (seeds m^{-2}) (mean ± SE) between the North and South aspect of trees of four *Acacia* species in the Kruger National Park, South Africa (*n* = 8; d.f. = 7).

	A. grandicornuta	*A. nilotica*	*A. senegal*	*A. tortilis*
P	0.1	0.4	0.5	0.5
t	1.3	0.2	0	0.1

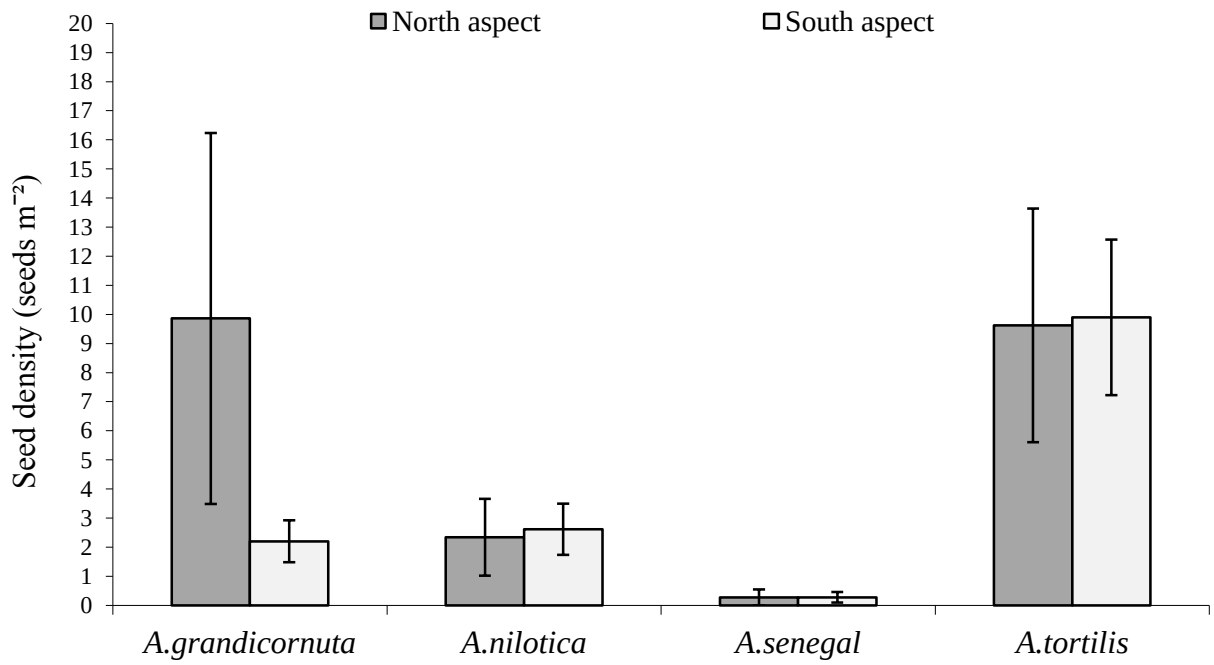

Figure 11. Seed density (mean ± SE) for the North and South aspects of trees of four *Acacia* species in the Kruger National Park, South Africa (*n* = 8).

One-way ANOVAs showed a significant difference in overall seed density between species ($P < 0.05$; $F = 3.01$; d.f. = 3, 28). Overall seed density (seeds m^{-2}) decreased between the

four species as follows: *A. tortilis* (19.53 ± 6.4), *A. grandicornuta* (12.08 ± 6.86), *A. nilotica* (4.95 ± 1.80) and *A. senegal* (0.55 ± 0.42) (Figure 12).

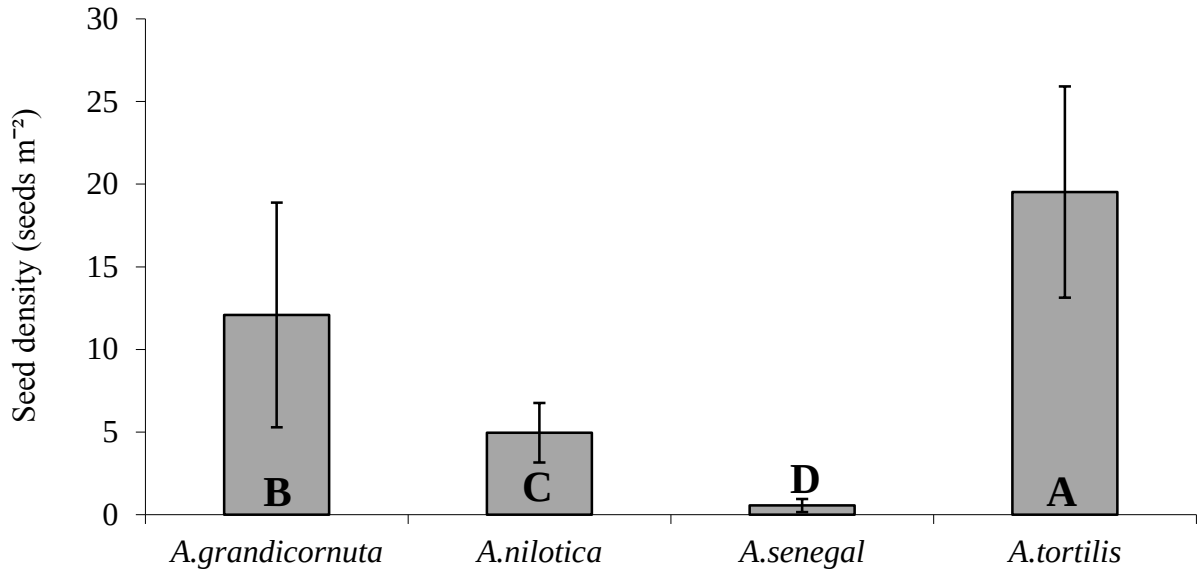

Figure 12. Overall seed density (mean ± SE) of four *Acacia* species in the Kruger National Park, South Africa (*n* = 8). Bars with highlighted letters are significantly different at the *P* = 0.05 level.

There was a significant positive relationship between seed density and tree height for *A. senegal* (r^2 = 0.84; *P* = 0.0013) and *A. tortilis* (r^2 = 0.61; *P* = 0.02) as well as between seed density and tree canopy volume for *A. nilotica* (Table 14). There were no significant relationships between any other associated tree characteristic and seed density for any other species. There was a significant negative relationship between soil compaction and seed density for *A. senegal* (Table 14) but no other significant relationship was found between seed density and soil compaction or seed density and herbaceous biomass for any of the other species.

Table 14. Relationship of five associated tree characteristics and three micro-site characteristics with seed density (*P*- & r^2- revalues) of four *Acacia* species in the Kruger National Park, South Africa. Means that are significantly different at the *P* = 0.05 level are highlighted in bold.

		A. grandicornuta (n = 8)	*A. nilotica* (n = 8)	*A. senegal* (n = 8)	*A. tortilis* (n = 8)
Tree height (m) and seed density	r^2	0.02	0.02	0.84	0.61
(seeds m^{-2})	*P*	0.72	0.72	**0.001**	**0.02**
Stem diameter (cm) and seed	r^2	0.02	<0.001	0.005	0.09
density (seeds m^{-2})	*P*	0.74	0.99	0.87	0.47
Bark thickness (in) and seed	r^2	0.13	0.01	0.12	0.001
density (seeds m^{-2})	*P*	0.38	0.84	0.39	0.93
Tree canopy area (m^2 $tree^{-1}$) and	r^2	0.16	0.38	0.03	0.08
seed density (seeds m^{-2})	*P*	0.33	0.12	0.68	0.49
Tree canopy volume (m^3 $tree^{-1}$) and	r^2	0.12	0.62	0.23	0.02
seed density (seeds m^{-2})	*P*	0.39	**0.02**	0.68	0.73
Over-storey canopy shading and	r^2	0.27	0.15	<0.001	0.09
seed density (seeds m^{-2})	*P*	0.19	0.34	0.98	0.46
Soil compaction (kg cm^{-2}) and seed	r^2	0.04	0.07	0.72	0.36
density (seeds m^{-2})	*P*	0.61	0.53	**0.01**	0.11
Herbaceous biomass (kg ha^{-1}) seed	r^2	0.02	0.23	0.25	0.07
density (seeds m^{-2})	*P*	0.72	0.23	0.21	0.51

There was a significant difference in seed mass, volume, density, seed sphericity and Corey Shape Factor (CSF) between all four species (Table 15). The seeds of *A. senegal* were significantly less spherical than any of the other species, while *A. nilotica* seeds were the most spherical. Seed mass decreased significantly between species as follows: *A. senegal* > *A. grandicornuta* > *A. nilotica* > *A. tortilis*. *A. nilotica* seeds were significantly less dense than all the other species, while *A. tortilis* seeds had a significantly smaller volume than all the other species (Table 15).

In comparison with seeds sampled from pods, *A. grandicornuta* and *A. tortilis* seeds sampled from *in situ* soil seed banks had smaller masses, volumes and densities (Table 15). Seeds of *A. nilotica* from the soil had a smaller mass and volume than seeds from pods, but a similar density. Seeds of *A. senegal* from the soil had a smaller volume but a similar mass and density to seeds sampled from pods. The sphericity and CSF of seeds sampled from pods and those sampled from seed banks were similar for all four species but seed bank seeds for *A. senegal* were slightly more spherical than pod seeds (Table 2 and 15).

Table 15 a) Comparisons of mass, volume, density, sphericity and Corey Shape Factor (CSF) (mean ± SE) of seeds sampled from *in situ* soil seed banks between four *Acacia* species in the Kruger National Park, South Africa. Means with different superscript letters are significantly different at the *P* = 0.05 level.

	Seed mass (g)	Seed volume (mm^3)	Seed density ($g\ mm^{-3}$)	Sphericity	CSF
A. grandicornuta (*n* = 87)	0.06 ± 0.003^{B}	49.39 ± 2.33^{B}	0.0012 ± 0.00006^{A}	0.12 ± 0.004^{B}	0.34 ± 0.012^{B}
A. nilotica (*n* = 36)	0.04 ± 0.007^{C}	48.85 ± 7.36^{B}	0.0005 ± 0.00008^{B}	0.04 ± 0.006^{D}	0.38 ± 0.053^{B}
A. senegal (*n* = 4)	0.11 ± 0.036^{A}	69.29 ± 2.38^{A}	0.0015 ± 0.00005^{A}	0.21 ± 0.015^{A}	0.22 ± 0.018^{C}
A. tortilis (*n* = 142)	0.03 ± 0.001^{D}	18.92 ± 0.64^{C}	0.0013 ± 0.00004^{A}	0.07 ± 0.002^{C}	0.48 ± 0.013^{A}

b) One-way ANOVA results for comparisons of mass, volume, density, sphericity and Corey Shape Factor (CSF) of seeds sampled from *in situ* soil seed banks between four *Acacia* species (d.f. = 3, 423).

	Seed mass (g)	Seed volume (mm^3)	Seed density ($g\ mm^{-3}$)	Sphericity	CSF
P	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
F	42.8	45.7	17.6	89.9	115.4

In comparison with seed viability of a sample of seeds taken from pods (Figure 4), the percentage of viable seeds sampled from *in situ* soil seed banks was lower. Tetrazolium staining of seeds sampled from the seed bank showed that no *A. senegal* seeds were viable, while 58% of 87 *A. grandicornuta* seeds, 61% of 36 *A. nilotica* seeds and 77% of 142 *A. tortilis* seeds were viable (Figure 13). There were more viable seeds in the leaf litter than in the 0-2 cm and 2-5 cm soil depth category for both *A. nilotica* (62, 30 and 8%, respectively) and *A. grandicornuta* (68, 23 and 9%, respectively), while more viable seeds in the 0-2 cm soil depth category (59%) than in the leaf litter (32%) and 2-5 cm soil depth category (9%) for *A. tortilis* (Table 16). For all three species, viability decreased with distance from the centre of the tree canopy (Table 16).

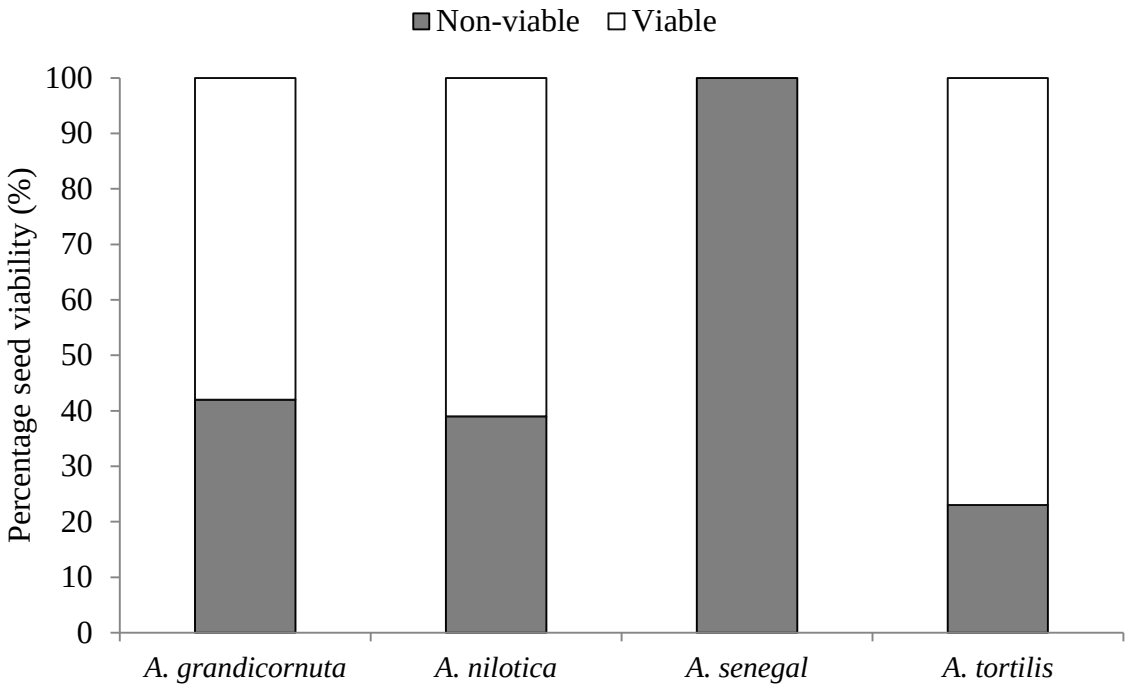

Figure 13. Percentage of viable seeds of the soil seed banks of *A. grandicornuta* (*n* = 87), *A. nilotica* (*n* = 36), *A. senegal* (*n* = 4) and *A. tortilis* (*n* = 142) in the Kruger National Park, South Africa.

Table 16. Percentage (%) of viable seeds sampled from *in situ* soil seed banks at different depth categories and micro-sites for three *Acacia* species, *A. grandicornuta, A. nilotica* and *A. tortilis* in the Kruger National Park, South Africa. *A. senegal* was not included as no viable seeds of this species were sampled from seed banks. The total number of seeds sampled per micro-site per species is in brackets next to the percentages, while the number of viable seeds sampled per species is listed below the species name.

	A. grandicornuta (*n* = 50)	*A. nilotica* (*n* = 22)	*A. senegal* (*n* = 0)	*A. tortilis* (*n* =109)
Under micro-site				
Litter	40 (29)	36 (9)	- (1)	14 (17)
0-2 cm deep	28 (18)	5 (3)	- (0)	45 (53)
2-5 cm deep	4 (3)	5 (1)	- (0)	6 (18)
Half-under micro-site				
Litter	16 (10)	23 (6)	- (1)	12 (14)
0-2 cm deep	0 (5)	14 (6)	- (0)	11 (12)
2-5 cm deep	0 (3)	0 (2)	- (0)	1 (4)
Edge micro-site				
Litter	2 (3)	5 (1)	- (1)	5 (7)
0-2 cm deep	2 (2)	5 (3)	- (0)	3 (5)
2-5 cm deep	4 (6)	5 (2)	- (1)	2 (7)
Beyond micro-site				
Litter	4 (5)	5 (1)	- (0)	2 (3)
0-2 cm deep	0 (3)	0 (1)	- (0)	0 (1)
2-5 cm deep	0 (0)	0 (1)	- (0)	0 (1)

2.5. Discussion

2.5.1. Pre-dispersal seed predation by bruchid beetles

In this study, a preliminary investigation of the pre-dispersal seed predation of a sample of current (newly produced) seeds removed from pods showed that bruchid beetle infestation occurred in all four species, with the amount of predation of 100 seeds varying between species as follows: *A. nilotica* (15.8%), *A. senegal* (5.5%), *A. grandicornuta* (2.5%) and *A. tortilis* (2.4%). There was no distinct pattern in bruchid beetle seed predation between indehiscent (*A. nilotica* and *A. tortilis*) and dehiscent seeds (*A. senegal* and *A. grandicornuta*). Larvae of species of the Bruchidae family feed exclusively on the seeds of flowering plants, more specifically Leguminosae (Ernst *et al.* 1990). Coe and Coe (1987) noted that bruchid

beetles are predominant *Acacia* seed predators. A study by Argaw *et al.* (1999) on woody plant demographics in an Ethiopian *Acacia* woodland showed that *A.* s*enegal* seeds (a dehiscent species, see van Wyk 1984) were favoured over *A. tortilis* seeds (an indehiscent species, see Coe and Coe 1987) by bruchid beetles. However, a study on bruchid beetle predation of savanna tree seeds such as *A. nilotica, A. tortilis* and *A. karroo,* showed that indehiscent *Acacia* seeds had a greater degree of bruchid beetle infestation than dehiscent seed species (Ernst *et al.* 1990). Johnson's (1981) investigation on bruchid beetle seed predation showed that 88% of bruchid beetle species investigated fed almost exclusively on seeds in indehiscent pods. Walters and Milton's (2003) study on seed ecology of two African *Acacias* showed less bruchid infestation of seeds in all stages of pod development of *A. karroo* (a dehiscent species, see van Wyk 1984) than *A. nilotica* (an indehiscent species, see Thomas and Grant 1997).

Coe and Coe (1987) suggest that because dehiscent seeds are often thinner and more discoid in shape, they are targeted by a smaller range of bruchid beetles than the larger and deeper indehiscent seeds. Szentesi and Jermy (1995) suggest that seeds that are more spherically shaped, or have a larger relative volume are often more susceptible to attack and infestation by bruchid beetles. A study by Garner and Witkowski (1997) on pre-dispersal predation in current seeds of three savanna species showed that more than four times more *A. nilotica* than *A. tortilis* seeds were predated by bruchid beetles. Additionally, seeds predated on had a smaller volume and predated *A. tortilis* seeds were less spherical than unpredated seeds.

In this study *A. nilotica* seeds, which were the most spherical of all four species and had a significantly larger volume than both *A. tortilis* and *A. grandicornuta* seeds had the highest percentage of bruchid beetle infestation. Although *A. senegal* seeds were the least spherical of all four species, the mean volume was also significantly larger than *A. tortilis* and *A. grandicornuta* seeds and again, *A.* s*enegal* had a higher percentage of bruchid beetle infestation than these two species. From the results it appears that a combination of high sphericity and large seed volume is the predominant factor influencing bruchid beetle infestation of current seeds.

Within a sample of current seeds removed from pods, the percentage of intact and aborted seeds varied between the four species. Eighty-seven percent of *A. tortilis* seeds were intact, followed by 81% of *A. grandicornuta*, 71% of *A. senegal* and 55% of *A. nilotica* seeds. Only 11% of *A. tortilis* seeds were aborted, followed by 16% of *A. grandicornuta*, 23% of *A. senegal* and 29% of *A. nilotica* seeds. In a study on the breeding systems of a number of *Acacia* species in Senegal and Kenya, it was found that seed abortion was lower in indehiscent seed species such as *A. tortilis* and *A. nilotica,* and the pattern of high seed abortion in dehiscent species such as *A. senegal* showed indications of selective seed abortion (Tybirk 1993). Some species may selectively abort ovules or fruit of inferior quality (Fenner and Thompson 2005). It has also been suggested that fruit may be aborted if subject to bruchid beetle oviposition (Forget *et al.* 1999). Indeed, in this study, species with a higher percentage of bruchid beetle infestation (e.g. *A. nilotica)* also had a higher percentage of seed abortion, while species with a lower percentage of infestation (e.g. *A. tortilis)* had a lower percentage of abortion.

2.5.2. Influence of seed characteristics on soil seed bank density

Current *A. nilotica* and *A. senegal* seeds collected from pods were similar in mass, volume and density to one another. The seeds of *A. tortilis* had the smallest mass and volume, followed by *A. grandicornuta.* The seeds of these two species had similar densities (see Table 2). The seeds of *A. nilotica* were the most spherical, followed by *A. tortilis*, *A. grandicornuta* and *A. senegal.* In comparison, seeds extracted from soil seed banks ('old' seeds) (Table 15) had slightly different characteristics. The seeds of *A. senegal* had the largest mass and volume; however, *A. grandicornuta* seeds were significantly heavier than *A. nilotica* seeds while, again, *A. tortilis* seeds had the smallest mass and volume. The seeds of both *A. grandicornuta* and *A. nilotica* had similar volumes, while *A. nilotica* seeds were significantly less dense than those of the other three species. Once again, *A. nilotica* seeds were the most spherical, followed by *A. tortilis, A. grandicornuta* and *A. senegal.* The seeds of *A. tortilis* from pods showed a positive relationship of sphericity with seed volume and mass; generally smaller *A. tortilis* seeds were also more spherical. No other species showed any such relationship, positive or negative. Generally seeds from pods were heavier and less spherical, with a larger volume than seeds from soil seed banks for all species except *A. senegal*, where both types of seeds had similar characteristics.

For *A. grandicornuta* and *A. tortilis*, seeds sampled from soil seed banks were smaller in mass, volume and density in comparison to seeds sampled from pods. The reason for this may be that smaller seeds are able to enter cracks in the soil surface as opposed to

larger seeds that would remain on the surface to be eaten or removed.

Thompson *et al.* (1993) stated that the size and shape of a seed can indicate whether a seed would persist in the soil or not. Smaller, more compact seeds are more easily buried by being washed into and penetrating cracks in the soil and therefore usually form persistent soil seed banks, while seeds forming transient soil seed banks are usually longer, flatter and elongated (Thompson *et al.* 1993). In a study of the soil seed banks of three savanna species, Garner and Witkowski (1997) also found more spherically shaped seeds buried at greater depths. Large seeds may be found in proportionally higher numbers above ground as their size prevents them from entering the soil due to the smaller size of cracks in the soil and of soil particles (Guo *et al.* 1998).

2.5.3. Influence of associated tree characteristics on soil seed bank density

In this study, *A. senegal* trees were significantly shorter in height (1.75 ± 0.10 m) than the three other species, with a significantly smaller tree canopy area (16.73 ± 2.50 m^2 $tree^{-1}$) and volume (20.07 ± 3.27 $m^3 tree^{-1}$). There was a significant positive relationship between tree height and seed bank density for *A. senegal,* indicating that a decrease in tree height would result in a decrease in soil seed bank density. The trees of *A. tortilis* also showed a significant positive relationship between height and seed bank density and were relatively tall (2.46 ± 0.08 m) with a large tree canopy area (90.76 ± 13.04 m^2 $tree^{-1}$) and volume (148.10 ± 20.35 m^3 $tree^{-1}$). In a study on the spatial distribution of soil seed banks in *A. tortilis, A. nilotica* and *Dichrostachys cinerea,* Witkowski and Garner (2000) showed that total seed store per parent plant increased with plant size for all species. In this study, only *A. nilotica,* with relatively short trees (2.30 ± 0.09 m) and a smaller tree canopy area (52.83 ± 7.73 m^2 $tree^{-1}$) showed a significant relationship between soil seed bank density and tree canopy volume (79.67 ± 11.03 m^3 $tree^{-1}$), indicating soil seed bank density would increase with canopy volume. The tallest trees were *A. grandicornuta* (2.61 ± 0.11 m), with a relatively large tree canopy area (59.98 ± 6.63 m^2 $tree^{-1}$) and volume (103.61 ± 11.36 $m^3 tree^{-1}$), but no relationship was found between these characteristics and soil seed bank density. There was no relationship of soil seed bank density with stem diameter, tree canopy area or bark thickness for any of the species. In a study on the vertical and horizontal distribution of desert plant seed banks, Guo *et al.* (1998) found that seed densities in the soil under desert shrubs was not clearly related to canopy size of the plants, but was related to seed abundance in the canopy.

In a comparison with van Wyk's (1984) description of these four *Acacia* species, the trees analysed in this study were all relatively small. Van Wyk (1984) describes *A. senegal* as a small tree (5 m in height) with a spreading dense crown, while *A. tortilis* trees are medium sized to large (11 m in height) with a wide spreading canopy. The smallish *A. nilotica* trees (7 m in height) are described as having a roundish crown and *A. grandicornuta* trees, which are described as small (9 m in height), have a spreading roundish crown.

2.5.4. Micro-site characteristics of associated tree environments

There was a significant negative relationship between soil compaction and herbaceous biomass for both *A. grandicornuta* and *A. tortilis*, which indicates that a decrease in soil compaction would influence an increase in herbaceous biomass. There was no relationship between soil compaction and herbaceous biomass for *A. senegal* or *A. nilotica.* There was no relationship of seed bank density with herbaceous biomass, soil compaction or over-storey canopy shading for any of the species except for *A. senegal,* which had a negative relationship between soil compaction and seed density.

Soil compaction was lower below tree canopies than in the open for all species and generally increased for all species with distance from the centre of the canopy. The soils around *A. senegal* were significantly more compacted than the three other species (3.7 ± 0.05 kg cm^{-2}) and herbaceous biomass was significantly lower for *A. senegal* (1739 ± 73 kg ha^{-1}) than the three other species. This may be because of the open and sparse canopy of *A. senegal* trees, which do not provide shade against heat and light.

Soil hardness and compaction has been cited as part of a group of factors that may reduce seed density in the soil (Garner and Witkowski 1997). The highest herbaceous biomass was found for *A. tortilis* (6272 ± 230 kg ha^{-1}) followed by *A. nilotica* (4285 ± 135 kg ha^{-1}) and *A. grandicornuta* (3035 ± 204 kg ha^{-1}). Significantly less compacted soil (0.9 ± 0.04 kg cm^{-2}) was found for *A. grandicornuta* than the other three species, probably as a result of the heavy rains experienced a few days before sampling was undertaken *(pers. obs).* Both *A. nilotica* and *A. tortilis* had similarly compacted soils (2.7 ± 0.07 kg cm^{-2} and 2.5 ± 0.06 kg cm^{-2}, respectively). The over-storey canopy shading of *A. senegal* trees was significantly less than the three other species (68.2 ± 2.1%), with *A. tortilis* having the most over-storey canopy shading (87.1 ± 1.9%), followed by *A. grandicornuta* (81.1 ± 53.9%) and *A. nilotica* (80.6 ± 5.8%).

In the KNP, granitic landscapes are characterized by a very distinctive catena, which comprises sandy soils on crests and clayey soils on footslopes, separated by a thin band of

grey hydromorphic soils called the seepline (Venter *et al.* 2003). The areas at the footslopes are called sodic sites. These are areas with soils that have accumulated minerals and fine particles and often have vegetation patterns that differ from normal savannas (Pickett *et al.* 2003). Sodic sites often have high sodium content, with hard and compact soils and usually border the riparian zone. Dye and Walker (1980) discuss the relatively shallow surface layer and largely impermeable B horizon characteristic of sodic soils. In this study, all trees sampled occurred within 900 m of a drainage line, except two *A. senegal* and two *A. tortilis* trees (Figure 14), and it is possible that the soils in the vicinity of the drainage lines are the hard compact sodic soils described above. Whether the trees sampled for seed banks occur in sodic soil does, however, warrant further investigation. Nonetheless, *A. grandicornuta* stands are common on these lower-lying sodium-rich soils (Joubert 2007) and often occur in brackish soils near drainage lines and rivers with *A. nilotica* and *A. tortilis* trees (Thomas and Grant 1997). Additionally, *A. senegal* is commonly found on alluvial soils (Schmidt *et al.* 2002)

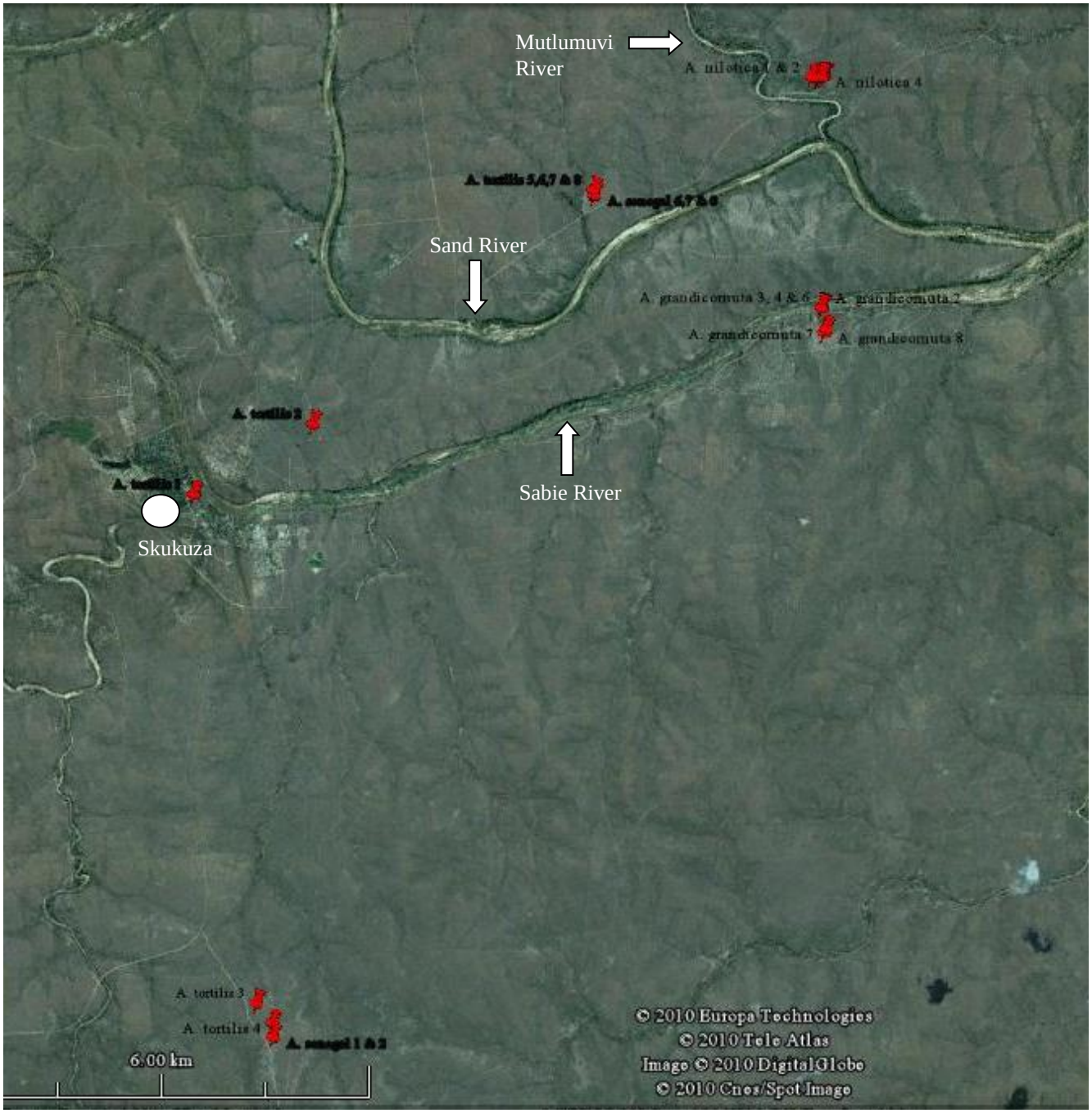

Figure 14. Sites of trees sampled for *in situ* soil seed banks. Red markers indicate individual trees sampled for soil seed banks, arrows indicate rivers and drainage lines and the circle indicates the Skukuza village in the Kruger National Park, South Africa (adapted from Google Earth).

This study showed that there was no significant difference in herbaceous biomass between the North and South aspects of trees of any species except for *A. grandicornuta*, where herbaceous biomass was significantly higher on the South aspects of trees. Herbaceous biomass was also slightly higher on the South aspects for *A. nilotica*, while slightly higher on the North

aspects for *A. senegal* and *A. tortilis.* There was a significant difference in soil compaction between the North and South aspects for all species, where the North aspects had a higher soil compaction, except for *A. nilotica,* where soil compaction was slightly higher on the South aspects. There was no significant difference in over-storey canopy shading between aspects for all species, although shading was slightly higher on the South aspects. Light intensity is generally greater on the North aspect of trees (Witkowski and Garner 2000). If the light intensity is greater and the over-storey tree canopy is sparser with less shading, it is possible that soil will become dry, hard and more compact. However, soil covered by herbaceous biomass may be less compacted, as the vegetation will retain soil moisture and shield the soil from heat and light *(pers. obs.).*

2.5.5. In situ soil seed bank density

The most dense soil seed banks was found for *A. tortilis* (19.5 ± 6.4 seeds m^{-2}), which produces indehiscent pods (see Coe and Coe 1987), while another indehiscent species, *A. nilotica,* formed relatively small seed banks (4.95 ± 1.80 seeds m^{-2}). Dehiscent pods are produced by *A. grandicornuta,* which had a relatively dense soil seed bank (12.08 ± 6.86 seeds m^{-2}). Although a small number of *A. senegal* seeds were found in the soil (0.6 ± 0.4 seeds m^{-2}), none of them were viable and therefore cannot constitute a soil seed bank. All of the seeds found in the soil were from the previous fruiting season (2005 or earlier) as seed bank sampling took place before the 2006 fruiting season started, and seeds from the soil were thereby classified as 'old' seeds. Additionally all seeds were brown in colour and were free from their pods. In a study on soil seed bank density of *A. nilotica* and *A. tortilis* in the Nylsvley Provincial Nature Reserve (NPNR) by Witkowski and Garner (2000), thirty-eight times more *A. nilotica* seeds from the previous fruiting season ('old' seeds) were found than those from seed banks sampled from the KNP in this study, while thirty-seven times more 'old' *A. tortilis* seeds were found in the NPNR than in this study. Seeds sampled from seed banks in this study were heavier than those collected in the NPNR (Garner and Witkowski 1997), and this may account for the smaller seed bank size (Table 17). Both *A. nilotica* and *A. tortilis* seeds sampled in this study were also less spherical than those of the same species from the NPNR (Table 17).

Table 17. Seed mass and sphericity (mean ± SE) for *A. nilotica* and *A. tortilis* and soil seed bank size comparisons for those species, between this study in the Kruger National Park and the study in the Nylsvley Provincial Nature Reserve (Garner and Witkowski 1997; Witkowski and Garner 2000), both in South Africa.

	KNP	NPNR
Mass (g)		
A. nilotica	0.11 ± 0.004	0.09 ± 0.004
A. tortilis	0.04 ± 0.002	0.03 ± 0.001
Sphericity (variance in seed dimensions)		
A. nilotica	0.06 ± 0.003	0.05 ± 0.003
A. tortilis	0.11 ± 0.002	0.06 ± 0.003
Seed bank density (sample size)		
A. nilotica	36	1382
A. tortilis	142	5220

A number of seed bank studies have shown a higher density of seeds in the upper layers of soil and also show that soil seed bank density decreases with distance from the centre of the associated tree canopy on a horizontal scale (Guo *et al.* 1998; Argaw *et al.* 1999; Witkowski and Garner 2000; Wilson and Witkowski 2003). In this study, *A. tortilis* trees had significantly denser soil seed banks in micro-sites under the tree canopy (12.2 ± 3.8 seeds m^{-2}), while there was no significant difference in seed density between micro-sites for any of the other species. However, there was a general decrease in seed bank density with an increase in distance from the centre of the tree canopy for all species except *A. senegal.* In a study on the germination strategy of *Acacia tortilis*, Loth *et al.* (2005) found that soil seed banks were denser under *Acacia* tree canopies than in the open. Over-storey canopy shading also influences seed persistence in the soil and seed banks in the open are expected to be less dense as a result of heat from the sun (Witkowski and Garner 2000).

Most seeds of all species were generally found amongst the leaf litter and the second soil depth category (0-2 cm), but there was no significant difference in seed densities between different depth categories for any of the species, perhaps due to the overall high variability between samples and the relatively low number of replicates *(n* = 8 trees/species). In a soil seed bank study conducted in the Nylsvley Provincial Nature Reserve, seeds in the litter were comprised mostly of current seeds (seeds from the current season's seed rain) for *A. nilotica*,

A. tortilis and *D. cinerea* (Witkowski and Garner 2000). Seeds in the seed bank sampled in this study were assumed to be from the previous year's fruiting season ('old' seeds), as sampling was done before the new fruiting season. No 'current' seeds were sampled, and a slightly higher percentage of 'old' seeds were found in the leaf litter than in the soil layer 0-2 cm deep for *A. grandicornuta* and *A. nilotica,* while a higher percentage of 'old' seeds were found in the soil layer 0-2 cm deep for *A. tortilis* than in the litter layer. Argaw *et. al.* (1999) attribute the trend of seed density decreasing with depth in the soil to the relatively large size of *Acacia* seeds; larger seeds may not penetrate the soil as easily or deeply as smaller seeds. Rotundo and Aguiar (2005) suggest that leaf litter may present a mechanical barrier to burial of seeds in the soil, although at the same time increasing the longevity of seeds.

Auld and Denham (2006) hypothesize that a reduction in seed germination of *Acacia* seeds at depths in the soil greater than 5 cm is a result of a combination of the following factors: reduced ability of fire cues that break seed dormancy, a declined ability of seeds to emerge from such depths successfully and a lower abundance of seeds in the soil at such depths. Therefore, it may not benefit regeneration for these *Acacia* seeds to be buried deeper than 5 cm below the surface of the soil. However, heavy disturbances may bring deeply buried seeds to the surface of the soil.

Soil seed bank density was significantly greater on the North aspect of *A. grandicornuta* trees (9.86 ± 6.37 seeds m^{-2}) than on the South aspect (2.2 ± 0.72 seeds m^{-2}). There was also less herbaceous biomass on the North aspects of trees of this species. Herbaceous biomass may prevent seeds from reaching the surface of the soil, reducing the chance of seeds becoming incorporated into the soil. For *A. tortilis* and *A. nilotica,* seed bank density was lower on the North aspects of trees, while for *A. senegal* seed bank density was the same on both aspects. As mentioned above, light intensity is often greater on the North aspect of trees (see Witkowski and Garner 2000). This may also explain the lower seed bank density on the North aspect of trees for *A. nilotica* and *A. senegal,* as a greater light intensity may cause more compacted soil, which makes incorporation of seeds into the soil more difficult.

2.5.6. Seed viability

There were no viable *A. senegal* seeds sampled from the seed bank, indicating that *A. senegal* seeds are possibly unable to persist in the soil for up to eight months (minimum age of old' seeds in the soil seed bank). However, 77% of *A. tortilis*, 61% of *A. nilotica* and 58% of *A. grandicornuta* seeds from the seed bank were viable. Wilson and Witkowski (2003) mentioned that low seed viability of *Burkea africana* Hook. seeds in the soil (viability of 45%) is

typical for a resprouting species. Most *Acacia* species are able to resprout and usually tall single-stemmed trees represent reseeders, while short multi-stemmed trees represent resprouters (Midgley and Bond 2001). The absence of viable seed bank seeds in *A. senegal* may then be typical of a resprouter, as this tree is usually short and multi-stemmed. However, *A. grandicornuta*, which is a taller single-stemmed tree, had a lower percentage of viable seeds in the soil than the shorter multi-stemmed *A. tortilis*, while the similarly short and multi-stemmed *A. nilotica* had a similar percentage of viable seeds to *A. grandicornuta*. It must be noted that the four *Acacia* species disperse their seeds in June and July. As seed bank samples were done between February and April, all seeds sampled from soil seed banks were at least eight months old and had survived the dry season and most of the following wet season. This means that any seeds sampled from the soil would have persisted for at least eight months and possibly more than a year, indicating that species with viable seeds in the soil are possibly able to form short-term persistent seed banks.

In this study, twice the percentage of viable *A. grandicornuta* and three times the percentage of viable *A. nilotica* seeds were found in the leaf litter than at 0-2 cm depth in the soil, while almost twice the percentage of viable *A. tortilis* seeds were found at 0-2 cm depth in the soil than in the litter layer. Relatively low percentages of viable seeds were found at 2-5 cm depth in the soil for all three species. No viable *A. senegal* seeds were found. For all three species with viable seeds the percentage of viable seeds decreased with distance from the tree canopy centre and seeds persisted in the soil under tree canopy cover. This may be a result of the protection from heat that tree canopy shading provides. Witkowski and Garner (2008) found that seed viability of an alien invasive species, *Solanum mauritianum* (bugweed) increased with depth of burial in the soil and was higher under canopy shade. In a study of seed banks of another African savanna tree, *Burkea africana*, Wilson and Witkowski (2003) noted that seeds on the soil surface had a lower viability than buried seeds. When analysing the viability of seed bank seeds of three savanna species, including *A. tortilis* and *A. nilotica*, Witkowski and Garner (2000) found that seed viability increased with depth of burial in the soil, except for *A. nilotica* at a depth of 3-5 cm. They attribute the lack of viability of *A. nilotica* seeds at a greater depth to seeds that have aged to senescence.

In comparison with seeds in the soil, viability of a sample of seeds collected from pods was higher for all species, namely 81.2% of *A. grandicornuta*, 71.1% of *A. senegal* and 87.1% of *A. tortilis*, except for *A. nilotica*, of which only 55.2% of seeds from pods were viable.

2.5.7. A brief analysis of the sampling method

A few studies have used the 'germination-method' to test the presence and composition of seed banks in the soil (e.g. Thompson and Grime 1979; Bigwood and Inouye 1988; Pugnaire and Lazaro 2000), which is allowing all the seeds in a sample of soil to germinate. However, as pointed out by Thompson *et al.* (2003) this method fails in determining the presence and composition of dormant seed species, as it is possible that germination is delayed by dormancy. Often only a fraction of the seeds available in the soil germinate as seedlings (Fenner 1987). Extracting naturally buried seeds and determining viability is the least ambiguous method of estimating longevity of seeds (Thompson *et al.* 2003). Accordingly, this study did not use the germination method but employed a method whereby samples of soil were sifted for seeds allowing all seeds, dormant or not, to be counted as being present in the soil seed bank. Seeds were then tested for viability. However, it is possible that because *Acacia* seedlings are shade-intolerant (Smith and Shackleton 1988) that sampling for seed banks under tree canopies becomes problematic, as these are poor sites for seedlings (Midgley and Bond 2001). Midgley and Bond (2001) further suggest that germination of dispersed seeds is more likely than that of seeds undispersed beneath the tree canopy. This study showed that seed bank density generally decreased with distance from the centre of the canopy and fewer seeds were found in the soil at micro-sites away from tree canopy shading. Other demographic factors and their effects on seed bank dynamics in a species also need to be analysed, such as germination, as this affects the number of seeds removed from a seed bank, and seedling establishment, to fully understand recruitment and regeneration in a plant species.

2.5.8. Conclusion

The results of this study suggest that there are a number of characteristics of seeds, trees and micro-sites that may influence soil seed bank density. It is possible that seed characteristics such as volume, mass and sphericity affect entry of seeds into the soil and the density of soil seed banks but pod type may not. Although *A. tortilis* had the lowest percentage of bruchid beetle predation and the densest soil seed banks, no other similar pattern was found for the other three species, indicating that predation may have a relatively small effect on seed bank density. *A. senegal* and *A. tortilis* tree height and *A. nilotica* tree canopy volume were weakly related to the density of soil seed banks, while other characteristics such as bark thickness, stem diameter and tree canopy area were not related to seed bank density for any of the four species. There were no significant results that showed herbaceous biomass, soil compaction or over-storey canopy had any effect on soil seed bank density for any of the species except for

A. senegal, for which seed bank density decreased with an increase in soil compaction. Most seeds of all species, except *A. senegal,* were found in the litter layer and 0-2 cm soil depth category as well as at micro-sites under the tree canopies (half-under and under canopy micro-sites). Three species, namely *A. grandicornuta, A. nilotica* and *A. tortilis* had viable seeds in the seed bank, indicating that these species are possibly able to form short-term persistent seed banks (seeds persist in the soil for between one and five years). No viable *A. senegal* seeds were found, indicating that these seeds are transient and do not form persistent seed banks. Species such as *A. nilotica and A. tortilis* are capable of forming at least short-term persistent seed banks, as seen in the Nylsvley study (Garner and Witkowski 1997; Witkowski and Garner 2000).

Although understanding the dynamics of soil seed banks of *Acacia* trees is essential in building knowledge of recruitment in these trees, there are other aspects of recruitment such as seed dispersal, germination and seedling establishment that are important to understand population dynamics of selected trees (Burke 2006). In order to fill in the knowledge gaps of *Acacia* tree recruitment and aid management of these species in southern African savannas, further studies on the aspects of these demographics listed by Burke (2006), such as seed dispersal, germination and seedling establishment, is required.

2.6. References

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CHAPTER 3

Persistence of seeds of four *Acacia* species under experimental field and greenhouse burial trials

Abstract

If seeds are able to persist in the soil for extended periods of time without germinating, a seed bank of dormant seeds may be formed, which can benefit plant regeneration in unpredictable and harsh environments. Two seed burial trials were conducted to determine seed persistence of four *Acacia* species from the Kruger National Park (KNP), South Africa. A field seed burial trial was conducted in the Nkhuhlu exclosures, near Skukuza, Kruger National Park, South Africa, from December 2005 to April 2007 and a greenhouse seed burial trial was conducted at the University of the Witwatersrand, Johannesburg, South Africa from April 2006-March 2007.

Four hundred seeds/species were used in the field burial trial. Fifty seeds each of *A. grandicornuta, A. senegal* and *A. tortilis* and 100 *A. nilotica* were destroyed by fire during the trial. Of the remaining seeds, 15% of *A. senegal,* 19% of *A. grandicornuta,* 34% of *A. nilotica* and 66% of *A. tortilis* remained intact after 16 months in the field. Of these, 65% of *A. tortilis,* 27% of *A. nilotica,* 5% of *A. grandicornuta* and no *A. senegal* seeds were still viable. The percentage of remaining intact, viable seeds was highest under tree canopy cover and buried for *A. tortilis* (86%), *A. nilotica* (39%) and *A. grandicornuta* (6%), but the micro-site placement of seeds had a significant effect on viability for *A. nilotica* only (d.f. = l; $\chi^2 = 7.5$; $P = 0.006$). For *A. nilotica* and *A. grandicornuta* there were no viable seeds in the open (no canopy cover) and on the soil surface, while 27% of *A. tortilis* seeds were viable. The number of intact, viable seeds in the open and buried was low for all species, namely 58% of *A. tortilis,* 17% of *A. nilotica* and 5% of *A. grandicornuta* seeds, while 55% of *A. tortilis,* 24% of *A. nilotica* and 5% of *A. grandicornuta* seeds under canopy cover and on the soil surface were viable.

The percentage of 450 seeds/species that germinated after one year in the greenhouse seed burial trial was low, namely, 2.9% of *A. grandicornuta,* 0.7% of *A. senegal* and 0.2% of both *A. nilotica* and *A. tortilis* seeds. *A. tortilis* had the highest percentage of remaining intact, viable seeds (92.2%), followed by *A. nilotica* (58.3%), *A. grandicornuta* (57.6%) and *A. senegal* (0%).

Eight seeds of all species combined germinated when watered with the highest rainfall, six seeds with the lowest and four seeds with the average rainfall. The number of remaining intact, viable seeds was highest when watered with the average rainfall (327 seeds), followed by

the highest (314 seeds) and the lowest rainfall (296 seeds). There was no association between rainfall treatments and the number of remaining intact, viable seeds for any of the species, except *A. grandicornuta* where the number of remaining intact, viable seeds increased significantly with the average rainfall.

A. tortilis, A. nilotica and *A. grandicornuta* seeds are able to persist in the soil to form at least short-term persistent soil seed banks (seeds that persist in the soil for 1-5 years). *A. senegal* seeds are transient and do not form soil seed banks. Understanding seed persistence in soil seed banks will assist KNP management in filling gaps in understanding of *Acacia* plant demography in the conservation of this southern African savanna.

Keywords: persistence, seed banks, *Acacia,* seed burial trials, germination.

3.2. Introduction

Seed burial trials are used to determine the longevity and dormancy of seeds and are usually carried out under laboratory or field conditions that are closely monitored. Seed burial trials have been used in many studies to investigate seed characteristics and movement and seed fate in soil seed banks (Kalisz 1991; Auld 1995; Hill and Vander Kloet 2005; van Mourik *et al.* 2005). Seeds used for burial trials are stored or buried under particular conditions and exhumed after a specific time period (Fenner and Thompson 2005). In designing seed burial trials, the investigator is able to manipulate environmental characteristics to fulfill the objectives of the study. Soil type, ambient temperature, humidity, rainfall, seed species and seed density in the soil can all be manipulated under laboratory conditions. Unfortunately, such specific conditions do not occur in nature and consequently, laboratory results regarding seed dormancy and persistence in the soil may differ to those obtained from *in situ* soil seed banks. The simplification of environmental conditions must be considered when analysing results of laboratory seed burial trials. Often only intact seeds are selected for burial trials, while not all seeds that enter the natural seed bank will be viable or intact. Van Mourik *et al.* (2005) suggest that analysing rates of seed movement and fate with seed burial trials may skew results as seed densities in experimental bags are usually higher than densities found naturally, thereby potentially elevating levels of pathogen activity in the bags.

Understanding the theories concerning seed dormancy and persistence is important to determine the potential of seeds to form soil seed banks. Dormancy and persistence must be accurately defined in order to understand how these processes affect seeds and seed fate. Dormancy is described by Taylorson and Hendricks (1977) as the long-term persistence of viable

seeds, or the arrest in development of seed embryos under conditions otherwise suited for growth. When viable seeds (seeds with the potential to germinate) experience a state of desiccation and do not germinate even when placed in an environment normally conducive to germination, they are considered dormant (Roberts 1972; Mayer and Poljakoff-Mayber 1975; Baskin and Baskin 1989). If seeds are able to remain dormant in the soil for many years, a genetic reserve will begin to accumulate, which is beneficial to the regeneration of plant communities in unpredictable and harsh environments. There are a number of advantages of seed dormancy to plant populations: seeds only germinate when environmental conditions favour seedling survival, dormant seeds create a seed bank to ensure that not all seeds of a species germinate in a single year, and the dormancy of seeds can allow the synchronization of germination at a particular time of year (Hartmann *et al.* 2002).

There are different types of dormancy described by seed ecologists. When dormancy occurs at a time when the external environment may appear favourable for seed metabolic, synthetic or morphogenetic activities, it is assumed that dormancy is endogenously controlled (Koller 1972). The failure to germinate primarily because of factors within the embryo is called endogenous dormancy (Hartmann *et al.* 2002). Physical dormancy that results from the presence of hard seed coats is called exogenous physical dormancy or seed coat dormancy (Hartmann *et al.* 2002). Hard seed coats act as mechanical barriers to germination and allow seeds to remain potentially viable for several decades without germinating (Taylorson and Hendricks 1977; Mayer and Poljakoff-Mayber 1975; Coe and Coe 1987; Bradbeer 1988; Murdoch and Ellis 1992). Physical dormancy is common in plants from the Leguminosae family, such as the woody *Acacia* species (Coe and Coe 1987).

Hard seed coats are very resistant to abrasion and are covered in a wax-like layer, which causes the seed to be impermeable to water (Mayer and Poljakoff-Mayber 1975) so that imbibition does not occur and germination is prevented (Baskin and Baskin 1989). This impermeability to water is a result of both structural and chemical changes that take place during the late stages of hard-coated seed maturation (Kigel 1995). The lack of imbibition of water keeps the embryo dry, which is crucial for the maintenance of dormancy (Fenner and Thompson 2005) and consequently, African *Acacia* seeds that are kept dry are able to remain dormant for many years (Tybirk *et al.* 1992). Coe and Coe (1987) conducted a germination experiment with *Acacia heteracantha* (Burch.) Brenan seeds and noted that germination events of the seeds were affected by factors that influence the permeability of the testa, such as seed coat thickness. Large hard-coated seeds such as those of the Leguminosae are usually long-lived while smaller soft-coated seeds are not (Chambers and MacMahon 1994). African *Acacias* are classified into two

distinct groups according to the method by which the seeds dispersed (Coe and Coe 1987). In dehiscent pods the fruit's pericarp splits and seeds are dispersed by wind or gravity, while indehiscent seeds are not released from the pericarp, rather the whole fruit along with the seeds remains intact and on the tree (or on the ground) until browsed or removed (Bradbeer 1988). Seeds from indehiscent pods are thick and robust, while seeds from dehiscent pods are thin and discoid in shape (Coe and Coe 1987).

Although non-dormant seeds are often less persistent in the soil than dormant seeds, it is not realistic to predict persistence of seeds in the soil simply based on a seed's ability to remain dormant (Thompson *et al.* 2003). There are many other factors that occur together to influence the persistence of seeds in the soil. Persistence is when a seed remains viable in the soil and seeds may be persistent even though they are non-dormant. However, dormancy is the characteristic that allows a seed to remain viable and ungerminated. A persistent seed is therefore not necessarily a dormant one. Thompson *et al.* (1993) note that persistence in the soil can be determined by the seed's germination requirements, dormancy mechanisms and resistance to pathogens. Thereby dormancy and persistence are only very weakly correlated and a species does not require dormant seeds to form a persistent seed bank (Thompson *et al.* 2003).

There are a number of ways to define soil seed banks. Price *et al.* (2010) noted that classification of soil seed banks is one of the most important demographic assessments that can be made for a plant community. Thompson and Grime (1979) suggested that persistent seed banks contain seeds that persist in the soil for at least one year. Csontos and Tamás (2003) defined two main types of seed banks: persistent and transient. They suggested that persistent seed banks are formed by seeds that remain dormant and viable in the soil for more than a year once they have been dispersed, while a seed bank is transient if seeds remain dormant and viable for less than a year. Thompson *et al.* (1993) and Fenner and Thompson (2005) described seeds persisting in the soil for less than a year as transient, seeds persisting in the soil for between 1 and 5 years as forming short-term persistent seed banks and those persisting in the soil for over five years as forming long-term persistent seed banks.

The main aim of this study was to analyse the persistence of seeds of four *Acacia* species, from the Kruger National Park, in the soil. The following objectives were developed to fulfill the aim of this study:

1. To investigate the ability of seeds to remain persistent in the soil at different horizontal and vertical micro-site positions, under natural field conditions for over a year.

2. To investigate the ability of seeds to persist ungerminated while buried in the soil for one year when watered according to three different rainfall records from Skukuza in the Kruger National Park.
3. To further understand the persistence, germination and viability of seeds of four *Acacia* species in the soil over various periods of time and environmental conditions.

3.3. Materials and methods

3.3.1. Study site

Seeds used in the seed burial trials were collected from trees in the vicinity of Skukuza in the Kruger National Park (KNP). Skukuza falls under the Skukuza land system, which is 19.6% (382 045 ha) of the KNP's total area (Venter *et al.* 2003). This land system is underlain by granitic rock and vegetation is broad-leaved bushveld dominated by moderately dense *Combretum apiculatum* Sond. subsp. *apiculatum* (red bushwillow) and *Combretum zeyheri* Sond. (large-fruited bushwillow) bushveld, and fine-leaved savannas with open *Acacia gerrardii* Benth. var. gerrardii (red thorn) and *Euclea divinorum* Hiern (magic guard) shrubveld with prominent *Combretum hereoense* Schinz (russet bushwillow) and *Acacia nigrescens* Oliv. (knob thorn) vegetation. Skukuza receives an average of 537 mm of rain annually and daily temperatures range between 20°C and 32°C in the hot summer months and 5°C and 26°C in the cooler winter months (Venter *et al.* 2003). Further details regarding the study site can be referenced from Chapter 2.

The field seed burial trial was conducted in one of the six treatments of the Nkhuhlu exclosures, specifically the fully fenced area (70 ha) (see Siebert and Eckhardt 2008). The exclosures are located 18 km downstream of Skukuza, parallel to the Sabie River and cover a total area of 139 ha (Siebert and Eckhardt 2008). Soils of the exclosures are shallow and sandy on the crests and deep sandy to sandy loam at the river bank. Footslope soils are duplex and sodium-rich and because of their high nutrient status, are favoured by animals (Siebert and Eckhardt 2008). Woody plant species conspicuously present in the exclosures include *Acacia nigrescens, Combretum apiculatum, Grewia bicolor* Juss. (white-leaved raisin), *Dichrostachys cinerea* (L.) Wight & Arn. (sickle-bush), *Euclea divinorum, Acacia grandicornuta* Gerstn. (horned thorn) and *Spirostachys africana* Sond. (tamboti) (Siebert and Eckhardt 2008).

3.3.2. Field seed burial trial

Pods of four *Acacia* species were collected from mature fruit-bearing trees during July

2005 from the 2004-2005 growing season, namely *A. grandicornuta* Gerstn. (horned thorn), *A. nilotica* (L.) Willd. ex Del. subsp. *kraussianna* (Benth.) Brenan (scented-pod thorn), *A. senegal* (L.) Willd. var. *rostrata* Brenan (bushy three-hook thorn), and *A. tortilis* (Forssk.) Hayne subsp. *heterocantha* (Burch.) Brenan (umbrella thorn).

Pods were de-seeded and seeds were classified as intact (large and swollen with no indications of bruchid beetle predation), predated (seeds with bruchid beetle exit holes and with the inside partially or completely eaten) or aborted (seeds that are flatter, less plump, small and shrivelled in comparison to intact seeds) (see Wilson and Witkowski 2003). Only intact seeds were selected for use in the field seed burial trial. Fifty intact seeds of each species were mixed in with a small amount of soil and placed in nylon bags of approximately 600 cm^2 in size. Eight nylon bags were made per species. One i-button (data-loggers used to record temperature at specific intervals) was placed in each bag. Each bag contained seeds from a single species only. High quality 2 mm nylon mesh was used to make the bags as this mesh size is small enough to retain seeds, but large enough to allow movement of small soil animals and soil moisture (Auld 1995). The soil for the bags was obtained on site and taken from the small holes that were dug to bury the nylon bags. The soil was sieved before being used in the burial trial to remove all seeds that may already be present. Once filled with a mixture of soil and seeds, each bag was tied closed and marked with bright red wire, which would facilitate easy retrieval of bags at a later stage. No insecticide or fungicide was added to the seed burial trial.

The burial trial was established in the Nkhuhlu exclosures on the 5th and 6th of December 2005 (Plate 1). A section of the exclosures is fully fenced (Figure 2), which prevents all large mammals from entering the area and this eliminated any disruptions to the burial trial as a result of large herbivore activity. In the initial experimental design, it was intended for nylon bags to be placed at various positions in the environment associated with the *Acacia* tree species of the seeds in the bag. However, on inspection of the tree species within the exclosure it was found that there were no *A. tortilis* or *A. nilotica* trees evidently present in the fully fenced area of the exclosure. Siebert and Eckhardt (2008) record that *A. tortilis* occurs sparingly through the exclosures in dry sodic savanna, tall bushveld and riverbank scrub. One of the dominant tree species in sodic bushveld and sodic patches is *A. grandicornuta.* These patches are plant sub-communities conspicuously present in the fully-fenced section of the exclosures. Additionally, *A. nilotica* and *A. senegal* also occur in sodic bushveld plant communities (Siebert and Eckhardt 2008). Consequently, bags containing *A. grandicornuta*, *A. nilotica* and *A. tortilis* seeds were all placed in the environment associated with *A. grandicornuta* trees, while bags containing *A. senegal* seeds were placed in the environment associated with *A. senegal* trees. For each

species, four nylon bags were placed at different micro-sites within the environment of two trees (Figure 1). Therefore, there were thirty-two replicates overall. The following micro-sites were used:

1. In the open (2 m from the edge of the associated tree canopy) and on the surface of the soil.
2. In the open (2 m from the edge of the associated tree canopy) and buried at a depth of 3-5 cm below the surface of the soil.
3. Under the canopy of the associated tree (next to the base of the trunk) and on the surface of the soil.
4. Under the canopy of the associated tree (next to the base of the trunk) and buried at a depth of 3-5 cm below the surface of the soil.

The position of each associated tree was recorded using a GPS (Global Positioning System) and each tree was marked with red tape. A metal peg was inserted into the ground along with the bags so that a metal detector could be used to accurately locate the bags at each site, as over time these bags tend to become completely buried (E.T.F. Witkowski, *pers. comm.*). The peg also ensured the bags placed on the ground surface were secured.

The bags were excavated from the exclosure on the 14th and 15th of April 2007. They were then taken back to the laboratory where the soil was sieved and any remaining seeds removed. Seeds were counted and categorized as intact, decomposed or predated. Viability tests were done on intact seeds using a 1% tetrazolium salt staining solution (2,3,5-triphenyl-tetrazolium chloride; Moore 1985; Mbalo and Witkowski 1997). Seeds only partially stained were assumed to have low viability and were recorded as non-viable (Mbalo and Witkowski 1997).

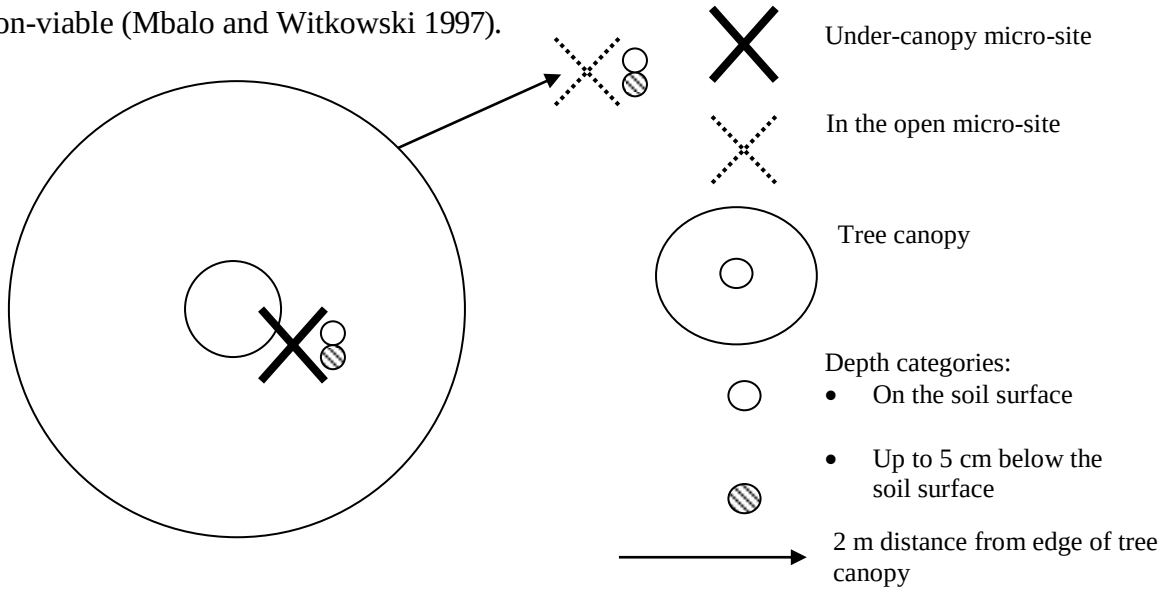

Figure 1. Micro-site and depth placement of nylon bags for the field seed burial trial in the fully fenced section of the Nkhuhlu exclosures near Skukuza, Kruger National Park, South Africa.

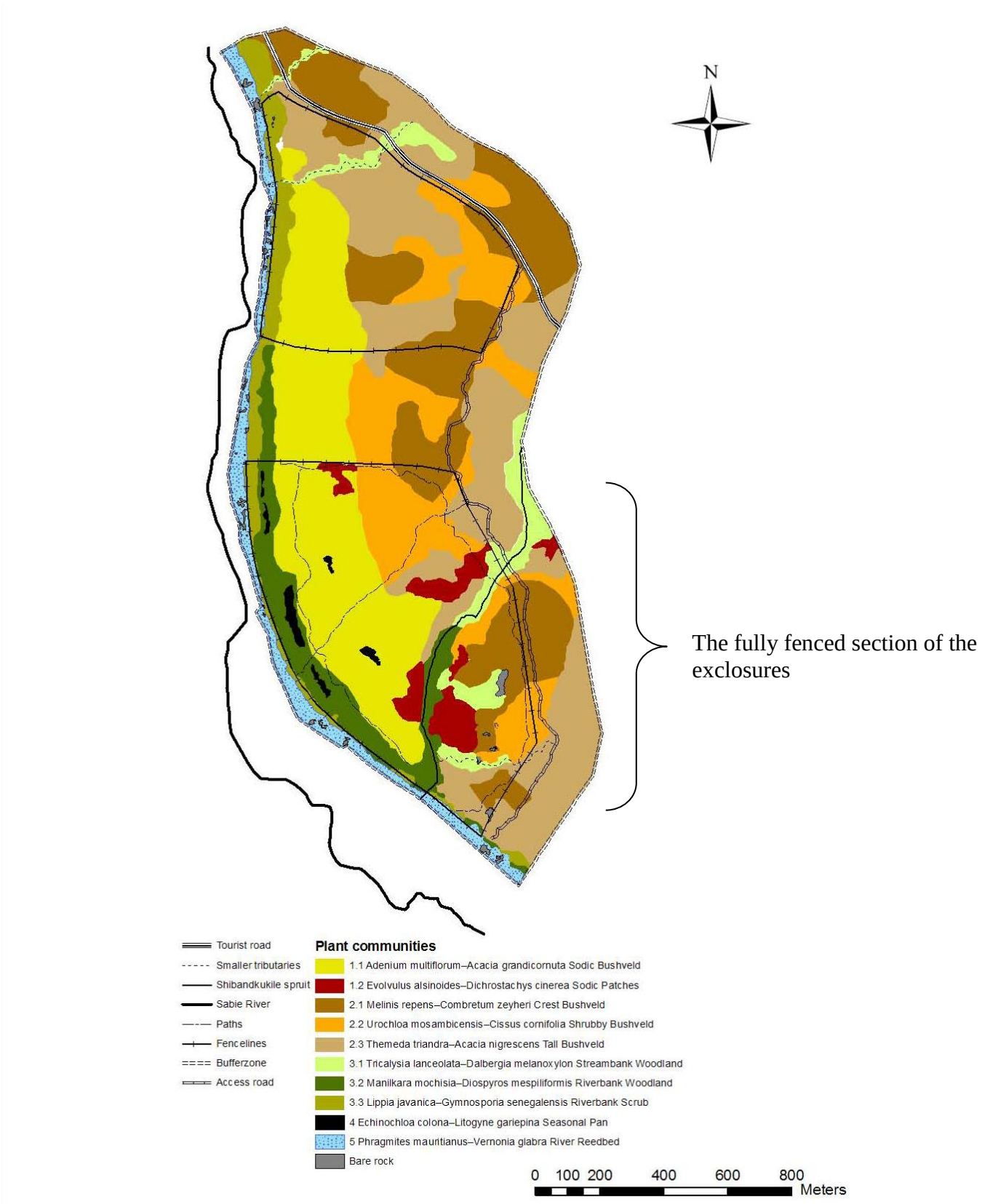

Figure 2. Vegetation map of the Nkhuhlu exclosures near Skukuza in the Kruger National Park, South Africa, showing the section that is fully fenced (adapted from Siebert and Eckhardt 2008). The fully fenced exclosure was used for the field seed burial trial.

3.3.2.1 Data and statistical analyses

Contingency table χ^2 tests with Yates corrections were done to test the effect of placement of seeds at different micro-sites, namely associations between shading (canopy cover vs. in the open) and burial (buried 3-5 cm underground vs. placed on the soil surface) for a) all intact seeds remaining and b) seeds remaining intact and viable, after completion of the field seed burial trial, as well as associations between a) seed viability and the effect of canopy shading and b) seed viability and the effect of burial in the soil, for each species. Two sets of χ^2 tests were done for all intact seeds remaining, namely a) including seeds destroyed by fire and b) excluding seeds destroyed by fire. All data are presented as percentages. Analyses of data were done using Microsoft Office Excel 2007.

Plate 1. Images of the field seed burial trial conducted in the fully fenced portion of the Nkhuhlu exclosures near Skukuza, Kruger National Park, South Africa, from December 2005 -April 2007. Clockwise from top left: digging holes to bury nylon bags; counting seeds for nylon bags; a completed nylon seed bag ready for placement; preparing nylon bags; and *A. senegal* seeds in a nylon seed bag.

3.3.3. Greenhouse seed burial trial

Permission was granted by relevant authorities from the Kruger National Park to harvest approximately 600 kg of granitic soil from a landfill site near Skukuza. The soil was sieved with a standard 2 mm soil sieve, which has pore sizes small enough to allow retention of any *Acacia* seeds but large enough to allow the majority of the soil to pass through (Witkowski and Garner 2000). This was done to remove any *Acacia* seeds that may already have been present in the soil and which would affect the burial trial results.

During July 2005 pods were sampled from mature fruit-bearing trees of the four *Acacia* study species, namely *A. grandicornuta, A. nilotica, A. senegal* and *A. tortilis.* Pods were de-seeded and seeds were categorized as intact, predated or aborted. Only intact seeds, which are large and swollen with no indications of predation (Wilson and Witkowski 2003), were used in the seed burial trial. The trial was set up in the greenhouse at the University of the Witwatersrand (hereon Wits University), Johannesburg, South Africa, from the 22nd - 30th March 2006 (Plate 2). Three tables of twelve 30 cm x 30 cm in area x 10 cm in depth black plastic seed trays filled with the sieved granitic soil were set up in the greenhouse (Figure 3). The trays were arranged in a pattern of four rows and three columns on each table. Each row corresponded to a separate *Acacia* species and the columns represented replicas of the experiment for each species. Fifty intact seeds of each species were buried at a depth of 4-5 cm in the soil at regular intervals in the seed trays. This means that there were 450 replicates overall per species. As seed density of most species declines with depth in the soil (Fenner and Thompson 2005) and as previous studies of similar savanna species have shown that the majority of seeds are found in the top 5 cm of the soil (Garner and Witkowski 1997; Witkowski and Garner 2000), seeds were not buried deeper than 5 cm. The seeds were not scarified before being buried. The position of each seed was marked with a toothpick.

The daily amount (mm) of rainfall in Skukuza from the year 1911 to 2005 was obtained from the KNP's records. The annual rainfall for each year was calculated, as was the mean annual rainfall over 94 years. The data were compared to find the years with the highest annual rainfall (1122.8 mm annum^{-1} in 1999/2000) and lowest annual rainfall (237.4 mm annum^{-1} in 1991/1992), as well as the year with the most similar annual rainfall (523.5 mm annum^{-1} in 1971/1972) to the overall mean annual rainfall (Appendix 1). The amount of daily rainfall recorded per year for the three selected years was used to water the three tables of seeds, in order to compare seed viability and persistence under three different rainfall treatments. Firstly, the

total area (m^2) of seed trays per table was calculated. Each seed tray was 0.09 m^2 in size; therefore twelve seed trays covered an area of 1.08 m^2. Secondly, rainfall in millimetres per day was converted to water in millilitres per day as follows: 1 mm of rain over one square metre equals one litre of water (100 cm x 100 cm = 1 m^2, while 100 cm x 100 cm x 0.1 cm of rainfall = 1000 cm^3, where 1000 cm^3 is one litre) (N. Zambatis *pers. comm.).* For example, the record for the highest annual rainfall received (1999/2000) showed that on the 02nd April 1999 Skukuza received 4.0 mm of rain. This is 4.0 litres of water over 1 m^2. However, as the area of the table requiring water is 1.08 m^2, the figure in litres was multiplied by 1.08, to give a total of 4.32 litres of water required for watering the seeds in the trays on that table.

Watering of the greenhouse seed burial trial commenced on the 1st April 2006 and continued for one year, until the 31st March 2007. When there was a recorded rainfall event from any of the three rainfall treatments on a specific day, that table was watered on the corresponding day in 2006/2007 with the volume of water measured according to the calculation described above. The exact volume of water was measured out with a measuring jug and poured gently and evenly over the trays on the corresponding table using a fine-spray watering can, at approximately 10:00 am on the specific day and month.

The seed burial trial was monitored on average twice a week, often at the same time that tables required watering. In the event of germination (emergence of a seedling above the soil surface, i.e. the plumule of the seed emerging from the soil), the corresponding toothpick and seedling were removed and the date of the event recorded. The trays were weeded where necessary to remove any other plants and grasses that may have emerged from the soil. At the conclusion of the trials on the 28th of March 2007, the soil from the trays was sieved and all remaining seeds removed from the soil. Seeds were counted and classified as intact or decomposed. Intact seeds were tested for viability using tetrazolium staining (2,3,5-triphenyl-tetrazolium chloride; Moore 1985; Mbalo and Witkowski 1997).

3.3.3.1 Data and statistical analyses

Contingency table (3 x 2) χ^2 tests were done to test the association between a) the number of remaining intact, viable seeds and b) the number of remaining non-viable seeds, and rainfall, using three different rainfall treatments, at the conclusion to the trial for all species with remaining viable seeds. The Germination Index (GI) of each species within each treatment (i.e. highest, lowest and average rainfall) was calculated. The GI is provided by a composite expression that combines both speed and completeness of germination; the higher the value, the better the quality of the seed lot (Czabator 1962). It takes into account the fact that a normally

vigorous seed may be slow to germinate because it is partly dormant (Czabator 1962) and can be used to compare between fast and slow germinating seeds within a species. Germination Index (GI) is calculated as follows:

$$GI = PV \times MDG$$

where *PV* is the peak value, which is the mean daily germination of the most vigorous component of the seed lot (the highest germination percent relative to the time elapsed from the start of the test), and expresses speed or rate of germination (Czabator 1962). This is an important element of the equation as it takes into account slower or faster germinators. *MDG* is the mean daily germination (the average number of seeds that germinate per day over the entire test period), an index that expresses both relative seed vigour and length of the test period. The *MDG* value also expresses completeness of germination (Czabator 1962). All data are presented as percentages. Analyses of data were done using Microsoft Office Excel 2007.

3.3.4. Analysis of weather data

Temperature data were obtained for the Wits University greenhouse from Chantal Helm (PhD candidate) at Wits University and weather data (temperature and rainfall) for Skukuza were obtained from KNP weather records for the study period April 2006 - March 2007. Skukuza's weather data were taken from weather station number 0596179 3 in Skukuza. Comparisons of temperature data were done using paired student *t*-tests and oneway ANOVAs with post-hoc Tukey Studentized Range tests (α, $P = 0.05$). For all statistical analyses, Microsoft Office Excel 2007 and STATISTICA Release 9 Statsoft, Inc., USA (2010) were used.

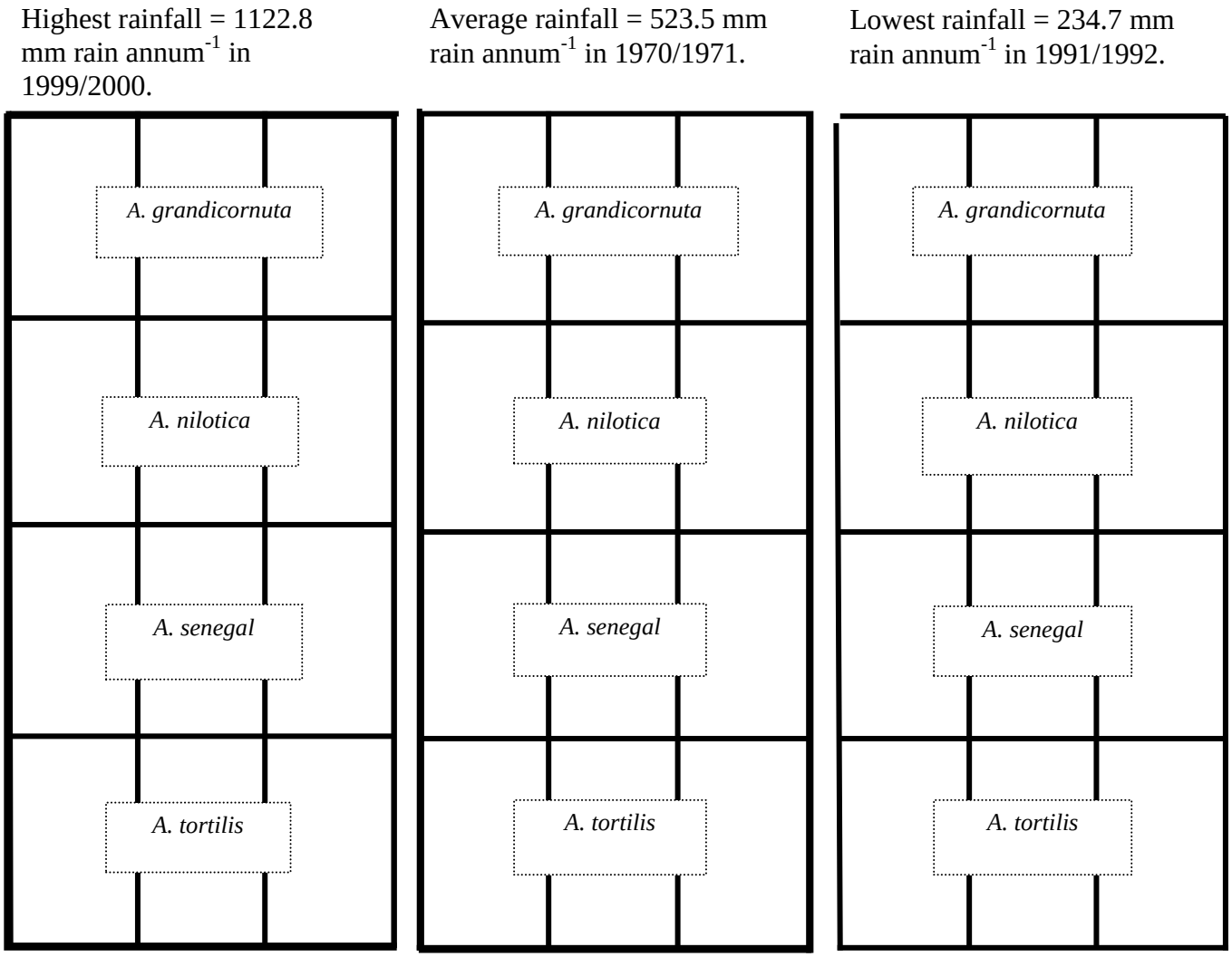

Figure 3. Diagrammatic representation of the greenhouse seed burial trial at the University of the Witwatersrand, Johannesburg, South Africa, from April 2006-March 2007. Headings correspond to the rainfall treatments used for each table and species names denote the seeds buried in each tray.

3.4. Results

3.4.1 Field seed burial trial

Only twenty-seven of the original thirty-two seed bags were retrieved from the field seed burial trial after 16 months. One bag each of *A. grandicornuta*, *A. senegal* and *A. tortilis* seeds and two of *A. nilotica* seeds were destroyed by a fire in a portion of the exclosures. The bags that were destroyed by the fire had been placed at micro-sites in the open (away from the shade of a tree canopy) and on the soil surface. Evidence of burnt bags was found in the form of pieces of degraded mesh, burnt red wire and tape and metal markers. The Nkhuhlu exclosures consist of

six treatments, one of which is a fully fenced area excluding all herbivores, and are divided into a burn and no-burn block (Siebert and Eckhardt 2008). All bags were placed in the fully fenced area of the exclosures, while five bags (one each of *A. grandicornuta*, *A. senegal* and *A. tortilis* seeds and two bags of *A. nilotica* seeds) were placed in the vicinity of fire breaks in the fully-fenced area of the exclosures due to the location of suitable trees. The rest of the bags were placed in the no-burn portion of the fully fenced area, also due to the location of suitable trees. The i-buttons were used incorrectly and as a result no data were obtained.

Of the initial four hundred seeds used in the burial trial per species, 13% (n = 50 seeds/species/micro-site) of *A. grandicornuta, A. senegal* and *A. tortilis* and 25% (100 seeds) of *A. nilotica* seeds were destroyed in the fire. It is uncertain as to when the fire destroyed the seeds and bags, but it is assumed that it occurred within one to two months before completion of the experiment (this assumption is based on the observation that there was little re-growth of new green grass when bags were retrieved, which occurs after a burnt area has received rain).

If the seeds destroyed by the fire are excluded from the analysis, the percentage of seeds remaining intact in the burial trial after 16 months varied between species, namely 66% of *A. tortilis* (n = 350), 34% of *A. nilotica* (n = 300), 19% of *A. grandicornuta* (n = 350) and 15% of *A. senegal* seeds (n = 350) (Table 1). Seeds no longer present in the bags are assumed to have germinated, or become decomposed or predated. Of the remaining intact seeds, the percentage of viable seeds was higher for *A. tortilis* (65%) and *A. nilotica* (27%) than non-viable seeds, while the percentage of viable *A. senegal* (0%) and *A. grandicornuta* (5%) seeds was lower than non-viable seeds (Table 1).

Plate 2. Images of the greenhouse seed burial trial conducted at the greenhouse at the University of the Witwatersrand, Johannesburg, South Africa, from April 2006-March 2007. Clockwise from top left: the high rainfall (1122.8 mm rain annum^{-1} in 1999/2000) seed trays; trays after a 'rainfall' event; germination of grass and other herbaceous seeds after a 'rainfall' event; watering of seed trays with a fine-spray watering can; and the germination of an *Acacia* seed.

Table 1. The overall percentages of seeds remaining intact and viable, seeds remaining intact and non-viable and seeds no longer present (excluding seeds destroyed by fire), of four *Acacia* species at the conclusion of a 16-month field seed burial trial in the Kruger National Park, South Africa.

	Seeds remaining after 16 months (%)		Seeds no longer present
	Intact and viable	Intact and non-viable	(%)
A. grandicornuta (n = 350)	4.6	14.4	81.0
A. nilotica (n = 300)	26.7	7.3	66.0
A. senegal (n = 350)	0	14.9	85.1
A. tortilis (n = 350)	64. 6	1.7	33.7

There were no seedlings found in any of the burned bags. There were no viable *A. senegal* seeds remaining at any of the micro-sites. The percentage of remaining intact, viable seeds was greatest at the micro-sites under tree canopy cover and buried at a depth of 3-5 cm for *A. tortilis* (86%), *A. nilotica* (39%) and *A. grandicornuta* (6%) (Table 2). For *A. nilotica* and *A. grandicornuta* there were no viable seeds found at the micro-sites in the open (no canopy cover) and on the soil surface, while only 27% of *A. tortilis* seeds at this micro-site were viable. The number of remaining intact, viable seeds in the open and buried was lower for all species, namely 58% of *A. tortilis*, 17% of *A. nilotica* and 5% of *A. grandicornuta*, while 55% of *A. tortilis*, 24% of *A. nilotica* and 5% of *A. grandicornuta* seeds under canopy and on the soil surface were viable (Table 2).

There was a significant association between depth of burial and placement under tree canopy shading of the number of remaining and intact *A. grandicornuta* and *A. nilotica* seeds, excluding seeds destroyed by fire (Table 3). While no significant association occurred when seeds destroyed by fire were included for *A. grandicornuta*, there was still a significant association for *A. nilotica* (Table 3). There was no association between burial and shading of the number of intact and remaining seeds for *A. senegal* or *A. tortilis* when the seeds destroyed by fire were excluded from the analysis, while the association was significant for both species when seeds destroyed by fire were included in the analysis (Table 3).

There were no remaining intact, viable seeds for *A. senegal* so no analysis could be done for this species. There was a significant association between canopy shading and seed viability for *A. grandicornuta* where more viable seeds were found under canopy cover, but not for *A. nilotica* or *A. tortilis.* There was no significant association between burial in the soil and seed viability for *A. grandicornuta,* but there was a significant association for *A. tortilis* where more viable seeds were found buried in the soil than on the soil surface (Table 4). The association tended to be different for *A. nilotica* (Table 4).

Table 2. Percentages of seeds destroyed by fire, seeds remaining intact and viable, seeds remaining intact and non-viable and seeds no longer present, at different micro-sites, after 16 months of a field seed burial trial for four selected *Acacia* species in the Kruger National Park, South Africa *(n* = 100 seeds/species for each treatment combination).

	Seeds destroyed by fire (%)	Seeds remaining after 16 months (%)		Seeds no longer present (%)
		Intact and viable	Intact and non-viable	
In the open, on the soil surface				
A. grandicornuta	50	0	3	47
A. nilotica	100	0	0	0
A. senegal	50	0	24	26
A. tortilis	50	27	2	21
In the open, buried below the soil surface				
A. grandicornuta	0	5	33	62
A. nilotica	0	17	5	78
A. senegal	0	0	9	91
A. tortilis	0	58	0	42
Under canopy cover, on the soil surface				
A. grandicornuta	0	5	7	88
A. nilotica	0	24	12	64
A. senegal	0	0	12	88
A. tortilis	0	55	4	41
Under canopy cover, buried below the soil surface				
A. grandicornuta	0	6	7	87
A. nilotica	0	39	5	56
A. senegal	0	0	7	93
A. tortilis	0	86	0	14

Table 3. Contingency table (2 x 2) χ^2 results for four selected *Acacia* species in the Kruger National Park, South Africa where the association was tested between canopy cover (open vs. under canopy) and burial (surface vs. buried) for a) remaining intact seeds and b) remaining intact, viable seeds, for treatments where the effect of fire was included, and where it was excluded (*n* = 100 seeds/species for each treatment combination, d.f. = 1 with Yates correction).

		A. grandicornuta	*A. nilotica*	*A. senegal*	*A. tortilis*
Including fire					
Remaining intact seeds	χ^2	0.471	28.359	6.058	7.457
	P	0.493	< 0.0001	0.014	0.006
Remaining intact and viable seeds	χ^2	1.529	7.527	-	0.910
	P	0.216	0.006	-	0.340
Excluding fire					
Remaining intact seeds	χ^2	12.411	13.393	0.166	0.957
	P	0.0004	0.0003	0.683	0.328
Remaining intact and viable seeds	χ^2	1.529	7.527	-	0.910
	P	0.216	0.006	-	0.340

Table 4. Contingency table (2 x 2) χ^2 results for four selected *Acacia* species in the Kruger National Park, where the association was tested between a) canopy shading (open vs. shading) and seed viability (viable vs. non-viable) and b) burial in the soil (surface vs. buried) and seed viability (viable vs. non-viable) (*n* = 100 seeds/species per treatment combination, d.f. = 1 with Yates correction).

		A. grandicornuta	*A. nilotica*	*A. senegal*	*A. tortilis*
Canopy shading					
Viable vs. non-viable seeds	χ^2	9.043	0.022	-	0.046
	P	0.003	0.881	-	0.831
Burial in the soil					
Viable vs. non-viable seeds	χ^2	0.123	3.541	-	7.554
	P	0.726	0.059	-	0.006

3.4.2. Greenhouse seed burial trial

After one year, only 1% of the initial 1800 *Acacia* seeds planted (450 seeds per species) had germinated, namely 13 *A. grandicornuta* (2.9%), 3 *A. senegal* (0.6%), 1 *A. nilotica* (0.2%) and 1 *A. tortilis* (0.2%) seed (Figure 4). Eight seeds germinated in the trays watered with the highest rainfall (1122.8 mm annum^{-1} in 1999/2000), namely one *A. nilotica* seed and seven *A. grandicornuta* seeds, six seeds in trays watered with the lowest rainfall (237.4 mm annum^{-1} in

1991/1992), namely one *A. tortilis* seeds, one *A. senegal* seed and four *A. grandicornuta* seeds, and four seeds in trays watered with the average rainfall (523.5 mm annum^{-1} in 1970/1971), namely two *A. senegal* and two *A. grandicornuta* seeds (Figure 4). All germination events occurred between November 2006 (late spring) and February 2007 (late summer) (Figure 5 and Table 7), which is effectively the first rainy season after planting.

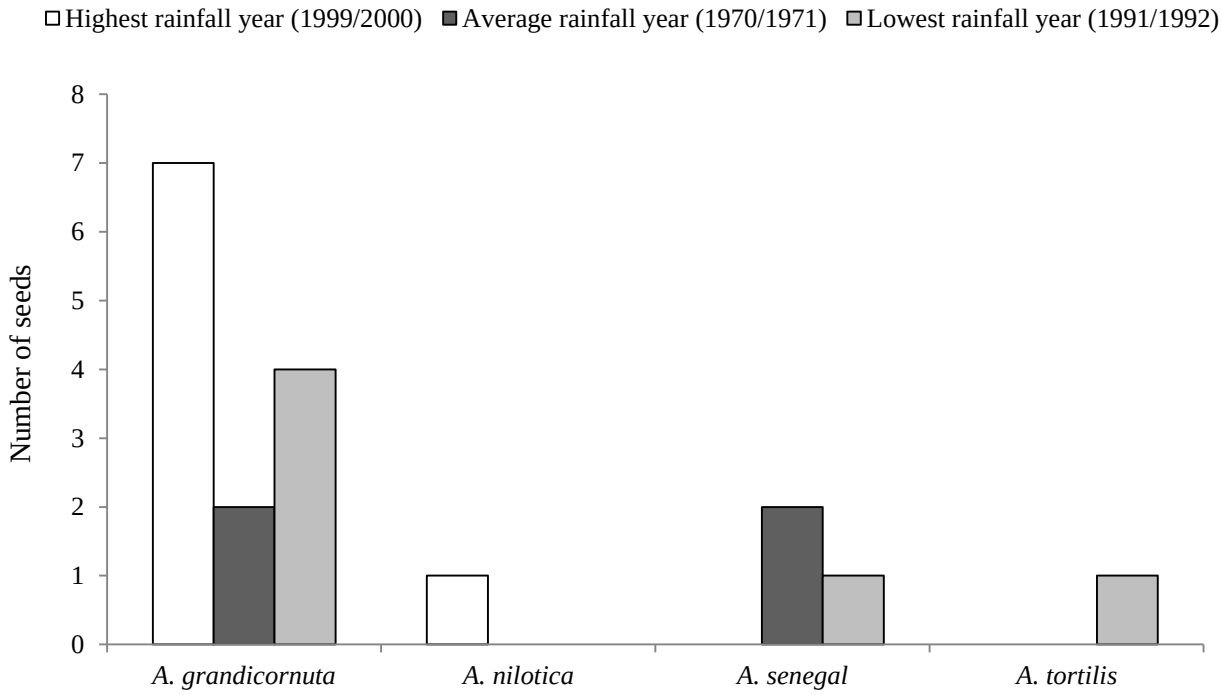

Figure 4. Number of seeds that germinated when watered with three different rainfalls, which correspond with three rainfall records from Skukuza (years in parentheses), Kruger National Park, South Africa, for four *Acacia* species in a greenhouse seed burial trial (*n* = 18).

Of 450 seeds initially planted per species, *A. tortilis* had the highest overall percentage of remaining intact, viable seeds (92.2%) followed by *A. nilotica* (58.3%), *A. grandicornuta* (57.6%) and *A. senegal* (0%) (Table 5). The overall percentage of remaining intact, non-viable seeds decreased between species as follows: *A. nilotica* (21.1%), *A. grandicornuta* (20.4%), *A. senegal* (18.2%) and *A. tortilis* (0%). The percentage of decomposed seeds decreased per species as follows: *A. senegal* (81.1%), *A. nilotica* (20.4%), *A. grandicornuta* (19.1%) and *A. tortilis* (7.6%).

The percentage of decomposed seeds (n = 600 seeds/treatment) was similar with the lowest rainfall (37.0%), and the highest rainfall (31.4%) and slightly less for seeds watered with the average rainfall (24.4%). Overall the percentage of remaining intact, viable seeds and remaining intact, non-viable seeds was similar for all rainfall treatments, namely, seeds watered with the average rainfall (54.5 and 20.5%, respectively), the highest rainfall (52.3 and 15.0%, respectively)

and the lowest rainfall (49.3 and 12.7%, respectively) (Table 5).

Table 5. Percentages (%) of decomposed, germinated, remaining intact, viable and remaining intact, non-viable seeds, after one year of a greenhouse seed burial trial watered with three different rainfall treatments, for four *Acacia* species (*n* = 150 seeds/species/rainfall treatment).

	A. grandicornuta	*A. nilotica*	*A. senegal*	*A. tortilis*
Highest rainfall (1122.8 mm annum^{-1} in 1999/2000)				
Decomposed	1.3	23.3	90	10.7
Germinated	4.7	0.7	0	0
Intact and viable	66	54	0	89.3
Non-viable	28	22	10	0
Average rainfall (523.5 mm annum^{-1} in 1970/1971)				
Decomposed	42	24.7	90	5.3
Germinated	1.3	0	1.3	0
Intact and viable	45.3	57.3	0	94.7
Non-viable	11.4	18	8.7	0
Lowest rainfall (237.4 mm annum^{-1} in 1991/1992)				
Decomposed	14	13.4	63.3	6.7
Germinated	2.7	0	0.7	0.7
Intact and viable	62	63.3	0	92.6
Non-viable	21.3	23.3	36	0
Overall (*n* = 450)				
Decomposed	19.1	20.4	81.1	7.6
Germinated	2.9	0.2	0.7	0.2
Intact and viable	57.6	58.3	0	92.2
Non-viable	20.4	21.1	18.2	0

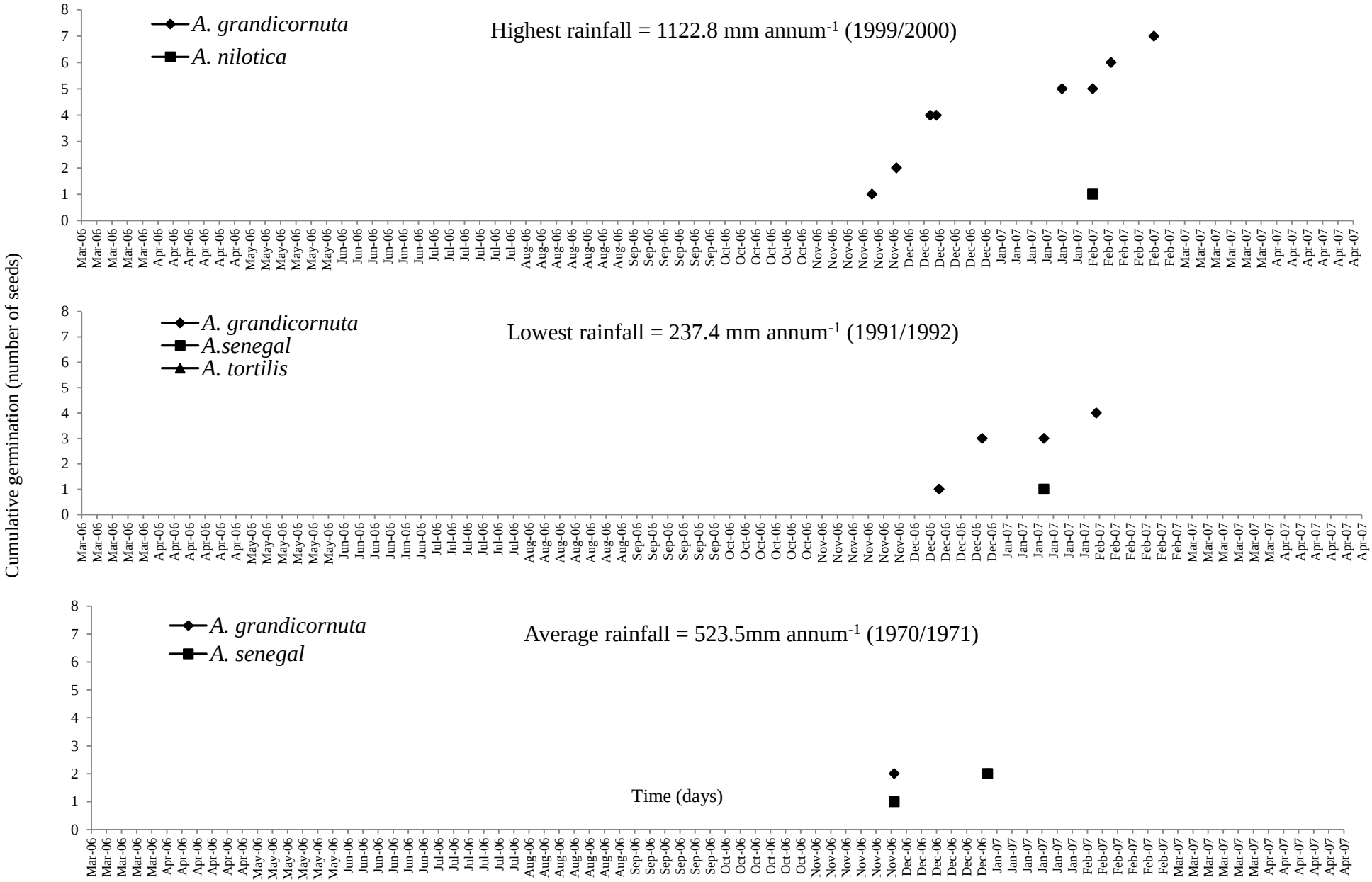

Figure 5. Cumulative germination of seeds of four *Acacia* species watered with three different rainfall treatments in a greenhouse seed burial trial conducted at the University of the Witwatersrand from April 2006-March 2007

There was no association between rainfall treatments and the number of remaining intact, viable seeds for *A. nilotica,* while the association tended to be different for *A. tortilis* (Table 6). However, there was a significant association for *A. grandicornuta,* where the number of remaining intact, viable seeds increased significantly with the average rainfall (Table 6).

Table 6. Contingency table (3 x 2) χ^2 results for the association between three different rainfall treatments (highest (1122.8 mm annum^{-1} in 1999/2000), lowest (237.4 mm annum^{-1} in 1991/1992) and average (523.5 mm annum^{-1} in 1970/1971)) and the number of remaining intact, viable seeds for four *Acacia* species (*n* = 150 seeds/species for each rainfall treatment, d.f. = 2).

		A. grandicornuta	*A. nilotica*	*A. senegal*	*A. tortilis*
Remaining intact, viable	χ^2	14.8	3.0	-	2.8
seeds	*P*	0.001	0.219	-	0.078

It must be noted that although the bottom of the seed trays were lined with paper, drainage holes in the trays allowed an amount of water to drain away during watering. The frequency of large rainfall events (> 25 mm rain per day) was highest for the highest rainfall treatment, which also had the largest number of small rainfall events (0-4 mm rain per day), followed by the average rainfall and lowest rainfall treatment (Figure 6).

The Germination Index was lowest for *A. senegal* when watered with the average rainfall, followed by *A. grandicornuta* when watered with the highest rainfall and then *A. grandicornuta* when watered with the lowest rainfall (Table 7). As only single seeds germinated for *A. nilotica* when watered with the highest rainfall, and for *A. senegal* and *A. tortilis* when watered with the lowest rainfall, the Germination Index was not calculated. Two *A. grandicornuta* seeds germinated on the same day when watered with the average rainfall. Overall, for all species and rainfall treatments, the germination rate was very low as was the relative vigour and capacity of the seed lot (Table 7).

Table 7. Indices of seed germination rate and vigour after one year for four *Acacia* species in a greenhouse seed burial trial, where seeds were treated with three different rainfall treatments, namely the highest (1122.8 mm rain annum^{-1} in 1999/2000), lowest (234.7 mm rain annum^{-1} in 1991/1992) and average (523.5 mm rain annum^{-1} in 1970/1971) rainfall *(n* is the total number of seeds that germinated per species per rainfall treatment).

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	Number of days to first day of germination	Number of days to last day of germination	1st, 2nd and 3rd important calendar months of germination	Mean days to germinate	Peak Value of germination	Time (days) for 50% germination	Mean Daily Germination (MDG)	Germination Index (MDG X PV)
Highest rainfall (1122.8 mm rain annum^{-1} in 1999/2000)								
A. grandicornuta (*n* = 7)	236	328	November, December, January	279	0.014	255	0.013	0.00018
A. nilotica (*n* = 1)	314	314	February	314				
Lowest rainfall (234.7 mm rain annum^{-1} in 1991/1992)								
A. grandicornuta (*n* = 4)	256	306	December, January, February	277	0.007	270	0.007	0.00005
A. senegal (*n* = 1)	290	290	January	290				
A. tortilis (*n* = 1)	256	356	December	356				
Average rainfall (523.5 mm rain annum^{-1} in 1970/1971)								
A. grandicornuta (*n* = 2)	244	244	November	244				
A. senegal (*n* = 2)	244	275	November, December	260	0.005	244	0.004	0.00002

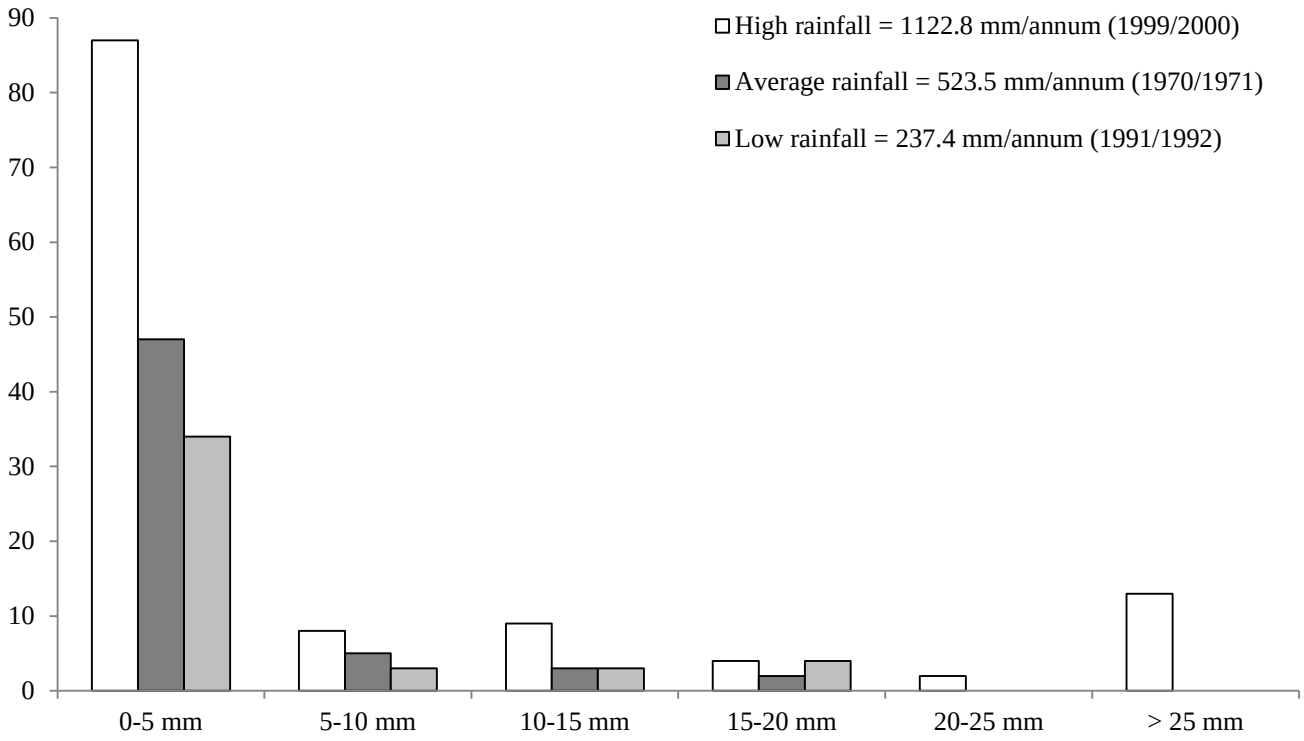


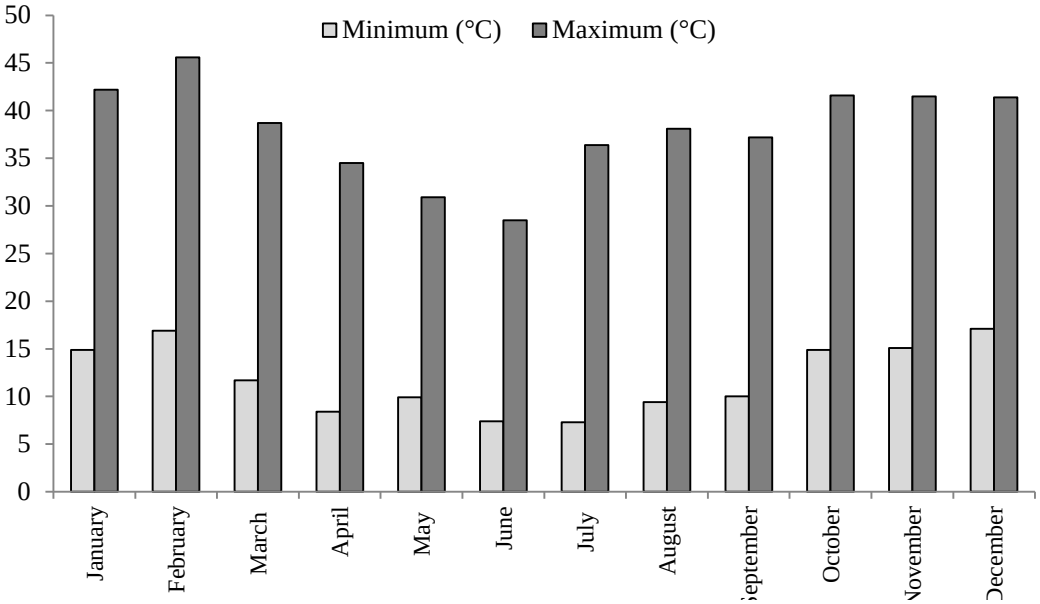
Figure 6. Frequency distribution of rainfall events for three different rainfall treatments used to water seeds in a greenhouse seed burial trial conducted at the University of the Witwatersrand, South Africa, from April 2006-March 2007.

3.4.3. Rainfall and temperature comparisons between Johannesburg and Skukuza

There was no significant difference in the monthly average temperature *(t*-test, $t = 0.6$, $P = 0.271$, d.f =11) between the Wits University greenhouse in Johannesburg, and Skukuza. However, the mean monthly maximum temperature in the Wits University greenhouse in Johannesburg was significantly higher than that in Skukuza (*t*-test, $t = 3.7$; $P = 0.002$; d.f. = 11), (Figure 7).

The greenhouse burial trial experienced three different simulated rainfalls, namely the highest (1122.8 mm rain annum^{-1} in 1999/2000), lowest (237.4 mm rain annum^{-1} in 1991/1992) and an average (523.5 mm rain annum^{-1} in 1970/1971) rainfall, with a mean monthly rainfall of 93.6 ± 34.8 mm rain month^{-1}, 20.3 ± 5.9 mm rain month^{-1} and 43.8 ± 14.4 mm rain month^{-1}, respectively. The field burial trial near Skukuza experienced natural rainfall of 29.1 ± 10.4 mm rain month^{-1}. One-way ANOVAs showed a significant difference between the monthly average rain for the three rainfall treatments and the natural rain experienced in Skukuza over the same time period (April 2006-March 2007) (Table 8), with the highest rainfall treatment having a significantly higher monthly rainfall than the other two treatments as well as the Skukuza rainfall. The monthly rainfall for Skukuza and the lowest rainfall treatment were significantly lower than both the highest and average rainfall treatments but were similar to one another (Table 8).

a)



b)

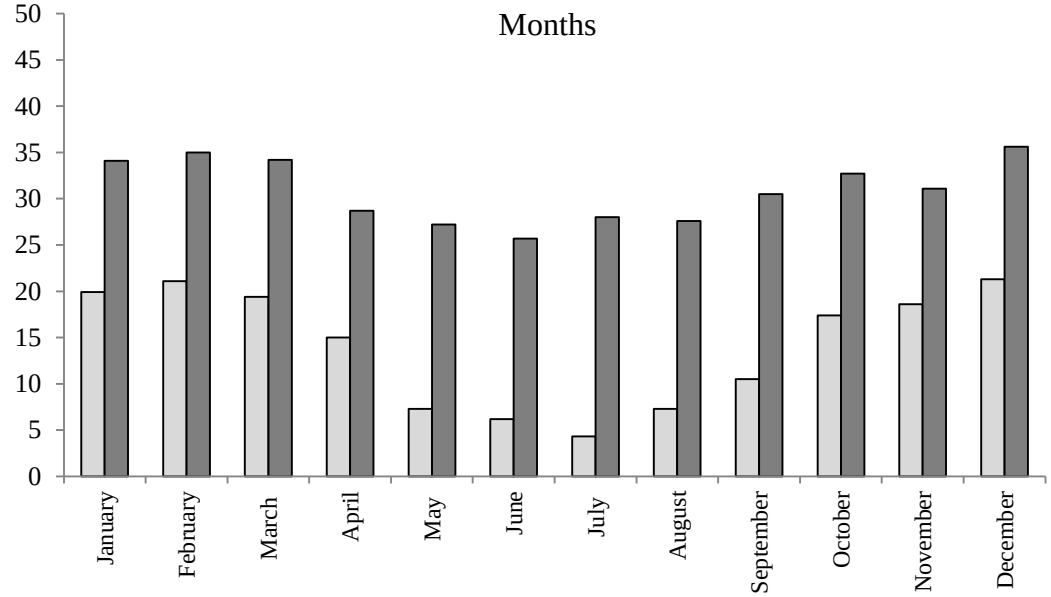


Figure 7 a) Maximum and minimum monthly temperatures (°C) for the period January-December 2008 for the University of the Witwatersrand greenhouse, Johannesburg (see Helm 2011). b) Maximum and minimum monthly temperatures (°C) for the period April 2006-March 2007 for Skukuza, Kruger National Park, South Africa.

Table 8. Comparisons of monthly average temperature and rainfall (mean ± SE) between Johannesburg (University of the Witwatersrand greenhouse) and Skukuza, Kruger National Park, South Africa, for the period April 2006 - March 2007 *(n* = 12). Student *t*-tests were used to calculate significant differences (d.f. = 11), as were one-way ANOVAs (d.f. = 3, 44). Means with different superscript letters are significantly different at the *P* < 0.05 level.

	Temperature (°C)			Rainfall (mm month^{-1})			
	Wits Greenhouse	Skukuza		Wits Greenhouse			Skukuza
				Highest	Average	Lowest	
t-test	21.2 ± 1.0	22.5 ± 1.4	One-way ANOVA	93.6 ± 34.8^{A}	43.8 ± 14.4^{B}	20.3 ± 5.9^{C}	29.1 ± 10.4^{C}
P	0.271		*P*	0.054			
t	0.630		*F*	2.738			

3.5. Discussion

3.51. Field seed burial trial

The exclosures were built to develop an understanding of the impact of fire and herbivory on heterogeneity patterns around the riparian zone of the Sabie River and consist of treatment areas divided into burn and no-burn blocks (Siebert and Eckhardt 2008). In 2005, 2006 and up until July 2007, the fully-fenced section of the exclosures did not undergo a fire treatment but firebreaks were burned (N. Govender *pers. comm.* 2010). The seed trial ran from December 2005 to April 2007, so it is possible that the fire-breaks in the exclosure were burned twice during the trial.

The percentage of seeds remaining intact after the 16-month trial period was relatively low for *A. senegal* (15%), *A. grandicornuta* (19%) and *A. nilotica* (34%), with a large percentage of seeds no longer present in the bags (excluding seeds that were destroyed by fire). Seeds of these species no longer present at conclusion to the trial may have either germinated, or become decomposed or predated. Over 66% of *A. tortilis* seeds remained intact in the soil, which suggests that these seeds may not decompose or germinate as readily as seeds of other species.

Auld (1995) conducted field seed burial trials for long-lived shrubs and trees in arid Australia; loss of seeds from the experimental trials of three *Acacia* species were attributed mainly to germination, as indicated by seedlings recovered from the mesh bags as well as empty seed testas. He also observed that seed decay in field experimental seed banks of *Acacia oswaldii* F.Muell. was very rapid, and within one year almost the entire seed lot had been 'lost'. *A. oswaldii* produces large seeds of up to 0.12 g in weight (Auld 1995), which is similar in mass

to species that suffered relatively high percentages of seed losses in this trial, namely *A. senegal* (0.10 ± 0.006 g), *A. nilotica* (0.11 ± 0.004 g) and *A. grandicornuta* seeds (0.09 ± 0.004 g) (see Chapter 2). *A. tortilis* seeds have a mass (0.04 ± 0.002 g) that is significantly lower than both *A. senegal* and *A. nilotica* seeds (see Chapter 2) and suffered smaller percentages of seed losses than the other three species. The large seeds of *A. oswaldii* have bright arils which attract seed dispersal by birds (Auld 1995). These seeds are most likely to be dehiscent if they are not dispersed by mammalian herbivores (see Coe and Coe 1987). If they are dehiscent they are likely to have thin seed coats (Coe and Coe 1987), which may be the reason they decayed rapidly in the field.

High percentages of seed loss in the trial may also be attributed to decomposition and fungal infections. This may be an effect of the experimental design: the density of seeds within experimental seed bags is usually much higher than the density of seeds occurring naturally in seed banks (van Mourik *et al.* 2005), which may encourage the spread of bacteria and fungi, facilitating higher rates of decomposition. In a study of seed burials of two *Acacia* species, *A. saligna* (Labill.) and *A. cyclops* A.Cunn. ex G.Don, Holmes and Moll (1990) found that seeds used in the trial showed extensive germination and decomposition, which they suggest implies that less hardy seeds are eliminated first, leaving behind a seed set more resistant to decay.

The largest percentage of remaining intact, viable seeds was found for *A. tortilis* (65%) after completion of the burial trial, followed by *A. nilotica* (27%) and *A. grandicornuta* (5%), while no remaining *A. senegal* seeds were viable. Smaller seeds tend to persist for longer in the soil (Garwood 1989; Thompson *et al.* 1993; Fenner and Thompson 2005) and *A. tortilis* seeds are significantly smaller than the other three species (see Chapter 2). The thickness of the seed coat may also affect persistence and viability of seeds in the soil. Both species that produce indehiscent seeds, namely *A. tortilis* and *A. nilotica,* had higher percentages of remaining intact, viable seeds than species that produce dehiscent seeds, namely *A. grandicornuta* and *A. senegal.* Indehiscent seeds are often thick and robust, while dehiscent seeds are thin and discoid in shape (Coe and Coe 1987).

Micro-site placement of nylon seed bags had a significant effect on the percentage of remaining seeds for *A. grandicornuta* when seeds destroyed by fire were excluded from the analysis; however when these seeds were included the association between burial and shading became non-significant, suggesting that if those seeds had survived to the completion of the burial trial micro-site placement would have had no effect on the number of seeds remaining.

For *A. nilotica* the association between burial and shading was significant both when

seeds destroyed by fire were excluded and included in the analysis, indicating that more *A. nilotica* seeds remained when buried in the soil under canopy cover. For *A. senegal* and *A. tortilis* there was no significant association between burial and shading and the number of remaining seeds when seeds destroyed by fire were excluded; however, the association became positive for both these species when these seeds were included. In this case, more *A. senegal* seeds remained when buried under-ground and in the open (no canopy shading), while more *A. tortilis* seeds remained when buried under-ground and under canopy cover.

The effect of canopy shading was significantly associated with seed viability for *A. grandicornuta* only, with more intact, viable seeds found under canopy cover. The effect of burial in the soil was significantly associated with seed viability for *A. nilotica* and *A. tortilis* only, which means that a larger percentage of viable seeds were found buried in the soil as opposed to being placed on the soil surface.

Generally the percentage of remaining intact, viable seeds was highest in nylon bags placed at micro-sites under tree canopy cover and buried 3-5 cm below the surface of the soil for all species, as opposed to micro-sites in the open and on the soil surface. Witkowski and Garner 2000) noted that seeds will persist in the soil for longer under canopy shading relative to the open. Studies have shown that seed viability and germination success of certain *Acacias* decreases in seeds exposed to high temperatures and low moisture (Wilson and Witkowski 1998; Loth *et al.* 2005) and seeds exposed on the soil surface (Argaw *et al.* 1999; Barnes 2001; Radford *et al.* 2001).

3.5.2. Greenhouse seed burial trial

The greenhouse seed burial trial allowed a comprehensive recording of the percentage of seeds that germinated, decomposed and remained viable as well as non-viable, after being buried in the soil for one year and exposed to different rainfall treatments. The percentage of seeds that germinated overall was below 3% for all species. In a previous study on soil seed banks of South African savanna trees (Garner and Witkowski 1997; Witkowski and Garner 2000), it was found that > 95% of seeds of *Acacia* species such as *A. tortilis* and *A. nilotica* were found in the litter and 0-5 cm soil depth. Less than 5% of *A. tortilis* seeds were found buried at depths of 6-7 cm and 8-9 cm. The depth range at which most of the ‘old’ (dispersed at least one season prior to the study) seeds were found was 2-4 cm, while the vast majority of recently dispersed seeds were still in the litter layer, with a few in the top 1 cm of soil (Garner and Witkowski 1997). In this trial seeds were buried in the soil at a depth of 4-5 cm, which may have prevented seeds from germinating and emerging successfully, as it is deeper than the average soil depth at which

seeds of similar species have been found (see Garner and Witkowski 1997). Often seeds that are buried too deeply in the soil do not germinate, as the soil temperature fluctuations sometimes required for successful germination decrease with soil depth (Hartmann *et al.* 2002). Loth *et al.* (2005) noted that *Acacia* seeds germinate readily under conditions of considerable temperature amplitude, which can affect imbibition rates and which is found closer to the soil surface.

The 'lethal' burial depth of seeds (i.e. the depth at which germination and seedling emergence become unsuccessful) is dependent on factors such as seed size, soil characteristics and germination cues and seeds buried too deeply may not germinate successfully (Witkowski and Garner 2000). Also, if a seed germinates too deeply beneath the soil surface, the shoot may not be able to reach the surface (Fenner and Thompson 2005). Generally, larger seeds show a greater rate of seedling emergence from greater depths than smaller seeds (Garner and Witkowski 1997; Grundy *et al.* 2003). Holmes and Moll (1990) found that the seeds of *A. saligna* (0.16 g) and *A. cyclops* (0.33 g) showed seedling emergence from a depth of burial in the soil of up to 100 mm. Grundy *et al.* (2003) found that weed seeds sown in controlled laboratory experiments emerged from much greater depths than seeds of the same species observed in the field and that the largest seeds (0.6 ± 10.8 g) of a weed, *Veronica hederifolia*, emerged from a depth of 8 cm below the surface of the soil.

Seeds with coat-imposed dormancy, such as legumes, are generally released from dormancy through scarification (Mbalo and Witkowski 1997; Wilson and Witkowski 1998). As this experiment looked at the ability of seeds to remain persistent in the soil under different rainfall treatments, seeds were not artificially scarified before being buried. If seeds are to remain dormant they need to be viable and ungerminated in the soil. Artificial scarification would break the coat-imposed dormancy, allowing the seed to imbibe water and initiate germination under artificial conditions. Orozco-Almanza *et al.* (2003) observed a high rate of germination of four legume species' seeds when the seed-coat imposed dormancy was interrupted by mechanical scarification, independent of the light conditions they were under. Results from an investigation into the germination of a number of woody plant species by Argaw *et al.* (1999) showed that the seeds of legume species such as *A. tortilis, A. seyal* and *Dichrostachys cinerea* need acid or mechanical scarification to make the hard seed coat permeable to water and to improve germination. The natural scarification of legume seeds often occurs due to fluctuating temperatures resulting from daily heating and cooling (Mbalo and Witkowski 1997).

The age of seeds may also affect successful germination. Seeds lose viability over time (Fenner and Thompson 2005) and older seeds in a seed bank generally germinate significantly later than younger seeds (Kalisz 1991). Valleriani and Tielborger (2006) suggest that due to the

staggering of germination over several years, seed banks become naturally age-structured. It can be assumed that in a relatively dense seed bank the majority of seeds are young and in a dense seed bank of young seeds, it is advantageous to have low germination rates to prevent high and unnecessary losses of seeds.

The percentage of seeds that decomposed in the greenhouse burial trial was highest for *A. senegal,* followed by *A. nilotica, A. grandicornuta* and *A. tortilis,* while the percentage of remaining intact, viable seeds was highest for *A. tortilis,* followed by *A. nilotica, A. grandicornuta* and *A. senegal.* These results suggest that dehiscent seeds decompose more readily and have a smaller percentage of viable seeds remaining in the soil after one year than indehiscent seeds. Argaw *et al.* (1999) noted that *A. senegal* seeds are able to germinate readily without scarification if provided with sufficient moisture and oxygen, possibly as a result of their thin seed coat. It is possible that indehiscent seeds are able to survive ungerminated and viable in the soil for longer periods of time as a result of hard seed-coat imposed dormancy, as suggested by Argaw *et al.* (1999) for *A. tortilis* seeds. In an investigation of the germination of four *Mimosa* species, Orozco-Almanza *et al.* (2003) observed that legume seeds with lighter, thinner seed coats germinated in greater numbers, without scarification. A study by Danthu *et al.* (2003) showed a possible lack of integumental dormancy in *A. senegal,* which may explain why *A. senegal* seeds did not persist in the soil in this trial.

Generally, slightly more seeds germinated (eight seeds overall) when exposed to the highest rainfall (1122.8 mm rainfall annum^{-1} in 1999/2000) than the average (four seeds) (523.5 mm annum^{-1} in 1970/1971) and lowest rainfall (six seeds) (237.4 mm annum^{-1} in 1999/1992) treatments. The highest and lowest rainfall treatments showed similar numbers of decomposed seeds (188 and 222 seeds, respectively), while slightly fewer seeds decomposed when watered with the average rainfall (146 seeds overall). The overall percentage of remaining intact, viable seeds was similar for all three rainfalls. Contingency table χ^2 results showed that there was no association between rainfall treatments and the number of remaining intact, viable seeds for *A. nilotica,* while the association tended to be different for *A. tortilis.* There was a significant association for *A. grandicornuta,* where the number of remaining intact, viable seeds increased significantly with the average rainfall.

The germination index (GI) was very low for all four species in the greenhouse burial trial. A lower germination index indicates a higher percentage of weak seeds in the lot and the potential for greater seed losses (Czabator 1962). However, as mentioned earlier there were a number of factors that may have limited germination, such as the lack of seed scarification and the soil depth at which the seeds were buried. As mentioned in the Methods above, a certain

amount of water drained through drainage holes in the trays during each watering event. Even though large rainfall events were experienced most often during the high rainfall treatment, this water would have drained away and would possibly become inconsequential to the experiment.

Germination events always occurred after periods of high and sustained levels of watering, which generally occurred between November (late spring) and February (late summer) during the wet season. In an investigation of water requirements for *A. nilotica* and *A. tortilis* seeds, Wilson and Witkowski (1998) found that imbibed seeds that have been scarified required the equivalent of 3 mm of rainfall every two days in order to germinate and cycles of imbibition and drying out would lead to a loss of viability and germinability (Wilson and Witkowski 1998). *Acacia* seeds require a moist environment in order to germinate successfully (Loth *et al.* 2005). Holmes and Moll (1990) found that survival of *Acacia saligna* was low at soil depths where soil was drier. It may be that for the low rainfall treatment, periods between watering events was long enough to allow the soil to dry out and cause a decrease in seed viability.

3.5.3. Temperature variables and comparisons between seed burial trial results

Heat and temperature variations can affect seed viability and germination. Fluctuating temperatures that result from daily heating and cooling of seeds can be responsible for scarification of legume seed coats and increased germination percentages (Taylorson and Hendricks 1977; Mbalo and Witkowski 1997). Alternating temperatures within the range of 10-40°C over a day, with time at lower temperatures exceeding that of time at higher temperatures, have been found to affect the breaking of seed dormancy (Taylorson and Hendricks 1977). Seeds were not scarified as the burial trials were conducted to test the ability of seeds to persist ungerminated, in other words, the ability of seeds to remain dormant in the soil for one year. Scarified seeds may have imbibed water and germinated, affecting the results of the experiment. Natural scarification, which occurs under natural field conditions, is probably less intense than laboratory scarification, and consequently imbibition in natural field conditions may take longer (Wilson and Witkowski 1998). The experiment aimed to simulate natural field conditions.

Although there was a significant difference in the mean monthly maximum temperatures between Skukuza (± 30.9°C) and the Wits Greenhouse (± 38.2°C), there was no significant difference in the mean monthly average temperatures (22.5 ± 1.4°C and 21.2 ± 1.0°C, respectively), possibly because there was no significant difference in the minimum monthly temperatures.

Seeds were buried in the soil at depths of 4-5 cm in the greenhouse seed burial trial, and 3-5 cm in the field seed burial trial, although a portion of seeds was also placed on the soil

surface in the field seed burial trial. It is possible that the effects of soil temperatures and fluctuations at these depths are not as great as those closer to the soil surface and would therefore not affect seed scarification or decrease seed viability.

Overall a smaller percentage of seeds remained intact and viable at the conclusion of the field seed burial trial than in the greenhouse seed burial trial for all species (no *A. senegal* seeds were viable for either trial). However, similar percentages of *A. senegal* seeds were no longer present in both the field (excluding seeds destroyed by fire) and greenhouse burial trials (85.1% and 81.1%, respectively). The percentage of germination of *A. senegal* seeds was low in the greenhouse burial trial, suggesting that they are possibly susceptible to decomposition in the soil. The percentage of intact, viable *A. tortilis* seeds was relatively high in both trials suggesting that these seeds are able to persist in the soil for one year to 16 months. Fifty-eight percent of *A. grandicornuta* seeds in the greenhouse trial remained intact and viable while only 5% of seeds remained so in the field, suggesting viability of these seeds in the soil may decrease over time. Similarly, twice the percentage of *A. nilotica* seeds remained intact and viable in the greenhouse trial (58%) compared with the field trial (27%). In the greenhouse trial, seeds were buried at a soil depth of 4-5 cm with no 'shading', while in the field trial one of the treatments was mesh seed bags buried at a soil depth of 3-5 cm and in the open (away from the shading of tree canopies). For these treatments and all four species, there were larger percentages of remaining intact seeds in the greenhouse trial than the field trial. Similarly, larger percentages of seeds of all species remained intact and viable in the greenhouse trial than the field trial. There was a significant difference in the mean monthly rainfall between the three rainfall treatments in the greenhouse and the natural rain experienced in Skukuza in the field trial, where the rainfall in Skukuza was similar to the lowest rainfall treatment, while significantly lower than both the highest and average rainfall treatments. While rainfall did not affect seed viability for *A. nilotica* or *A. tortilis* in the greenhouse burial trial, a significantly larger number of remaining intact, viable *A. grandicornuta* seeds were present with the average rainfall treatment. It may be that the low rainfall experienced in the field burial trial is partially responsible for the lower numbers of remaining intact, viable seeds.

3.6. Conclusion

Artificial burial nearly always over-estimates the potential of seed to persist in the soil and although seeds of certain species frequently become buried, others do so only rarely (Thompson *et al.* 1993). If seeds persist in seed burial trials, it does not necessarily mean they would be incorporated into the soil and persist under natural conditions. The probability of burial

is associated with a number of characteristics concerning dormancy, germination and defense chemistry (Thompson *et al.* 1993). As mentioned by Hill and Vander Kloet (2005), even though burial trials may be exposed to conditions very similar to those experienced naturally in the field, it is unlikely that these experiments can replicate the natural interactions between seeds and seed predators and fungi. Indeed, in this study the percentage of remaining intact, viable seeds was lower overall in the field trial than in the greenhouse trial for the three species with remaining intact, viable seeds. From these results it can be seen that greenhouse trials overestimate the persistence of seeds compared with field trials, which are done under more natural conditions.

Field and greenhouse seed burial trials showed that seeds of three *Acacia* species, namely *A. grandicornuta, A. nilotica* and *A. tortilis* have the potential to form short-term persistent seed banks (seeds that remain persistent in the soil for over one year but less than five, following Fenner and Thompson (2005)). Seeds were not scarified before being placed in the trial and the germination index of these species was low, which may indicate that these seeds can remain dormant for extended periods of time. *A. senegal* seeds appear to be transient and do not form persistent seed banks. In addition to the low percentage of *A. senegal* seeds that germinated, none of the seeds remained viable after one year in the greenhouse seed burial trial.

It is recommended that further seed burial trials are conducted for the three species with the potential to form short-term persistent seed banks, whereby trials are run over five years and more, and where seeds are retrieved at regular intervals and tested for viability. This will assist in clearly defining the types of seed banks formed by these *Acacia* species.

3.7. References

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Appendix 1

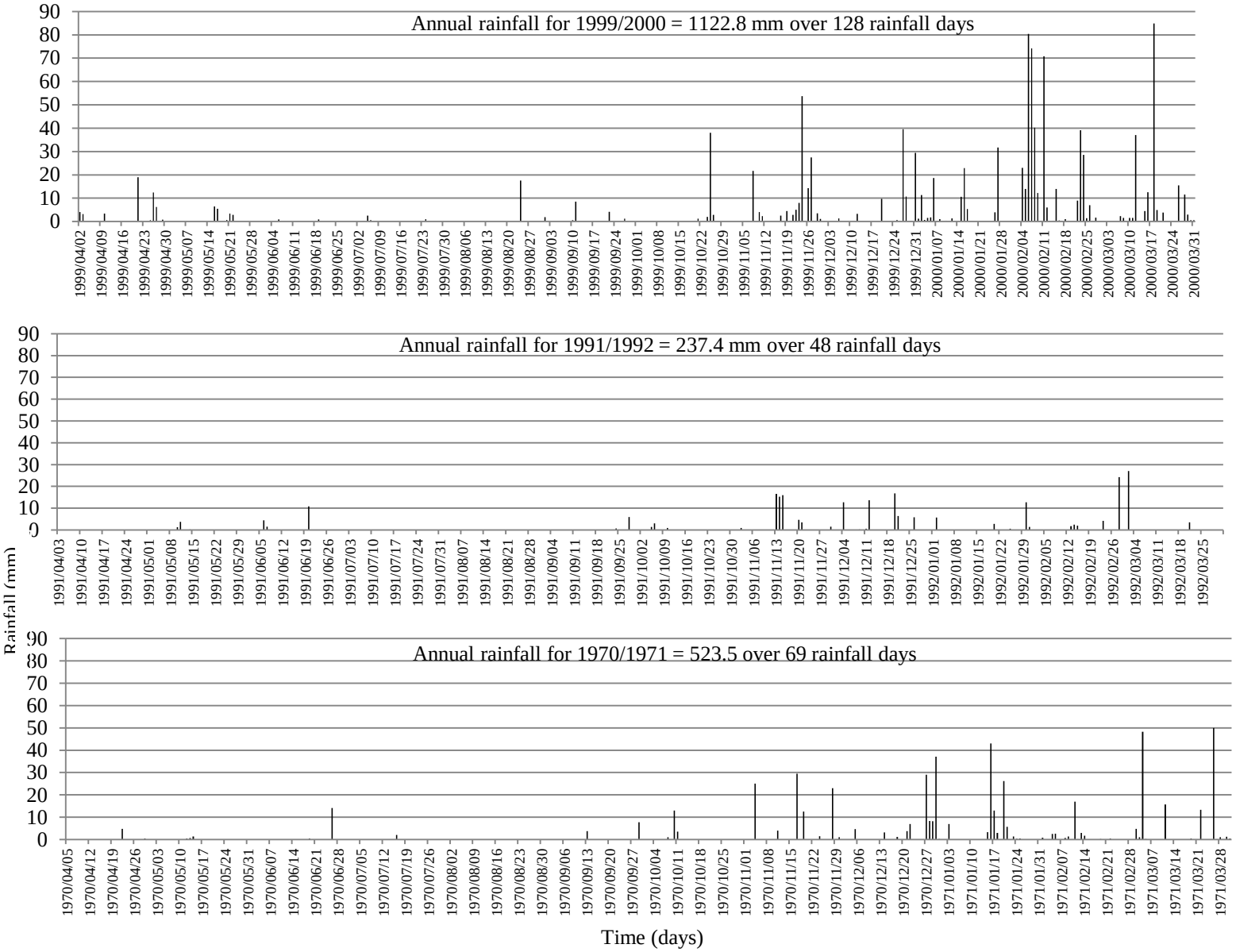

Figure. 9. Daily rainfall (mm) over one year for three different rainfall regimes in Skukuza, Kruger National Park, South Africa, a low, high and average rainfall regime. The amount of annual rainfall, the number of rain days and the year the rainfall was recorded is shown in the title of each Figure.

CHAPTER 4

The effect of seed predation by large herbivores, termites and rodents on seed fate

Abstract

Pre- and post-dispersal seed predation is often regarded as a threat to plant population recruitment. However, recent studies suggest that seed predators may facilitate the dispersal of seeds to 'safe sites'. This study examined the role of large herbivores, termites and rodents as seed predators, and the effect of large herbivore ingestion of *Acacia* and other savanna tree species seeds on seed viability and germinability. The study was conducted in six grids in the Skukuza land system in the Kruger National Park (KNP) during June/July 2006.

A. grandicornuta was the most dominant woody plant of all six study species, followed by *Dichrostachys cinerea, A. tortilis, A. nilotica, A. senegal* and *A. nigrescens.* Woody plant density in grids varied between 226 plants ha^{-1} (Grid 3) to 1618 plants ha^{-1} (Grid 5), with a mean (± SE) density of 862 ± 195 plants ha^{-1}. Forty-seven woody plant species were observed across the six grids. Overall, woody plant species diversity was low (Shannon Wiener Index, 1.8 ± 2.8; Evenness Index, 0.7 ± 0.02; Simpson's Reciprocal Index, 4.5 ± 0.6).

Large herbivores appeared to favour indehiscent seeds (55 *A. tortilis* seeds and 11 *A. nilotica* seeds ingested) over dehiscent seeds (1 *A. grandicornuta* seed ingested). From the 67 seeds extracted from herbivore dung, only 9% (five *A. tortilis* seeds and one *A. grandicornuta* seed) germinated after a six-week germination trial. Herbivore ingestion may have affected seed viability, as less than half the remaining ungerminated *A. nilotica* seeds (46%) and *A. tortilis* (40%) seeds tested viable. Generally, *A. nilotica* seeds from *in situ* soil seed banks were significantly lighter, with a smaller volume and density and rounder shape than seeds ingested by herbivores and seeds sampled from pods collected from tree canopies. Ingested *A. tortilis* seeds and seed bank seeds had similar characteristics while seed from pods collected from tree canopies were heavier, less spherical, denser and had a larger volume.

Twenty trees were recorded growing on termite mounds across all six grids, comprising *A. robusta* (85%), *A. grandicornuta* (10%) and *A. tortilis* (5%). No correlation was observed between the number of termite mounds recorded and the number of *Acacia* trees growing on termitaria (r = 0.07). Termite mounds occupied 0.0009 ± 0.0003 ha per grid matrix (0.8%).

Only four rodent species in total were recorded across all six grids namely, *Mastomys concha* (multimammate mouse), *Rhabdomys pumilio* (striped mouse), *Aethomys chrysophilus* (red veld rat) and *Tatera leucogaster* (highveld gerbil), thus species diversity was low (Shannon

Wiener Index, 0.6 ± 0.2; Evenness Index, 0.6 ± 0.2; Simpson's Reciprocal Index, 1.9 ± 0.3). Cafeteria trials showed a significant difference between seed species ($P < 0.05$; $F = 2.8$; d.f = 3, 236) in the percentage of seeds removed by rodents per tray. There was a significant difference in the percentage of seeds removed from trays placed under vegetation cover compared with trays in the open ($P = 0.034$).

This study showed that herbivores ingest a variety of woody plant seeds and that although rodents show a preference for certain seeds, their diet comprises a variety of seed types. Understanding how such seed predation affects plant population dynamics and species composition can assist effective conservation management of vegetation in the KNP, where an increase in the density and cover of woody plants on granite substrates was observed between 1940 and 1998.

Keywords: seed predation, rodents, termites, large herbivores, seed dispersal, *Acacias.*

4.2. Introduction

4.2.1. Seed fate and dispersal

Plant recruitment limited by the availability of seeds is known as seed-limited recruitment (Clark *et al.* 2007). Turnbull *et al.* (2000) define seed limitation as an increase in plant population size following seed addition. Seed limitation may be due to a failure of seeds arriving at sites suitable for successful germination and seedling establishment, possibly because of poor dispersal (Fenner and Thompson 2005). The precision at which a 'seed eater' processes a seed, as well as whether it is after the seed or fruit, will determine whether it is a predator or disperser (Janzen 1971); post-dispersal seed predation may also represent the next step in the seed dispersal process (Vander Wall *et al.* 2005). The effect of seed predation on plant population density will also depend on whether populations are seed- or establishment- limited (Clark *et al.* 2007). If a plant population is not seed-limited, moderate seed herbivory may have no effect on plant abundance (Crawley 1990).

Janzen (1971) describes the effect of seed predation on plant population recruitment as a game that is played by mobile predators and sessile prey. Both pre- and post-dispersal seed predators represent potential facilitators of seed mortality but differ greatly in their effect on plant recruitment (Szentesi and Jermy 2003). Consequently, Crawley (2000) suggests that the effects of pre- and post-dispersal seed predators on plant populations should be dealt with separately. Chambers and MacMahon (1994) developed a dispersal model that summarises the movement and fate of seeds. They describe 'Phase I dispersal' as any mechanism by which seeds

are moved from the parent plant to a surface, either actively by animals that select seeds or fruit, or passively when seeds or fruit are consumed incidentally with other food and 'Phase II dispersal' as further movement of a seed once it has arrived on a surface, either to a new location or incorporated into the soil (Chambers and MacMahon 1994). Pre-dispersal seed predation occurs when a seed is eaten before it is dispersed from the parent plant (Fenner and Thompson 2005) and affects 'Phase I dispersal'. Conversely, post-dispersal seed predation occurs after initial dispersal has taken place (Fenner and Thompson 2005) and affects 'Phase II dispersal'. Phase II dispersers may have an antagonistic (granivory) or mutualistic (dispersal) association with plants (Chambers and MacMahon 1994) and whether a seed eater is a predator or a disperser is related to whether the seeds are dispersed from the parent plant to a safe site (Janzen 1971). Studies have suggested that post-dispersal seed predation is responsible for the majority of seed losses in a system (Mittelbach and Gross 1984; Miller 1994a; Barnes 2001; Moles *et al.* 2003; Walters *et al.* 2005). Vander Wall *et al.* (2005) however, suggest that many of these studies focus too intently on seed dispersal as a proxy for seed predation and do not analyse the role that seed predators play as important dispersers in plant recruitment.

Once a seed is dispersed, a safe site needs to be reached and a plant needs to establish successfully before recruitment can occur (Strykstra *et. al.* 2002). Safe sites are defined as areas where the requirements for seed dormancy-breaking, germination and seedling establishment are present and the effects of predators, competitors and pathogens are decreased (Harper 1977; Green 1983).The importance of safe site availability is emphasized by Eriksson and Ehrlen (1992), who propose that an increase in the availability of safe sites can lead to an increase in population recruitment. Post-dispersal seed removal may facilitate movement of seeds to safe sites favourable to germination and seedling establishment (Fenner and Thompson 2005; Vander Wall *et al.* 2005). The probability of a seed being eaten by a predator can define whether an area is a safe site for seeds or not (Janzen 1971). A number of studies focus on dispersal of seeds to safe sites and the effect of this strategy on population recruitment (Green 1983; Wenny 2001; Rey *et al.* 2002; Strykstra *et al.* 2002), however there is generally no consensus on whether dispersers deliver seeds to safe sites for germination and establishment, or not (Fenner and Thompson 2005).

4.2.2. Large herbivores and Acacia seed ingestion and dispersal

Almost all *Acacia* tree species provide a valuable source of browsing material for a range of large herbivores such as elephants, giraffes, black rhinos and kudus (Coe and Coe 1987). Barnes (1982) suggests that African *Acacia* pods are favoured by herbivores because of their

relatively high crude protein content. The aromatic and highly palatable pods of African *Acacias* may be evidence that co-evolution occurred between large herbivores and these tree species, resulting in effective seed and pollen dispersal (Du Toit 1990). In the Kruger National Park (KNP), giraffe and the savanna tree *Acacia nigrescens* share a symbiotic relationship whereby giraffe play a role in the tree's pollination processes (Du Toit 1990). The pods of *A. nilotica* and *A. tortilis* appear to be favoured by giraffe as is evident by the visual impact on browse trees, with the shaping of trees by giraffe into a dumb-bell shape or trimming them at a height of around 6 m (Scholes *et al.* 2003; *pers. obs.*).

Coe and Coe (1987) suggest that certain *Acacia* seeds have evolved a thick seed coat to facilitate consumption (and dispersion) of seeds by herbivores, while another group of *Acacia* seeds retain a discoid shape to aid short-distance dispersal by wind. *Acacias* are thereby classified according to the method by which the seeds are dispersed (Coe and Coe 1987), namely, dehiscent pods, where the fruit's pericarp splits and seeds are dispersed by wind or gravity, and indehiscent pods, where the seeds remain in the pods in tree canopies or on the ground, until the pods are browsed or removed and seeds are released (Bradbeer 1988). A seed must be able to avoid mastication and endure the acidic juices of large herbivores' guts in order to be effectively dispersed (Coe and Coe 1987). Selection may even favour seeds that are hard enough to escape mastication and destruction by acidic gut contents, but are soft enough to allow scarification within the gut, which aids germination (Van Staden *et al.* 1989). However, if large herbivores chew seeds, digest or remove them or their pods from trees before the seeds are ripe it could result in a decrease in the number of viable seeds available for germination (Barnes 2001).

One of the most important positive effects of dispersal of *Acacia* seeds by animals is the removal of the seeds from the vicinity of the parent plant, as seedlings rarely develop under parent plant canopies (Tybirk *et al.* 1992). Seeds dispersed by ungulates may thereby be defecated in areas more suitable for germination (Tybirk *et al.* 1992). Coughenour and Detling (1986) suggest that dung heaps may provide an area of elevated nutrients and moisture for seeds that is favourable for germination. Miller (1995) reported elevated levels of germination in dung, of seeds that had been consumed by large herbivores, while Loth *et al.* (2005) showed that *A. tortilis* seeds germinated readily in dung as opposed to seeds uncovered and dried out by the sun, and plant population regeneration was higher away from tree canopies.

4.2.3. The dispersal of seeds by termites and ants

The dispersal of seeds by ants is a common and important ecosystem service and the food body or elaiosome attached to certain seeds attracts ants and facilitates this dispersal away from

the parent plant (Vander Wall *et al.* 2005; Wenny 2001). Dispersal can reduce parent-offspring competition (Ness *et al.* 2004) as many *Acacia* species are unable to establish under the canopy of large established trees (Smit *et al.* 1999). The dispersal of seeds by ants, called myrmecochory, is a form of mutualism in which the plant benefits by dispersal and the ant is rewarded with food (Wenny 2001; Fenner and Thompson 2005). Although seed dispersal by ants may not contribute to seed bank build-up, it contributes to regeneration success in myrmecochorous plant species (Dostal 2004). Myrmecochory, however, is generally not a feature of South African savannas (Garner and Witkowski 1997).

Termites play a crucial role in nutrient cycling and decomposition in the KNP, consuming up to 55 percent of all surface plant litter (Naiman *et al.* 2003) and termite mounds contain important micro-nutrients and key resources (Mills and Fey 2005; Mills *et al.* 2009). Termite mounds provide islands of fertility in nutrient-poor landscapes (Levick *et al.* 2010).While the nutrient-rich and protective features of a termite mound may suggest a favourable site for seed germination and seedling establishment, this may not be so. Dauber *et al.* (2006) suggests that species with seeds adapted to survival in moist and humid soils, seeds that are unable to grow up through mounds of soil (i.e. unable to germinate under a thick layer of soil) and larger seeds are found less frequently in ant mounds. Generally, there are apparent gaps in the literature regarding seed dispersal by termites, as opposed to ants. Termite mounds in miombo woodlands in Zimbabwe appear to increase vegetative biodiversity compared to surrounding areas (Fleming and Loveridge 2003) but the authors do not ascribe this observation to dispersal and removal of seeds to mounds by termites, but rather to termite mounds as nutrient hot-spots.

4.2.4. Post-dispersal seed predation by rodents

The role of granivorous rodents in plant demography is a contentious topic (Xiao *et al.* 2006) and because rodents act primarily as seed predators they are made accountable for a large majority of seed losses in a system (Vaughton 1998; Blaney and Kotanen 2001; Fedriani and Manzaneda 2005), especially to seed-limited tree species. There have been a number of studies documenting the removal of seeds by food-storing rodents (see Mittelbach and Gross 1984; Ostfeld *et al.* 1997; Lortie *et al.* 2000; Jansen *et al.* 2004) and although some seeds are consumed, a large portion is cached or scattered-hoarded by certain rodent species (Vander Wall 2002; Vander Wall *et al.* 2005). Rodents may also remove intact seeds to new and favourable micro-sites for germination (Mittelbach and Gross 1984; Miller 1995; Vander Wall *et al.* 2005). Typically, if a seed is removed by a rodent and remains intact and viable it will form part of the

dispersal process (Vander Wall *et al.* 2005).

Many studies focusing on seed dispersal and predation by rodents have shown that rodent seed preferences may be based on energy considerations (Emlen and Emlen 1975; Reichman 1977; Price *et al.* 2000), nutrients such as fat (Kerley and Erasmus 1991; Xiao *et al.* 2004), water content (Frank 1988; Murray and Dickman 1994), seed size (Lima 1985; Vander Wall 1995; Brewer 2001) or handling time (Tort *et al.* 1998). Optimal foraging theory suggests that small seed size in combination with a smaller food reward should result in a decrease in selection by rodents (Witkowski *et al.* 1991; Fenner 1995). Xiao *et al.* (2006) have shown that from a selection of different sized seeds, those with the highest fat concentration were harvested more quickly by rodents. Large seeds (with a mass of up to 14 g, see Xiao *et al.* 2006) produced in abundance prompt rodents to disperse rather than consume seeds; thereby seed dispersal increases and seeds are stored (Jansen *et al.* 2004; Xiao *et al.* 2006). Generally, the giving-up density (GUD) of seeds by rodents is lower when sheltered by vegetation, suggesting that the risk of predation, whether direct or indirect, is an important factor to seed selection by rodents (Hughes and Ward 1993; Mohr *et al.* 2003; Orrock *et al.* 2004; Xiao *et al.* 2005). The giving-up density method measures the particular density of food left in a certain feeding patch when the animal ceases to forage (Mohr *et al.* 2004).

4.2.5. Bush encroachment in savanna ecosystems

Between 1940 and 1998, the density and cover of woody plant species on granite substrates in the KNP increased. This trend is ascribed to decreased grass competition, which is possibly a result of overgrazing by wild herbivores (Eckhardt *et al.* 2000). The increase of woody plant density in savannas, known as bush encroachment, is a diverse and complex issue which can greatly reduce the grazing capacity of savannas (Smit *et al.* 1999) and can decrease biodiversity in ecosystems as a whole (Walters and Milton 2005).

Woody species such as *Dichrostachys cinerea* are often responsible for bush encroachment, which includes the thickening of existing woody species or their invasion into open grasslands (Stuart-Hill and Tainton 1999). As these small-leaved savanna species are normally shade intolerant and predominate in early successional communities, they act as initial invaders of rangeland (Wolfson and Tainton 1999). Various *Acacia* species such as *A. nilotica* and *A. karroo* are also responsible for bush encroachment and thicket formation in a number of African savannas (Garner and Witkowski 1997; Kriticos *et al.* 1999; Radford *et al.* 2001; Munkert 2009).

4.2.6. Aim and objectives

The aim of this study was to investigate the role of herbivores, termites and rodents as seed predators and/or dispersers of selected savanna woody plant species and their impact on seed fate in a portion of the Skukuza land system in the Kruger National Park (KNP). The following objectives were developed to fulfill the aim:

1. To determine *A. grandicornuta, A. nigrescens, A. nilotica, A. senegal, A. tortilis* and *Dichrostachys cinerea* density and diversity across the study sites.
2. To determine whether herbivores ingest seeds of *A. grandicornuta, A. nilotica, A. senegal* and *A. tortilis.*
3. To investigate the effect of passing through the gut of large herbivores of *Acacia* seeds on viability and germinability.
4. To examine the abundance of *A. grandicornuta, A. nilotica, A. senegal* and *A. tortilis* on termitaria.
5. To determine whether rodents show a preference for *A. nigrescens, A. senegal, A. tortilis* or *Dichrostachys cinerea* seeds.
6. To investigate the influence of factors such as vegetation cover and rodent species richness and diversity on seed preference for seeds of *A. nigrescens, A. senegal, A. tortilis* and *Dichrostachys cinerea.*

This study focused largely on four *Acacia* species, namely, *A. grandicornuta* Gerstn. (horned thorn), *A. nilotica* (L.) Willd. ex Del. subsp. *kraussianna* (Benth.) Brenan (scented-pod thorn), *A. senegal* (L.) Willd. var. *rostrata* Brenan (bushy three-hook thorn) and *A. tortilis* (Forssk.) Hayne subsp. *heterocantha* (Burch.) Brenan (umbrella thorn), in line with studies discussed in Chapter 2 and 3. The seeds of *Acacia nigrescens* Oliv. (knob thorn) and *Dichrostachys cinerea* (L.) Wight & Arn. subsp. *africana* Brenan & Brummitt (small-leaved sickle-bush) were used in the cafeteria trial to complement a PhD study running simultaneously on *D. cinerea* and *A. nigrescens* trees, and because of the occurrence of specific species in selected study sites.

4.3. Methods and materials

4.3.1. Study site

All field work was conducted within 15 km of the town of Skukuza in the Kruger National Park. Skukuza lies in the southwest of the Park and is characterized by moderately undulating plains underlain by granitic rocks of the Basement Complex (Joubert 2007). It falls

into the lowveld bushveld climatic zone, which receives an annual rainfall of 500-700 mm, strongly concentrated between October and April (Joubert 2007). Skukuza experiences high mean temperatures in summer and mild frost-free winters. It occurs in the landscape that is described as the thickets of the Sabie and Crocodile Rivers, and which is 1263 km^2 in size. This landscape is relatively flat with a dense growth of small thorny shrubs (Joubert 2007). A number of dominant woody plant species are found in the area, namely *Combretum apiculatum* Sond. subsp. *apiculatum* (red bushwillow), *Combretum zeyheri* Sond. (large-fruited bushwillow), *Acacia gerrardii* Benth. var. gerrardii (red thorn), *Euclea divinorum* Hiern (magic guard), *Combretum hereroense* Schinz (russet bushwillow) and *Acacia nigrescens.*

There are 1 903 known species of flora in the Park, which includes over 400 shrub and tree species. The diverse fauna is made up of 147 mammal species, including a number of important herbivores such as elephant (*Loxodonta africana* Blumenbach, 1797), impala (*Aepyceros melampus* Lichtenstein, 1812) and giraffe (*Giraffa camelopardalis* Linnaeus, 1758), amongst others (Eckhardt *et al.* 2000). There are 22 termite genera in the KNP (Meyer *et al.* 2000). Although the majority of termites live underground and are relatively inconspicuous they play a major role in nutrient cycling in the KNP, especially by providing nutrients where they can be easily accessed by plant roots (Braack and Kryger 2003). Seventeen species of rodents from the Family Muridae were described in a list of small mammals of the KNP compiled by Pienaar (1964). These include *Saccostomus campestris* Peters, (pouched mouse), *Steatomys pratensis* Peters, (fat mouse), *Dendromus melanotis* Thomas, (grey climbing mouse), *Tatera leucogaster* Peters, (bushveld gerbil), *Aethomys chrysophilus* de Winton. (red veld rat) and *Mastomys natalensis* A. Smith, (natal multimammate mouse). Other rodent species found in the region include *Mastomys coucha* (multimammate mouse) and *Rhabdomys pumilio* (striped mouse) (Stuart and Stuart 2001).

4.3.2. Seed ingestion by large herbivores

4.3.2.1. Woody plant density and diversity in a portion of the Skukuza land system

Using the GPS (global positioning system) co-ordinates of each tree sampled for *in situ* soil seed banks (see Chapter 2), a satellite map of the area was developed. This resulted in an area being defined of a 15 km radius around Skukuza, KNP (Figure 1). From these co-ordinates, grids of 0.5 ha in size were demarcated, whereby a minimum of three trees of at least one of the four *Acacia* study species occurred within 80 m of one another and where the grids were at least 80 m apart. This resulted in the demarcation of six separate grids. The grids were spaced at least 80 m apart to facilitate rodent trapping and to prevent the overlapping of rodents during foraging

and travelling behaviours. Studies conducted at the Hluhluwe-iMfolozi Park have shown that murid rodents such as *Mastomys* and *Aethomys spp.* travel on average 20-40 m depending on the presence of large herbivore mammals, in savanna vegetation (Hagenah 2006). A distance of 100 m between grids is usually acceptable to overcome pseudo-replication during rodent trapping, as rodent home ranges are usually between 0.5 and 2 ha (Meyer 2004).

Within each grid woody plant density was sampled using the Point-Centred Quarter (PCQ) method, which is a plot-less sampling technique used to determine the number of plants per unit area (see Cottam and Curtis 1956; Greig-Smith 1983). Fifty points were positioned along one side of each grid by measuring out five straight lines ten metres apart, with ten points along each line also ten metres apart (Figure 2a). Lines were measured out using a tape measure. At each 10 m point on the line, four quarters were defined, and the distance to the closest woody plant (> 1 m in height) in each quarter was measured with a tape measure (Figure 2b). Woody plants were identified to species level and the canopy area (CA) (determined by measuring the largest diameter$_1$ (D_1) and smallest diameter$_2$ (D_2)), lowest canopy height (LH-height at the bottom of the canopy) and height (H-height at the top of the canopy) were measured. The height of trees taller than 1 m was determined using the trigonometry method, where the recorder stood at a measured distance from the base of the tree (B) and using a clinometer (model No. 43830, Forestry Suppliers, Inc.), measured the angle in degrees between the horizontal (their eye) and the top of the tree (angle A). The height of the tree was therefore determined using tangent tables and the following formula:

Height of tree = *h* (height of recorder) + B *x* tan(A).

Woody plant density sampling took place on the 9th, 10th, 17th, 18th, 24th and 25th of July 2006. Woody plant density per grid was determined using the following formula:

Density (plants ha-^{1}) = $\frac{1}{r2}$, where r = mean point to plant distance.

The density of individual woody plant species was determined using the following formula:

The density of a species (plants ha^{-1}) = (total density (x/200)), where x = the number of occurrences of individuals of that species. Relative density, which is a comparison of density between specific species, was also calculated. The aerial cover of each tree was calculated using a spherical densiometer Model A with a convex mirror of 2.5 inches in diameter and 24 quarter inch squares (Lemmon 1957);

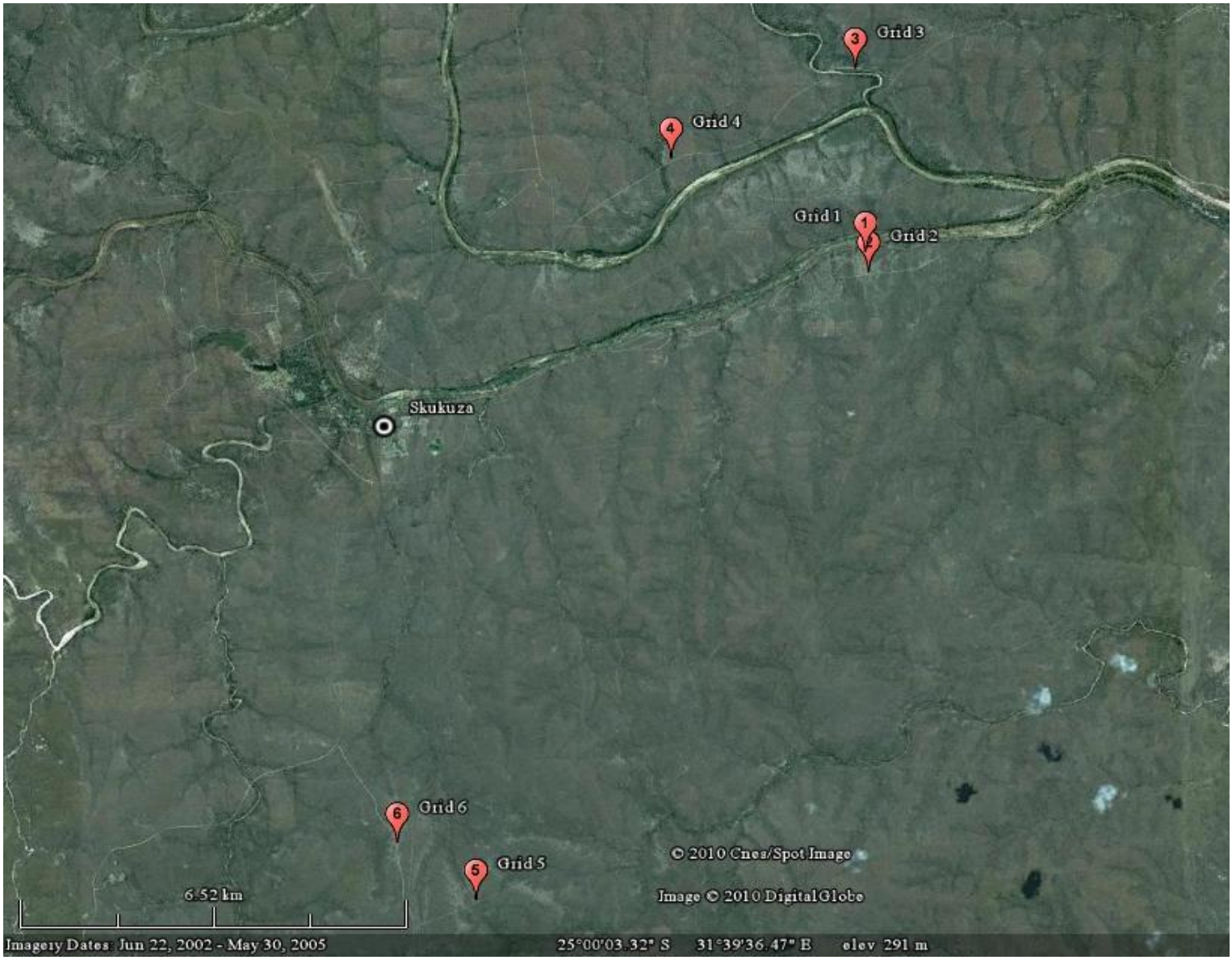

Figure 1. A satellite map showing grid locations for this Chapter.

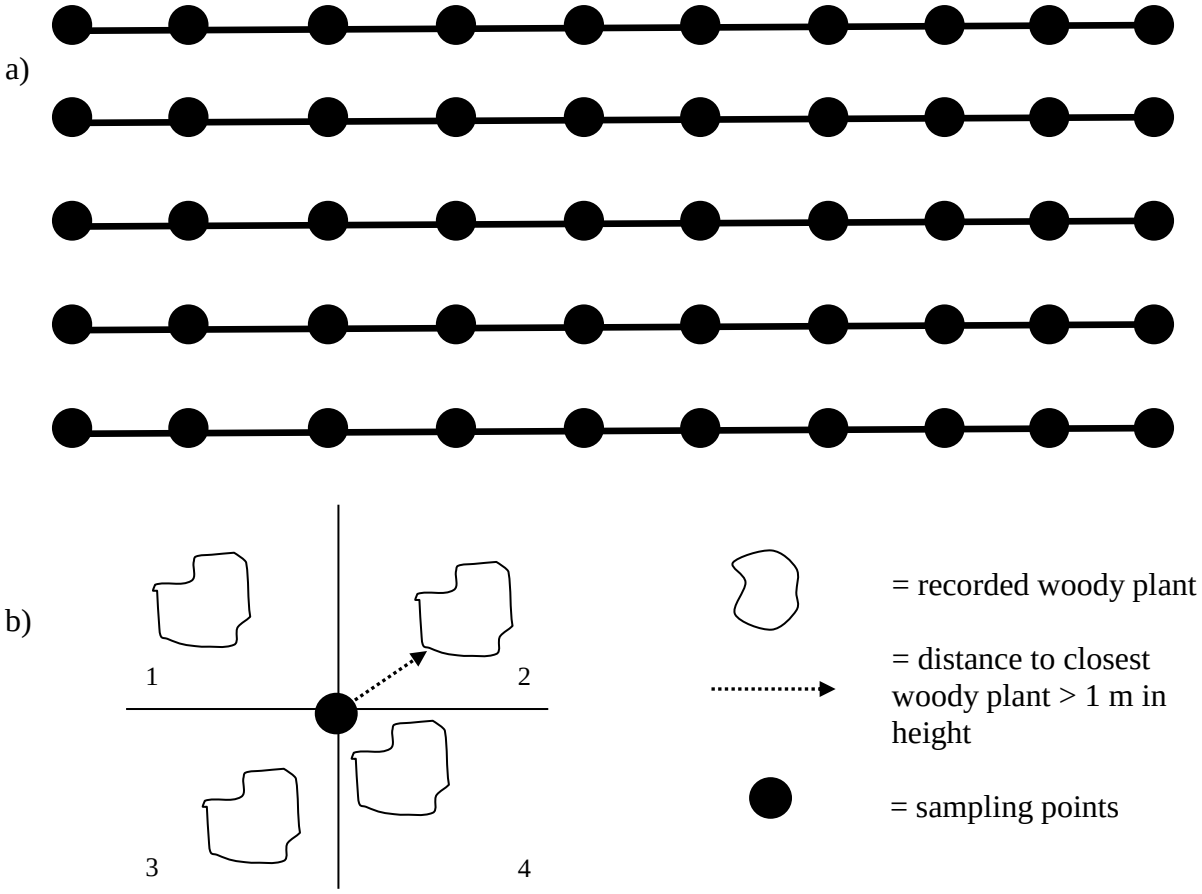

Figure 1. PCQ (Point-Centred Quarter) woody plant density sampling method.

a) The 50 sampling points, along five lines 10 m apart, with 10 points (10 m apart) on each of the lines.

b) Each point was divided into four quarters, where the distance to the nearest woody plant (> 1m in height) was recorded.

4.3.2.2. Acacia seed ingestion by large herbivores

At the same time that PCQ sampling was conducted, large herbivore dung was collected and recorded. At each of the fifty PCQ points, a 1 m x 1 m metal quadrat was thrown randomly within each of the four quarters (Figure 2b) and all herbivore dung situated in the quadrat was collected and placed in labelled bags according to species. It was further noted whether the quadrat was under tree canopy cover or not. Lastly, the percentage of herbaceous ground cover of each quadrat was subjectively estimated for each point. Subjective estimations of herbaceous ground cover were done by the same recorder throughout.

The bagged dung was taken back to the laboratory on the 31st July and 1st August 2006, where it was air-dried and gently crushed with a weight, care being taken not to crush any seeds present in the dung. *Acacia* seeds were separated from the dung with careful sorting using

forceps. Extracted seeds were then identified to species level (modified from Miller 1995). Seed characteristics such as sphericity (see Thompson *et al.* 1993) and Corey Shape Factor (CSF) (Corey 1949), volume, mass and density were recorded for each of the species, *A. grandicornuta, A. nilotica, A. senegal* and *A. tortilis.*

Once measurements were recorded all seeds were then used for a germination trial. Extracted seeds were placed in round Petri dishes (20 cm in diameter; 5 seeds per dish) under a thin layer of potting soil (± 1 cm deep), which was kept moist. Petri dishes were placed in the greenhouse at the University of the Witwatersrand (hereon Wits University) on the 7th August 2006 and monitored for six weeks (D. Mycock *pers. comm.*), on average twice a week, for any germination events i.e. emergence of the plumule above the soil surface. The greenhouse at Wits University is temperature-controlled and experiences similar temperatures to those of Skukuza (see Chapter 3). Seeds were not artificially scarified before the germination trial, as it is assumed that passage through the gut of large herbivores may facilitate scarification (following Miller 1995). Any ungerminated seeds were removed from the Petri dishes on the 18th September 2006 and tested for viability using a 1% tetrazolium salt staining solution (2,3,5-triphenyl-tetrazolium chloride; Moore 1985; Mbalo and Witkowski 1997). Seeds only partially stained were assumed to have low viability and were recorded as non-viable (Mbalo and Witkowski 1997).

4.3.2.3. Data and statistical analyses

For each grid, the Shannon-Weiner Index ($\hat{H}$), the Evenness Index (E) and Simpson's Reciprocal Index (1/D) were calculated (see Magurran 2004) for woody plants, as well as the total number of woody species occurring. For the six study species, woody plant density and density per species per ha was calculated, as was the relative density per species per ha and aerial cover (%). The percentage of quadrats from which dung was collected that were under tree canopy cover was determined. Unless otherwise stated, all data are presented as percentages with means ± standard errors (SE).

Seed characteristics examined (i.e. CSF, sphericity, volume, mass and density) from dung were compared to seeds of the same species sampled from *in situ* soil seed banks, in addition to seeds removed from pods collected from the canopies of trees (see Chapter 2). Comparisons of seed characteristics were done using one-way ANOVAs and post-hoc Tukey Studentized Range tests (α, $P = 0.05$). One-way ANOVAs denote significant differences between the conditions and the post-hoc Tukey test (denoted by superscript letters) specifies where these differences lie, in other words which species are significantly different. All data are

presented as means ± standard errors (SE). For all statistical analyses, Microsoft Office Excel 2007, STATISTICA Release 9 Statsoft, Inc., USA (2010) and SAS ® Institute Inc., USA were used.

4.3.3. Acacia trees and termitaria

The number of termitaria, their surface area and the number of *Acacia* trees growing on the mounds were recorded per grid. The surface area (S A) of each mound was determined by measuring the largest diameter$_1$ (D_1) and smallest diameter$_2$ (D_2) of each mound and using the formula SA = D_1 x D_2. Sampling was done on the 9th, 10th, 17th, 18th, 24th and 25th of July 2006.

4.3.3.1. Data and statistical analyses

Pearson's *r* correlation coefficient was used to analyse the relationship between the number of termite mounds and *Acacia* trees growing on the mounds per grid. For all statistical analyses, Microsoft Office Excel 2007 was used. Data are presented as percentages with means ± standard errors (SE).

4.3.4. Acacia seed predation by rodents

This project was approved by the University of the Witwatersrand Animal Ethics Screening Committee (AESC), clearance certificate number 2006/63/2A.

4.3.4.1. Rodent species diversity

Rodent trapping was done to determine rodent species diversity across all grids, over four consecutive nights per grid, namely on the 5th-8th, 13th-16th and 21st-24th July 2006 (Plate 1). Trapping was done using 100 Sherman live traps (29 cm x 6 cm x 7 cm). Ten lines with ten trapping stations per line were set up in each grid. Traps were placed on the ground in a straight line, ten metres apart, at points on the grids known as trapping stations (one trap/station). Traps were baited with a mixture of oats, raisins, salt, oil and peanut butter, which was placed inside the far end of the trap, and traps were covered with vegetation to buffer the fluctuating daily environmental temperature changes. All traps were checked twice daily, once in the morning (± 7:00) and once in the evening (± 18:00). Any rodent found in a trap was removed immediately and identified to species level. Following species identification, animals were marked by fur clipping and released directly at the station of capture. Fur clipping involves cutting some hair off the rump of the animal with a pair of scissors and is a temporary form of marking. This process does not harm the animal in any way and allows identification of individuals that may

have previously been trapped (N. Pillay *pers. comm.* 2005).

4.3.4.2. Seed preference testing

The seed preference of rodents was investigated using an *in situ* cafeteria trial (developed by N. Pillay 2003/4, unpublished). Due to the seasonal availability of seeds and the occurrence of specific *Acacia* species in the selected grids, only seeds of the following species were used in the cafeteria trial: *A. grandicornuta, A. nigrescens, A. tortilis* and *Dichrostachys cinerea.* Seeds of these species were collected from tree canopies of mature pod-producing trees in the 2005/2006 growing season. Between 200 and 1000 pods were collected per species, depending on the number of accessible pods in the canopy and once pods were de-seeded over 1000 seeds were collected per species. From this, one hundred seeds of each species were randomly selected and categorised as intact (large and swollen with no indications of bruchid beetle predation), predated (seeds with bruchid beetle exit holes and with the inside partially or completely eaten) or aborted (seeds that are flatter, less plump, small and shrivelled in comparison to intact seeds) (see Wilson and Witkowski 2003). Seed trays were prepared for the trial by gluing intact seeds onto pieces of thick cardboard that would be left overnight in grids. Gluing the seeds to cardboard reduced the likelihood of seeds being predated by insects but rendered their removal still possible by rodents. Ten seeds of each species were glued in a 10 cm diameter circle for each separate group, using Ponal Wood Glue, onto a 60 cm x 50 cm cardboard tray (n = 10 seeds/species/tray). Seeds were handled with latex gloves to prevent human odours from affecting the outcome of the experiment. The scent of the wood glue does not appear to affect the attraction of rodents to the seed trays (N. Pillay *pers. comm.* 2005).

The *in situ* cafeteria trial involved a matched-pair design of 5:5 trays, identically prepared, set up at two of the six grids (Figure 3). The trial was conducted in Grid 3 and Grid 4 only, as these grids exhibit vegetation patterns conducive to the experimental design of the trial i.e. dense herbaceous vegetation cover in parallel and within a minimum distance of two metres to an open area with no vegetation cover. Five trays were placed in a line 10 m apart under vegetation cover and five trays were placed in a parallel line (with each tray ± 2 m directly opposite its corresponding covered tray) in open conditions (i.e. no vegetation cover). Trays were weighed down by sticking a peg or nail through the middle, as well as with rocks and sand.

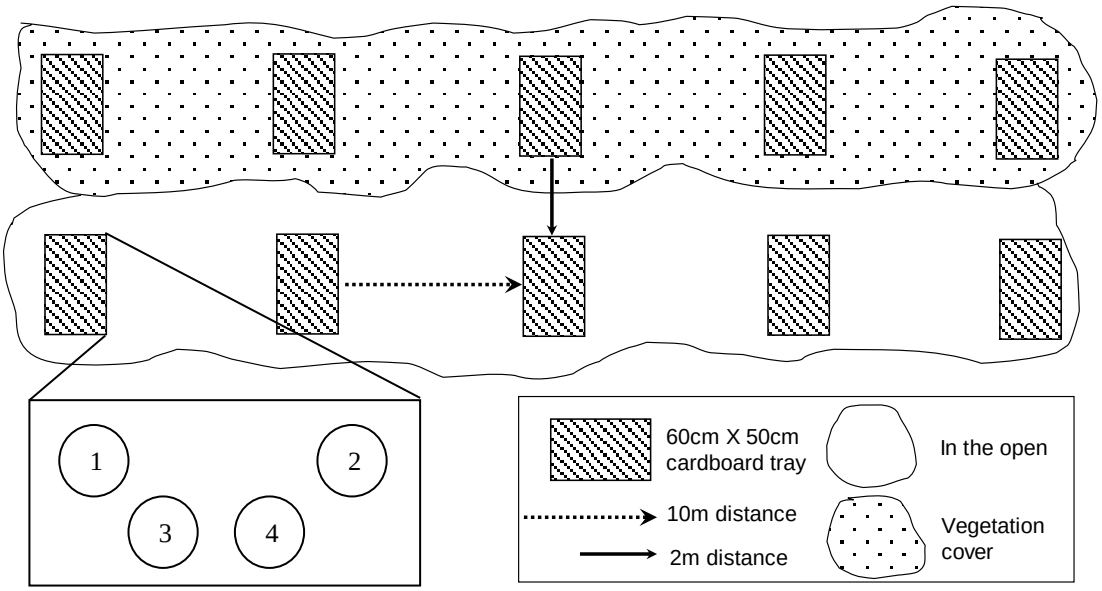

Figure 3. Placement of the *in situ* cafeteria trial seed trays in Grid 3 and Grid 4. Five trays were placed under vegetation cover and five trays were placed in the open, in each grid. Each seed tray was baited with ten seeds each of four savanna woody plant species, specifically 1) *A. nigrescens*, 2) *A. tortilis*, 3) *A. grandicornuta* and 4) *D. cinerea*.

Seed trays were placed in each grid after dusk (± 18:30) and removed just before dawn (± 05:00) to prevent diurnal birds from removing the seeds. New trays were set out every night. The trial ran for three consecutive nights, the 28th, 29th and 30th July 2006, simultaneously in Grid 3 and Grid 4. Each morning the number and type (species) of seeds removed from each tray were recorded as well as whether the tray was located under vegetation cover or in the open.

4.3.4.3. Data and statistical analyses

Pearson's *r* correlation coefficient was used to test the relationship between rodent and woody plant species richness and diversity in all six grids. Comparisons of the percentage of seeds removed from seed trays (per tray) in the *in situ* cafeteria trial between seed species was analysed using one-way ANOVAs and the post-hoc Tukey Studentized Range test (α, P = 0.05). The non-parametric Mann-Whitney U Test was used to compare the percentage of seeds removed per tray between Grid 3 and Grid 4. The Wilcoxon Paired Test was used to compare between trays left under vegetation cover and trays left in the open across both grids combined, as well as between trays under cover and in the open within each grid and between grids for trays in the open and under cover. Data are mostly presented as means ± standard errors (SE). For all statistical analyses, Microsoft Office Excel 2007, STATISTICA Release 9 Statsoft, Inc., USA (2010) and SAS ® Institute Inc., USA were used.

Plate 1. Images of the rodent trapping experiment and the *in situ* cafeteria trial conducted near Skukuza, Kruger National Park, South Africa. Clockwise from top left: checking traps for rodents; one live Sherman trap (29 cm x 6 cm x 7 cm) in position at a trapping station; retrieving trays from the *in situ* cafeteria trial left overnight in Grid 3; seed trays ready to be placed in position for the *in situ* cafeteria trial; a rodent removed from the Sherman live traps.

4.4. Results

4.4.1. Acacia seed ingestion by large herbivores

4.4.1.1. Woody plant density and diversity

A total of 47 woody plant species were recorded across all six grids (Appendix 1), with 18.2 ± 2.8 species observed per grid, within an area of approximately 0.5 ha per grid, which was 36 ± 5.5 species ha^{-1}. The Shannon-Wiener Index (1.8 ± 0.2), Evenness Index (0.7 ± 0.02) and Simpson's Reciprocal Index (4.5 ± 0.6) indicate that species diversity was relatively low across all six grids, specifically in Grid 6 (Table 1).

Table 1. The number of woody plant species recorded as well as the Shannon-Wiener Index (Ĥ), the Evenness Index (E) and the Simpson's Reciprocal Index (1/D) across six sampling grids in the Skukuza land system, Kruger National Park, South Africa (means ± SE).

Grid	Number of species recorded	Shannon-Wiener Index (Ĥ)	Evenness Index (E)	Simpson's Reciprocal Index (1/D)
1	14	1.7	0.7	3.9
2	13	1.4	0.5	2.6
3	29	2.4	0.7	6.6
4	19	2.0	0.7	4.3
5	22	2.2	0.7	5.5
6	11	0.7	0.7	3.7
Mean (± SE)	18. 2 ± 2.8	1.8 ± 0.2	0.7 ± 0.02	4.5 ± 0.6

Woody plant density per ha (Figure 4) was highest in Grid 5 (1618 plants ha^{-1}), followed by Grid 6 (1055 plants ha^{-1}), Grid 4 (1017 plants ha^{-1}), Grid 1 (646 plants ha^{-1}), Grid 2 (610 plants ha^{-1}) and Grid 3 (226 plants ha^{-1}).

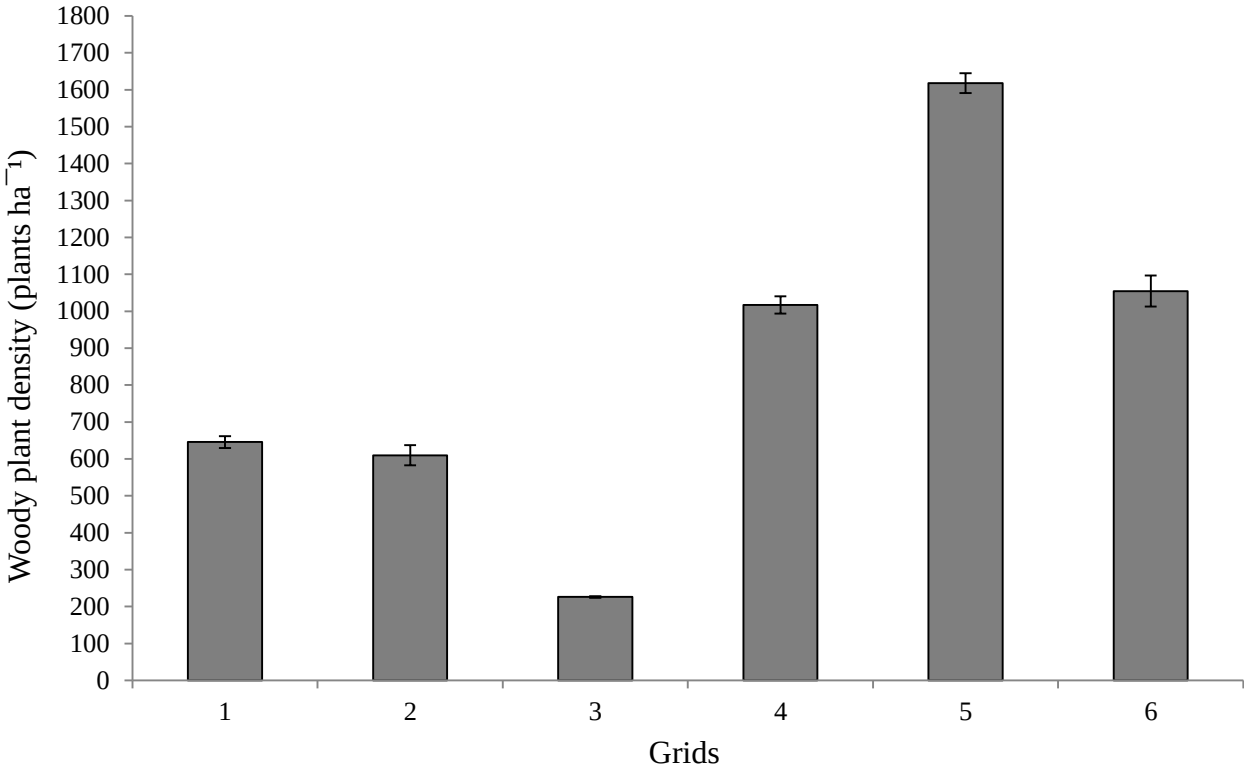

Figure 4. Woody plant density (mean ± SE) for six sampling grids in the Skukuza land system, Kruger National Park, South Africa determined using the Point-Centred Quarter (PCQ) sampling method.

The most densely occurring species overall was *A. grandicornuta* (169 ± 60 plants ha^{-1}), followed by *D. cinerea* (11 ± 63 plants ha^{-1}), *A. tortilis* (13 ± 10 plants ha^{-1}), *A. nilotica* (9 ± 5 plants ha^{-1}), *A. senegal* (2 ± 1 plants ha^{-1}) and *A. nigrescens* (1 ± 1 plants ha^{-1}). In Grid 3 five of the six study species were recorded, in Grids 2, 4 and 6 four study species were recorded, two study species were recorded in Grid 1, and one species was recorded in Grid 5 (Figure 5).
In Grids 1, 2, 4 and 6 *A. grandicornuta* was the most densely occurring study species, *A. tortilis* in Grid 3 and *D. cinerea* in Grid 5 (Figure 5).

Relative density of the six woody species is presented in relation to all woody species recorded in the PCQ-sampling method (Figure 5). The relative density of *A. grandicornuta* trees (> 1 m in height) was higher (24.2 ± 8.3%) than the other six species, and was followed by *D. cinerea* (7.0 ± 3.9%), *A. tortilis* (4.8 ± 4.5%), *A. nilotica* (1.3 ± 0.8%), *A. senegal* (0.7 ± 0.6%) and *A. nigrescens* (0.1 ± 0.1%).

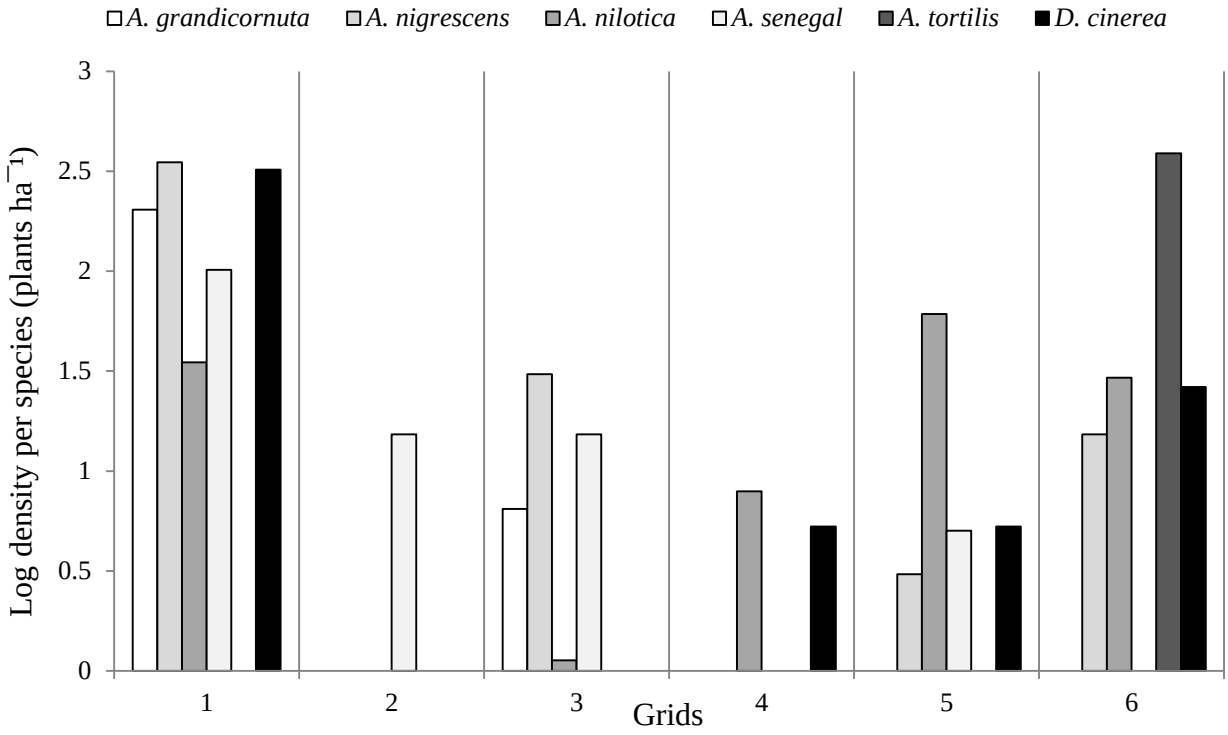

Figure 5. Density of six selected savanna woody plant species (log values) for six sampling grids in the Skukuza land system, Kruger National Park, South Africa, determined using the Point-Centred Quarter (PCQ) sampling method.

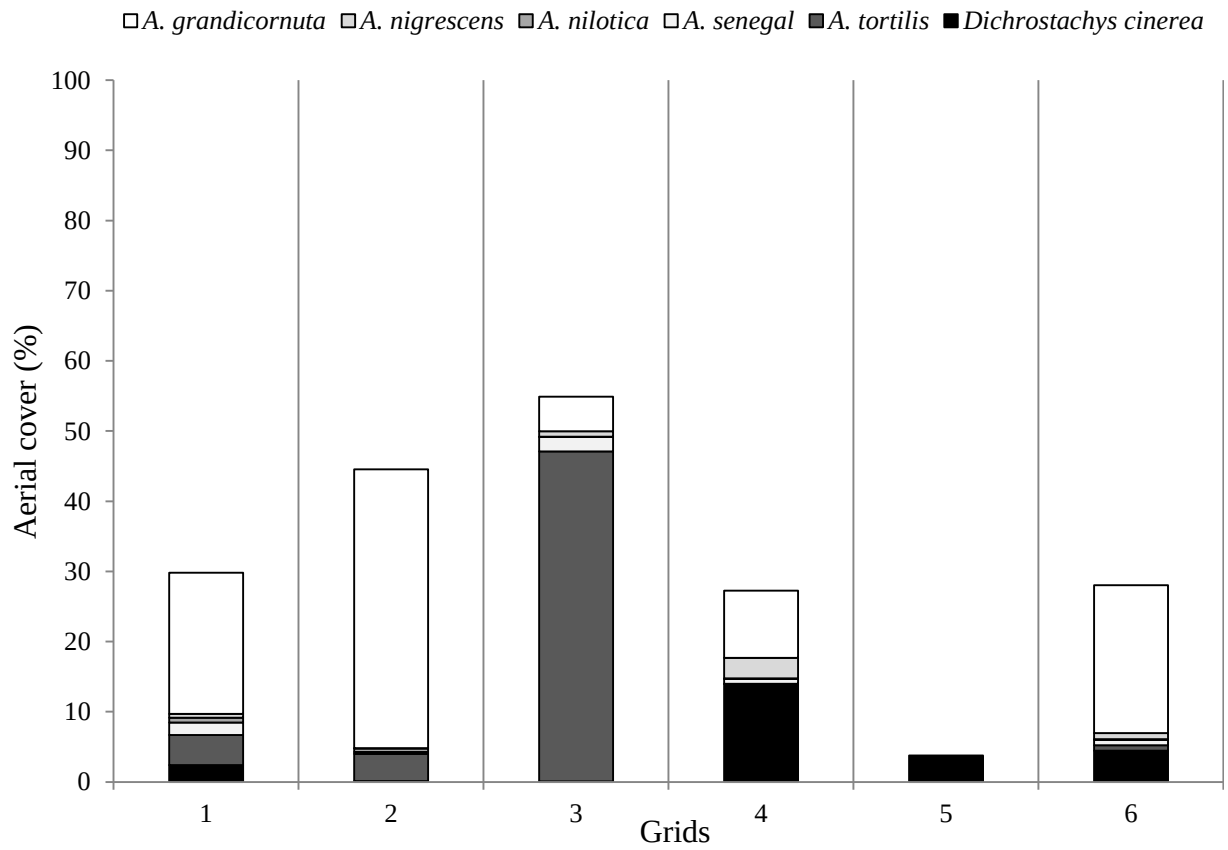

Figure 6. Aerial cover of six savanna woody plant species for six sampling grids in the Skukuza land system, Kruger National Park, South Africa.

Of the six selected woody plant species, *A. grandicornuta* had the largest aerial cover (15.9 ± 5.8%) (Figure 6), followed by *A. tortilis* (9.3 ± 0.3%), *D. cinerea* (4.1 ± 2.1%), *A. senegal* (0.9 ± 0.3%), *A. nigrescens* (0.8 ± 0.4%) and *A. nilotica* (0.2 ± 0.1%). Overall woody plant aerial cover (%) was highest in Grid 3 (77.7%), followed by Grid 1 (62.1%), Grid 2 (48.2%), Grid 6 (44.8%), Grid 4 (42.6%) and Grid 5 (24.6%).

4.4.1.2. Large herbivore seed ingestion

Nine different types of herbivore dung were collected across all six grids, these were: *Aepyceros melampus* (impala), *Loxodonta africana* (elephant), *Connochaetes taurinus* (wildebeest), *Phacochoerus aethiopicus* (warthog), *Sylvicapra grimmia* (duiker), *Raphicerus campestris* (steenbok), *Tragelaphus strepsiceros* (kudu), *Diceros bicornis* (black rhino) and *Giraffa camelopardalis* (giraffe).A total of 67 *Acacia* seeds were extracted from the dung collected across all six grids (Table 2). Of the ingested seeds 82.1% were *A. tortilis,* followed by *A. nilotica* (16.4%) and *A. grandicornuta* (1.5 %). No *A. senegal* seeds were extracted from dung. *Acacia* seeds were extracted from impala, elephant, wildebeest and giraffe dung only (Table 2). Of the 67 ingested *Acacia* seeds 53.8% were extracted from wildebeest dung, followed by elephant (29.9%), impala (13.4%) and giraffe dung (2.9%).

Table 2. Percentages (%) of seeds of four *Acacia* species extracted from the dung of four large herbivore species across six sampling grids in the Skukuza land system, Kruger National Park, South Africa (*n* = 67). Numbers in brackets indicate the grids from where dung and ingested seeds were collected.

	Seed species			
Large herbivore species (grid number)	*A. grandicornuta* (3)	*A. nilotica* (3,6)	*A. senegal*	*A. tortilis* (1,2,3,4,5,6)
Aepyceros melampus (impala)	1.5	0	0	11.9
Connochaetes taurinus (wildebeest)	0	14.9	0	38.8
Giraffa camelopardalis (giraffe)	0	1.5	0	1.5
Loxodonta africana (elephant)	0	0	0	29.9
TOTAL:	1.5	16.4	0	82.1

From the 67 seeds collected only six seeds germinated in the germination trial, one *A. grandicornuta* seed and five *A. tortilis* seeds (Table 3). Of the remaining sixty-one ungerminated seeds, five *A. nilotica* and twenty-two *A. tortilis* seeds tested viable. After the germination trial, there was a higher percentage of remaining intact, non-viable seeds than

remaining intact, viable seeds for both *A. nilotica* and *A. tortilis* (Table 3).

Table 3. Percentages (%) of germinated, remaining intact, viable, and remaining intact, non-viable seeds of three *Acacia* species extracted from dung after a six-week germination trial *(n* = 67). The fourth species, *A. senegal* was not included as no seeds of that species were found in dung.

	A. grandicornuta (n = 1)	*A. nilotica* (n = 11)	*A. tortilis* (n = 55)
Germinated seeds	100	0	10
Remaining intact, viable seeds	0	46	40
Remaining intact, non-viable seeds	0	54	50

Dung was recorded in a smaller percentage of quadrats under tree canopy cover as in the open. In Grid 2, 37% of quadrats recorded with dung were under tree canopy cover, followed by Grid 6 (35%), Grid 1 (31%), Grid 3 (27%), Grid 4 (25%) and Grid 5 (20%).

4.4.1.3. Comparison of seed characteristics between three different seed treatments

The three treatments of seeds comprised a) seeds sampled from pods from the canopy of mature trees (hereon, canopy seeds) (see Chapter 2), b) seeds sampled from *in situ* soil seed banks (see Chapter 2) and c) seeds ingested by large herbivores and extracted from dung. Comparisons of seed characteristics were done for *A. nilotica* and *A. tortilis* only, since only a single *A. grandicornuta* seed and no *A. senegal* seeds were extracted from dung.

One-way ANOVAs showed a significant difference in seed mass, volume, density and CSF (see Table 4 for statistics), while no significant difference in sphericity *(P* = 0.9), between three different treatments of *A. nilotica* seeds. Seed bank seeds were significantly lighter (0.04 ± 0.007 g), with a significantly smaller volume (48.9 ± 7.4 mm^3), density (0.0006 ± 0.00008 g mm^{-3}) and CSF (0.4 ± 0.05) than seeds from dung and canopy seeds (Table 4a and b).

There was a significant difference between treatments of *A. tortilis* seeds for mass, volume, density and sphericity (Table 4a and c). The CSF values between the three samples tended to be different (*P* = 0.07). Seeds of *A. tortilis* extracted from dung and seed bank seeds had similar characteristics, while canopy seeds were heavier (0.04 ± 0.002 g), less spherical (0.4 ± 0.01), more dense (0.0017 ± 0.00002 g mm^{-3}) and had a larger volume (26.0 ±1.1 mm^3) than the other two treatments of seeds.

Table 4 a) A comparison of seed volume, mass, density, CSF (Corey Shape Factor) and sphericity (mean ± SE) between three different treatments of seeds: a) seeds sampled from pods taken from the canopy of mature trees, b) seeds sampled from *in situ* soil seed banks, and c) ingested seeds extracted from large herbivore dung, for *A. nilotica* and *A. tortilis*. *A. senegal* and *A. grandicornuta* seeds were not included in this comparison, as not enough seeds of these species were extracted from dung (0 and 1 seed, respectively). Means with different superscript letters are significantly different at the *P* = 0.05 level, within columns.

Seed treatments	Volume (mm^3)	Mass (g)	Density ($g\ mm^{-3}$)	CSF	Sphericity
			A. nilotica		
Canopy seeds (n = 20)	90.3 ± 3.6^{A}	0.1 ± 0.004^{A}	0.0012 ± 0.00001^{A}	0.6 ± 0.01^{A}	0.06 ± 0.003
Seed bank seeds (n = 36)	48.9 ± 7.4^{B}	0.04 ± 0.007^{B}	0.0005 ± 0.00008^{B}	0.4 ± 0.05^{B}	0.06 ± 0.006
Dung seeds (n = 11)	88.5 ± 4.8^{A}	0.1 ± 0.007^{A}	0.0012 ± 0.00004^{A}	0.6 ± 0.03^{A}	0.06 ± 0.006
			A. tortilis		
Canopy seeds (n = 20)	26.0 ± 1.1^{A}	0.04 ± 0.002^{A}	0.0017 ± 0.00002^{A}	0.4 ± 0.01	0.11 ± 0.002^{A}
Seed bank seeds (n = 142)	18.9 ± 0.6^{B}	0.03 ± 0.001^{B}	0.0013 ± 0.00004^{B}	0.5 ± 0.01	0.07 ± 0.002^{C}
Dung seeds (n = 55)	19.3 ± 0.6^{B}	0.02 ± 0.001^{C}	0.0012 ± 0.00005^{B}	0.5 ± 0.01	0.09 ± 0.003^{B}

b) One-way ANOVA results for the comparison of seed volume, mass, density, sphericity and CSF between three different samples of seeds for *A. nilotica* (d.f. = 2, 64).

	Volume (mm^3)	Mass (g)	Density ($g\ mm^{-3}$)	CSF	Sphericity
P	0.00005	< 0.0001	< 0.0001	0.0079	0.9
F	11.7	28.1	23.2	5.2	0.1

c) One-way ANOVA results for comparisons of seed volume, mass, density, sphericity and CSF between three different samples of seeds for *A. tortilis* (d.f. = 2, 214).

	Volume (mm^3)	Mass (g)	Density ($g\ mm^{-3}$)	CSF	Sphericity
P	0.00008	< 0.0001	0.0003	0.07	< 0.0001
F	9.8	41.8	8.3	3.0	18.7

4.4.2. Termitaria and Acacia trees

No termitaria were recorded in Grids 1 or 4. Per grid, termitaria occupied 0.00091 ± 0.00034 ha of the grid matrix surface area. Three mounds were recorded in Grid 2 (0.00073 ± 0.0001 ha), one mound in Grid 3 (0.0011 ± 0 ha), seven mounds in Grid 5 (0.0016 ± 0.00071 ha) and four mounds in Grid 6 (0.002 ± 0.0016 ha). The percentage surface area of the grid matrix occupied by termitaria was low for all grids, namely 0.4% (Grid 2), 0.2% (Grid 3), 2.3% (Grid 5) and 1.6% (Grid 6).

There was no correlation *(n* = 15, 20; r = 0.07) between the number of termitaria recorded and the number of *Acacia* trees growing on termitaria. Only twenty *Acacia* trees were recorded growing on termitaria, comprised of *A. grandicornuta* (10%), *A. tortilis* (5%) and *A. robusta* (85%). One *A. grandicornuta* tree was recorded on a mound in Grid 2 and a mound in Grid 6, while one *A. tortilis* tree was recorded on a mound in Grid 3.

4.4.3. Acacia seed predation by rodents

4.4.3.1. Rodent species diversity

Four different species of rodents were recorded across all six grids, from the family Muridae (Stuart and Stuart 2001), namely *Mastomys coucha* (Smith 1834) (multimammate mouse), *Rhabdomys pumilio* (Thomas 1916) (striped mouse), *Aethomys chrysophilus* (de Winton 1897) (red veld rat) and *Tatera leucogaster* (Peters 1852) (highveld gerbil) (Appendix 2), with 2.3 ± 0.5 species recorded per grid.

The Shannon-Wiener Index (0.6 ± 0.2), Simpson's Reciprocal Index (1.9 ± 0.4) and Evenness Index (0.6 ± 0. 2) indicate a low rodent species diversity in the grids (Table 5).

Table 5. The number of rodent species recorded as well as the Shannon-Wiener Index (Ḯ), the Evenness Index (E) and the Simpson's Reciprocal Index (1/D) across six sampling grids in the Skukuza land system, Kruger National Park, South Africa (*n* = 6).

Grid	Number of species recorded	Shannon-Wiener Index (Ḯ)	Evenness Index (E)	Simpson's Reciprocal Index (1/D)
1	2	0.6	0.8	1.7
2	1	0	0	1.0
3	3	1.0	0.9	2.5
4	3	0.8	0.8	2.0
5	4	1.2	0.9	3.3
6	1	0	0	1.0
Mean (± SE)	2.3 ± 0.5	0.6 ± 0.2	0.6 ± 0. 2	1.9 ± 0.4

Eighteen rodents were recorded in Grid 5, nine in Grid 4, eight in Grid 3, four in Grid 1, two in Grid 2 and one in Grid 6 (Figure 7). Four rodent species were recorded in Grid 5, three in Grids 3 and 4, two in Grid 1 and one in Grids 2 and 6 (Figure 7).

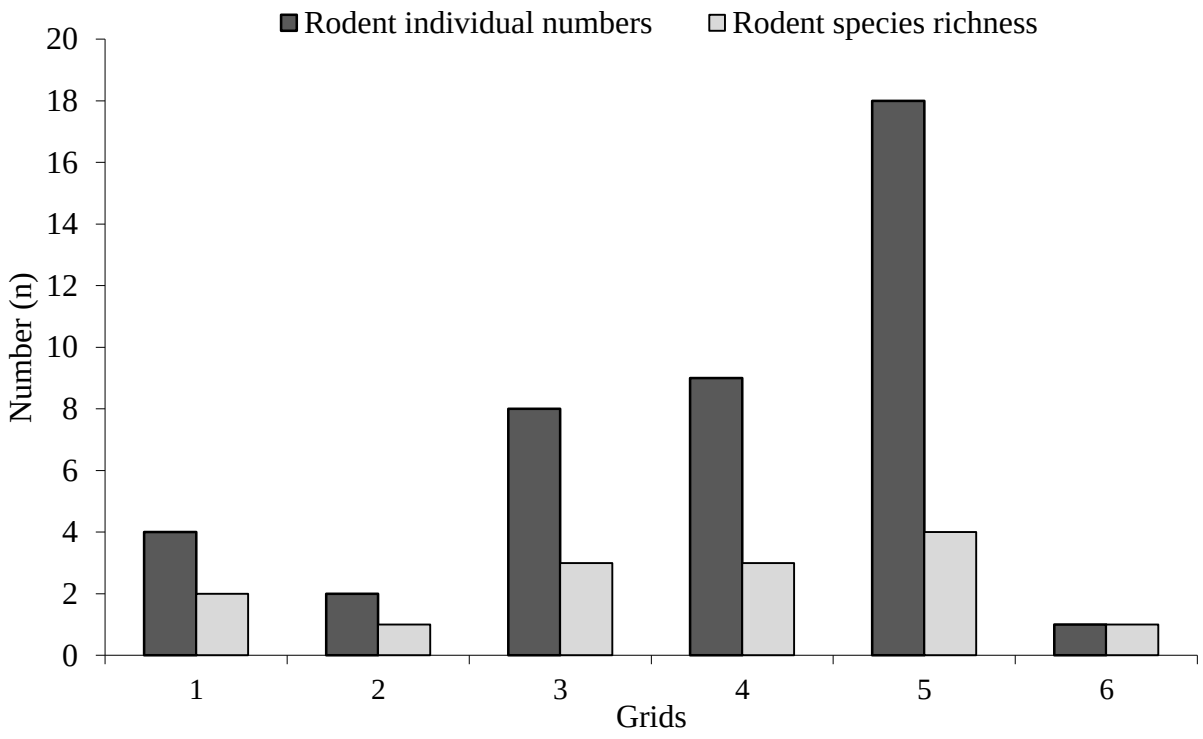

Figure 7. The number of rodent individuals and species recorded in the sampling grids 1-6 in the Skukuza land system, Kruger National Park, South Africa.

Pearson's *r* correlation coefficient showed a positive relationship between the number of rodent species and woody plant species per grid for Grids 1-6 (r = 0.8), whereas the number of woody plant species increased, the number of rodent species increased. A positive relationship was also found between rodent and woody plant diversity (r = 0.9).

4.4.3.2. In situ cafeteria trial

There was a significant difference in the percentage of seeds removed of each species from the trays (P = 0.04; F = 2.8; d.f = 3, 236). The post-hoc analysis showed that, from a sample of 10 seeds per species per tray, *A. senegal* seeds were removed significantly less (6.5 ± 1.2 %) compared to the other three species, where no differences occurred (14.3 ± 2.5 % *A. tortilis* seeds, 15.3 ± 3.1 % *A. nigrescens* seeds, and 15.3 ± 2.9 % *Dichrostachys cinerea* seeds). There was a significant difference in the percentage of seeds removed per tray overall between trays placed under herbaceous cover (13.5 ±1.7 %) and trays placed in the open (12.3 ± 1.9 %) (Wilcoxon Two-sample Test: P = 0.03; W= 13319) (Table 6). There was a significant difference in the percentage of seeds removed from trays between Grid 3 and Grid 4, with more seeds removed per tray from Grid 3 (Mann-Whitney U Test: P < 0.05; U = 10718.0). There was a significant difference in the percentage of seeds removed between Grid 3 and 4 for trays under cover (P < 0.05; W = 88.5) and in the open (P = 0.0008; W = 140). Within Grid 3, there was no significant difference in the percentage of seeds removed between trays in the open and under cover (P = 0.3; W = 636.5) while there was a significant difference in Grid 4 (P = 0.003; W = 205).

Table 6. Comparison of the percentage of seeds (%) (mean ± SE) removed per tray by rodents from seed trays placed in two sampling grids at two different micro-sites, namely in the open and under vegetation cover, in an *in situ* field cafeteria trial between four savanna woody plant species, in the Kruger National Park, South Africa (*n* = 10). Means with different superscript letters are significantly different at the *P* = 0.05 level.

	Grid 3		Grid 4		
Seed species	Open	Cover	Open	Cover	Mean ± SE
A. nigrescens	23.3	22.7	5.3	10.0	15.3 ± 3.1^{A}
A. senegal	9.3	10.0	0.7	6.0	6.5 ± 1.2^{B}
A. tortilis	31.3	16.7	1.3	8.7	14.3 ± 2.5^{A}
D. cinerea	22.0	27.3	5.3	6.7	15.3 ± 2.9^{A}
Mean ± SE	21.3 ± 3.4	19.2 ± 3.0	3.2 ± 0.9	7.8 ± 1.1	
Mean ± SE	20.3 ± 2.4^{A}		5.5 ± 0.7^{B}		

4.5. Discussion

4.5.1. Woody plant density and diversity

All grids were situated in the Sabie/Crocodile thorn thickets on granite (Figure 8) (Thomas and Grant 1997), which occurs in the Skukuza land system (Venter *et al.* 2003). At the landscape scale of the Skukuza land system, fertile and infertile habitats can be clearly defined where granitic catenas exhibit sandy, nutrient-poor crests and clayey nutrient-rich valleys (Figure 8). These nutrient-rich areas are dominated by fine-leafed *Acacia* vegetation, which has physical defences against herbivory, while the nutrient-poor areas exhibit broad-leafed woody vegetation chemically protected from herbivory (Scholes *et al.* 2003). Plant communities in alluvial sediments are generally species-rich and the extent of the effect of these soils increases with the size of the drainage line (Venter *et al.* 2003).

Woody plant density varied between grids where the highest woody plant density was recorded at grids located at a greater distance from drainage lines and at higher elevations, namely Grid 5 (1618 plants ha^{-1}) and Grid 6 (1055 plants ha^{-1}). Grid 5 (31°36'04.6 E, 25°04'18.1 S), which was located approximately 289 m above sea level and Grid 6 (31°35'56.5 E, 25°3'42.3 S), approximately 345 m above sea level, were both more than 5 km from the nearest drainage line, the Msimuku River (Figure 2).The number of species recorded in Grid 5 was relatively high, as was the species diversity indicated by various diversity indices. Dominant woody plant species in Grid 5 included *Grewia bicolor* Juss, *D. cinerea, A. exuvialis* and *Combretum apiculatum* Sond. These species are found on granite crests and well-drained sandy or rocky soils in the Sabie/Crocodile thorn thickets (Thomas and Grant 1997). Dominant

species in Grid 6, which had a relatively low number of species and diversity, included *Spirostachys africana* Sond., *A. grandicornuta, Euclea divinorum* Hiern. and *G. bicolor*. Although *S. africana* is more common along rivers and brackish flats, it occurs on all soil types, and although *G. bicolor* is also more common in alluvial soils along river courses (Thomas and Grant 1997), it is widely spread throughout the KNP (van Wyk 1984).

Grids located in closer vicinity to drainage lines (less than 500 m in distance), namely Grid 1, 2, 3 and 4, had lower woody plant densities. The footslope positions on the granitic catena of the Skukuza land system are characterized by more open and less dense small-leaved shrubveld (Venter *et al.* 2003). Grid 1 (31°40'38.9 E, 24°58'1.2 S) and Grid 2 (31°40'40.9 E, 24°58'12.4 S) were approximately 259 and 264 m above sea level, respectively (Figure 2). Both grids had similar woody plant density (646 plants ha^{-1} and 610 plants ha^{-1}, respectively) and number of species recorded (35 and 33 species ha^{-1}, respectively). Grid 1, which was approximately 120 m from the Sabie River, had dominant woody plant species such as *A. grandicornuta, Spirostachys africana,* and *G. bicolor* and low species diversity. All three of these species are common in brackish flats along river banks in the Sabie/Crocodile thorn thickets (Thomas and Grant). Grid 2, which had low species diversity, was located at a distance of 450 m from the Sabie River and woody plants dominating the area included *A. grandicornuta, G. bicolor, Balanites maughamii* Sprague and *A. nilotica. Balanites maughamii* is most common at valley bottoms, while *A. nilotica* is often found on brackish flats alongside river edges (Thomas and Grant 1997).

Grid 3 had the lowest woody plant density (226 plants ha^{-1}) and the highest number of species recorded, specifically 73 species ha^{-1}. Species diversity in Grid 3 was high compared to other grids. All six study species were recorded there, except *A. nigrescens.* Grid 3 (Figure 2) (31°40'32.6 E, 24°56'14.9 S), was situated 279 m above sea level and approximately 115 m from the Sand River. The five most abundant woody plant species recorded included *A. tortilis, A. grandicornuta, D. cinerea, G. bicolor* and *Pappea capensis* Eckl. & Zeyh. A combination of these species is often located in valley bottoms and brackish flats and all five species are often found growing close to river edges in the Sabie/Crocodile thorn thickets (Thomas and Grant 1997).

Grid 4 (31°38'41.4 E, 24°57'6.7 S) was approximately 130 m from the Nwanyikulu drainage line (Figure 2), at about 270 m above sea level. With a relatively high number of species recorded (48 species ha^{-1}), and high species diversity and woody plant density (1017 plants ha^{-1}), dominant woody plant species in this grid included *Euclea divinorum, A. grandicornuta, S. africana* and *P. capensis.* All of these species are commonly found on brackish flats along

river courses (Thomas and Grant 1997).

The Point-Centred Quarter (PCQ) woody plant density analysis showed that *A. grandicornuta* was the most abundant of the six study species (169 ± 60 plants ha^{-1}). This deciduous tree grows in arid bushveld, usually in deep clay soils near rivers and drainage lines and dehiscent pods split to release seeds from April to July (Schmidt *et al.* 2002). Although it was the dominant woody plant species in Grid 3, *A. tortilis* occurred relatively less densely overall (13 ± 10 plants ha^{-1}). This *Acacia* species is an arid bushveld tree that fruits during the period May-June (Schmidt *et al.* 2002) and grows along brackish flats alongside rivers (Thomas and Grant 1997). Neither *A. nilotica* (9 ± 5 plants ha^{-1}), which was recorded in small numbers in Grids 1, 2, 3 and 4, nor *A. senegal* (2 ± 1 plants ha^{-1}), which was only recorded in Grid 3 and 6, were dominant species in any of the grids. South of Tshokwane in the KNP, low-lying brackish areas show growth of *A.senegal*, while *A. nilotica* grows throughout the Park, also along rivers in low-lying brackish areas (van Wyk 1984). The woody plant *D. cinerea* occurred relatively densely overall (77 ± 63 plants ha^{-1}) and was absent from Grids 1 and 4 only, while only a single *A. nigrescens* tree was recorded in Grid 3. While *D. cinerea* often occurs in large groups close to rivers and on brackish flats, *A. nigrescens* favours clay soils often found in granitic valley landscapes but does not grow along permanent water courses (Thomas and Grant 1997).

The aerial cover of all woody plant species recorded in grids in this study was high in comparison to that recorded in three other savanna regions in South Africa (Scholes and Walker 2004; Eckhardt *et al.* 2000; Munkert 2009) (Table 7). In a study conducted on the granite transects of the south-central region of the KNP, woody vegetation cover increased significantly from 1940-1998 (Eckhardt *et al.* 2000) but was lower in 1998 than that recorded in this study. Overall woody plant cover on granite substrate in the KNP has increased over 58 years, possibly as a result of overgrazing by herbivores (Eckhardt *et al.* 2000). In this study, grids with higher aerial cover were recorded with a higher number of *Acacia* species, specifically *A. grandicornuta* and *A. tortilis.* African *Acacias* contribute greatly to the increase in the woody components of savannas, also known as bush encroachment (Walters and Milton 2003), which is a poorly understood phenomenon (Midgley and Bond 2001). In southern Africa, legumes from the subfamily Mimosoideae are often bush-encroaching species (Witkowski and Garner 2000).

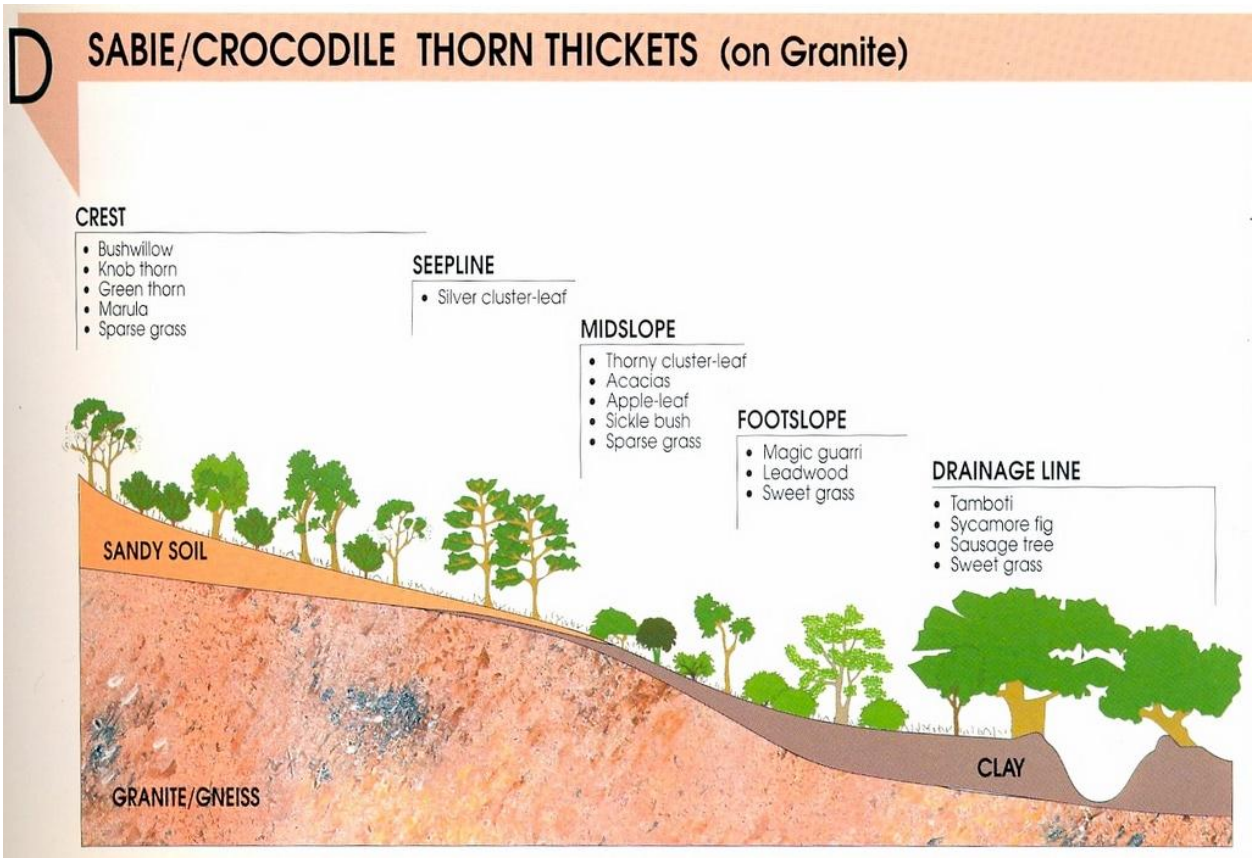

Figure 8. Diagrammatic representation of a granitic catena at the landscape scale, from the Sabie/Crocodile Thorn Thickets in the Kruger National Park, South Africa (adapted from Ecozone Map, Jacana Maps 1998).

Table 7. Comparison of woody plant aerial cover in four different savanna regions in South Africa.

	Skukuza land system, KNP (this study, 2006)	South-central KNP (Eckhardt *et al.* 2000)	NPNR (Scholes and Walker 2004)	Hluhluwe-iMfolozi Park (Munkert 2009)
Aerial cover (%)	49.9 ± 7.4	22.1	25.7 (fine-leaved) – 32.0 (broad-leaved)	23

4.5.2. Large herbivore seed ingestion and dispersal

Sixty-seven *Acacia* seeds were extracted from large herbivore dung collected from six grids in this study, indicating that seeds are ingested by large herbivores to some extent. The most important effect of the ingestion of seeds by mammals is the defecation in and dispersal of seeds to areas more suitable for germination (i.e. safe sites, see Fenner and Thompson 2005) and away from the threats induced in the environment around a parent plant such as high shade and seed predation (du Toit 1990; Tybirk *et al.* 1992; Wilson and Witkowski 1998; Midgley and Bond 2001). Various savanna *Acacia* characteristics such as mechanical defences and palatable

leaves indicate that African savannas may have co-evolved with mammalian herbivores (Du Toit 1990; Stuart-Hill and Tainton 1999). Miller (1996) showed that in the presence of large herbivores, seeds of *A. tortilis, A. karroo* and *A. nilotica* were freed from pods and dispersed away from the parent tree to open and non-shaded habitats. Poor dispersal may affect the probability of seeds arriving at appropriate micro-sites, essentially causing seed limitations to plant recruitment (Fenner and Thompson 2005).

The majority of seeds extracted from dung in this study were indehiscent species, namely *A. nilotica* (16.4%) and *A. tortilis* (82.1 %). Indehiscent seeds such as *A. tortilis* and *A. nilotica* are typically ungulate-dispersed (Tybirk *et al.* 1992). Although *A. nilotica* seeds have a strong smell thought to attract ungulates to eat them (Shayo and Udén 1998), almost five times more *A. tortilis* than *A. nilotica* seeds were extracted from herbivore dung in this study. In a study by Shayo and Udén (1998) on the ingestion of seeds by domestic herbivores, the intake of *A. tortilis* pods and seeds was significantly higher than the intake of *A. nilotica* pods and seeds. Only one *A. grandicornuta* seed was extracted from dung in this study, while no *A. senegal* seeds were recorded; both are dehiscent seed species.

Argaw *et al.* (1999) found that *Acacia* species in *Acacia* woodlands, such as the indehiscent *A. tortilis* and dehiscent *A. senegal,* accumulated seeds in and on the soil under tree canopies as ungulates usually dispose of faeces when they are idle i.e. resting in shade. However, in this study the majority of areas from where dung was collected was in the open and not under tree canopy cover (between 20-35% of quadrats), confirming similar results obtained by Miller (1996).

In a study on *Acacia* seed dispersal in a South African savanna ecosystem, it was found that impala dispersed more *A. tortilis* seeds, while giraffe dispersed more *A. nilotica* seeds (Miller 1996). In this study, the majority of seeds of *A. tortilis* (39 seeds) and *A. nilotica* (15 seeds) were extracted from wildebeest dung, suggesting that wildebeest may be the main consumers of seeds of indehiscent species such as *A. nilotica* and *A. tortilis.* It was not analysed whether ingestion of seeds and pods was accidental or deliberate. The single *A. grandicornuta* seed extracted from impala dung suggests that ingestion was possibly incidental. Impala are both grazers and browsers (Stuart and Stuart 2001) and it is possible the seed was picked up off the ground surface during grazing. Giraffe tend to browse *A. tortilis* pods from the canopy, while pods on the ground are removed by herbivores such as kudu, impala, steenbok and duiker (Miller 1994b). However, up to 75% of pods in taller *Acacia* tree canopies may be out of reach of giraffe, lessening the effect on overall seed fate depending on tree height (Barnes 2001).

In this study only 10% of ingested *A. tortilis* seeds and no ingested *A. nilotica* seeds germinated after a six-week germination trial. The single *A. grandicornuta* seed extracted from dung germinated after three weeks. Ingested *Acacia* seeds often do not show enhanced germination after passage through the gut of ungulates (Coe and Coe 1987; Shayo and Udén 1998; Loth *et. al.* 2005) even though it has been suggested that herbivore consumption can facilitate successful germination by rendering the seed permeable to germination cues through the process of scarification (Mayer and Poljakoff-Mayber 1975; Miller 1994b; Miller 1995; Barnes 2001; Wenny 2001). More than half the ingested seeds of both *A. nilotica* and *A. tortilis* were found to be non-viable. Although studies on seed consumption by herbivores show that the majority of seeds from indehiscent pods pass through the gut of herbivores undamaged (Coe and Coe 1987), ingestion may be detrimental to seeds as germination of species such as *A. tortilis* and *A. nilotica* is often reduced after seeds have been eaten by animals (Tybirk *et al.* 1992; Shayo and Udén 1998).

Although seeds in the germination trial were kept moist, were not buried too deeply in soil (± 1 cm deep) and were not exposed to temperatures significantly different to those seeds would experience naturally (see Chapter 3), low rates of germination may have been caused by certain laboratory conditions in the greenhouse which may not have been conducive to successful *Acacia* seed germination. The seeds of *A. nilotica* require specific levels of soil moisture up to two weeks before successful germination (Wilson and Witkowski 1998) and *A. tortilis* seeds may require considerable temperature amplitudes to germinate (Loth *et al.* 2005).

Ingested *A. tortilis* seeds, as well as seeds sampled from *in situ* soil seed banks (see Chapter 2) were lighter and more spherical than seeds removed from pods, while ingested *A. nilotica* seeds were similar in mass and sphericity to seeds from pods and were heavier than seeds sampled from seed banks. Traba *et al.* (2006) suggests there is a greater impact of post-dispersal seed predation on longer and/or heavier seeds. Herbivores will chew food before swallowing so only hard and small seeds (< 2 mm) will pass through the mouth and gut undamaged (Pakeman *et al.* 2002). This may explain the absence of larger seeds in the faeces of herbivores (Calviño-Cancela *et al.* 2008); larger seeds will be destroyed by herbivorous mastication.

4.5.3. Termites and Acacia seed dispersal

This preliminary investigation into *Acacia* seed-termite association showed no relationship between the number of termitaria recorded per grid and the number of *Acacia* trees

(specifically *A. grandicornuta, A. nilotica, A. senegal* and *A. tortilis)* growing on the mounds (r = 0.07). This suggests that either *Acacia* seeds are not dispersed and buried by termites in termite mounds, or buried seeds do not emerge successfully as seedlings. *Acacia* seed burial by termites was recorded in the Nylsvley Provincial Nature Reserve (Miller 1994a), a mainly broad-leafed savanna situated in the Northern Province of South Africa (Wilson and Witkowski 2003); dispersal and burial of the seeds within termite mounds specifically, however, is not discussed in that study.

Miller (1994a) observed that 97.5% of *A. nilotica* seeds and 92.8% of *A. tortilis* seeds removed from a selection experiment were buried by ants and termites, although she ascribed pod remains buried beneath the soil surface as evidence of termite and ant activity. Harvester ants in the Karoo shrubland were found by Dean and Yeaton (1992) to store seeds in their nest-mounds, whereby disturbance by aardvarks (ant-predators) effectively dispersed seeds amongst the disturbed soil of the mound where they remained to germinate after sufficient rains. Dostal (2004) found, in a study of seed dispersal by ants in mountain grasslands, that up to 11% of available seeds were removed even though the seeds were not adapted to ant-dispersal. However, literature considering possible *Acacia* seed dispersal and burial by termites in termite mounds and the subsequent effects of either seed bank formation or provision of safe sites for emerging seedlings in savanna ecosystems is limited.

4.5.4. Acacia seed removal and selection by rodents

4.5.4.1. Rodent species richness

Four species of rodent were recorded in the study region. *Tatera leucogaster* (bushveld gerbil) and *Mastomys coucha* (multimammate mouse) can be found in a wide variety of habitats and have a wide habitat tolerance, as does *Aethomys chrysophilus* (red veld rat), whose habitat includes savanna woodlands. *Rhabdomys pumilio* (striped mouse) is wide-ranging but requires a grassy habitat (Stuart and Stuart 2001).

This study showed that there was a positive relationship between the number of rodent and tree species recorded (r = 0.8), as well as between rodent and woody plant diversity (r = 0.9). As the rodents recorded in the grids feed primarily on seeds and plant material (see Stuart and Stuart 2001) a greater number of woody plant species may encourage rodent activity.

4.5.4.2. Selection by rodents of seeds of savanna woody plants

This study showed a significant difference in the percentage of seeds of different woody plant species removed by rodents in the *in situ* cafeteria trial. Flat, thin and discoid seeds were

selected less by rodents (6.5 ± 1.2% *A. senegal* seeds), while smaller and more spherical seeds were selected the most (15.3 ± 2.9% *D. cinerea* seeds and 14.3 ± 2.5% *A. tortilis* seeds). Although *A. nigrescens* seeds are similar in size and shape to *A. senegal* seeds (*pers. obs.*) a similar percentage of *A. nigrescens* seeds were selected (15.3 ± 3.1% seeds) to the smaller *D. cinerea* and *A. tortilis* seeds. Generally, larger seeds and seeds with high-fat content are harvested more frequently by seed predators (Xiao *et al.* 2006).

In this study, a significantly larger percentage of seeds were removed overall per tray from Grid 3 (20.3 ± 2.4%), which had higher number of woody plant species and diversity than Grid 4 (5.5 ± 0.7%). There was a significant difference in the percentage of seeds removed by rodents between trays under vegetation cover and trays in the open, with more seeds removed from trays under cover. These results confirm those of Fedriani and Manzaneda (2005) who studied the balance between food and safety while foraging by rodents. They found that in the post-dispersal phase of seed predation, more seeds were removed from areas that were sheltered, as opposed to open areas, suggesting that foraging is affected by the threat of predation. Review of the literature on foraging decisions by rodents (for example, Mohr *et al.* 2003; Orrock *et al.* 2004; Fedriani and Manzaneda 2005) suggest the existence of a variety of factors that influence foraging behaviour in rodents such as the risk of predation and the energy value of the food provided. In an investigation on the foraging behaviour of the multimammate mouse in East Africa, Mohr *et al.* (2003) noticed that mice seemed to prefer using chosen feeding sites rather than exposing themselves to predation by searching for new sites. Orrock *et al.* (2004) showed that rodents removed more seeds on nights with precipitation and when moon illumination was low. However, rodents prefer foraging close to cover regardless of the moon phase, indicating that the risk of predation in the open is a priority concern (Hughes and Ward 1993).

4.6. Conclusion

This study showed that large herbivores ingest *A. nilotica*, *A. tortilis* and *A. grandicornuta* seeds, although ingestion did not appear to facilitate germination. Additionally, a large portion of ingested seeds of *A. tortilis* and *A. nilotica* were found non-viable. While *A. grandicornuta* was relatively densely distributed through the study sites, the number of seeds ingested by large herbivores was small, while the seeds of the less densely distributed *A. tortilis* trees were ingested the most by herbivores. Evidence of removal of seeds of *A. nigrescens*, *A. senegal*, *A. tortilis* and *D. cinerea* by rodents was also observed, and the difference in the percentage of seeds removed between species was significant. There was also a significant

difference in seed removal by rodents between seeds placed under vegetation cover and seeds placed in the open. No relationship between the number *of Acacia* trees growing on termite mounds and the number of recorded termite mounds was found.

There is great uncertainty as to what proportion of dispersed seeds is actually consumed, survives intact or suffers other fates (Vander Wall *et al.* 2005). Seed selection by animals should no longer be viewed simply as seed predation until the overall effect of this selection on plant population dynamics is understood. Understanding how seed selection, dispersal and predation affect the process of bush encroachment by *Acacia* trees is important, specifically in the KNP, where an increase in the density and cover of woody plants on granite substrates has been observed since 1940 (see Eckhardt *et al.* 2000). The following suggestions are made for further investigations to supplement the results obtained here:

1. Determine the proportion of active pre- and post-dispersal selection and ingestion of *Acacia* seeds by herbivores (browsers and grazers).
3. Further analyse the effect of herbivore ingestion on seed fate.
4. Monitor seed selection and dispersal of *Acacia* seeds by termites in termite mounds.
5. Investigate whether *Acacia* seeds are able to germinate and establish successfully once buried in termite mounds.
6. Analyse the nutrient content of *Acacia* seeds and the effect of this and seed size on rodent seed selection, predation and dispersal.
7. Further investigate the foraging behaviour of rodents in the KNP in relation to vegetation cover and predation risk.

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4.8. Appendices

Appendix 1: Total number and species of trees with their abundance (N_i) sampled across six grids.

Tree species	N_i
Acacia borleae Burtt Davy (sticky thorn)	1
A.exuvialis I. Verd. (flaky-bark thorn)	24
A.grandicornuta Gerstner (horned thorn)	265
A.nigrescens Oliv. (knob thorn)	1
A.nilotica (L.) Willd. Ex Delile **subsp. Kraussiana** (Benth.) Brenan (scented-pod thorn)	16
A.robusta Burch. **susp. *clavigera*** (E. Mey.) Brenan (brack thorn)	11
A.senegal (L.) Willd. **var. *rostrata*** Brenan (bushy three-hook thorn)	8
A.tortilis (Forssk.) Hayne **subsp. *heterocantha*** (Burch.) Brenan (umbrella thorn)	57
A.welwitschii Oliv. s**ubsp. delagoensis** (Harms) J.H. Ross & Brenan (delagoa thorn)	1
Balanites maughamii Sprague (green thorn)	26
Bolusanthus speciosus (L. Bolus) Harms (tree wistaria)	1
Boscia albitrunca (Burch.) Gilg & Ben (sheperds-tree)	2
Combretum apiculatum Sond. **subsp. apiculatum (**red bushwillow)	21
C.hereroense Schinz (= *C.transvaalense, C. rhodesicum*) (russet bushwillow)	2
C.zeyheri Sond. (large-fruit bushwillow)	1
Commiphora pyracanthoides Engl. (firethorn corkwood)	5
Dichrostachys cinerea (L.) Wight & Arn. **subsp. *africana*** Brenan & Brummitt (small-leaved sickle-bush)	84
Diospyros mespiliformis Hochst. Ex A.D. (jackal-berry)	5
Ehretia amonea Klotzsch (=*E.coerulea*) (sandpaper-bush)	6
Elaeodendron transvaalense (Burtt Davy) R.H.Archer (bushveld saffron)	1
Euclea divinorum Hiern (magic guarri)	128
E. natalensis A.DC **subsp. *natalensis*** F.White (=*E.multiflora*) (hairy guarri)	1
Grewia bicolor Juss. (=*G.grisea, G.kwebensis, G.mossambicensis*) (white-leaved raisin)	167
G. flavescens Juss **var. *olukondae*** (Schinz.) (soft-leaved sandpaper raisin)	15
G. hexamita Burret (=*G.messinica*) (giant raisin)	2
G. monticola Sond. (grey raisin)	2
Gymnosporia buxifolia (L.) Szyszyl. (common spikethorn)	1
G. senegalensis (Lam.) Loes. (red spikethorn)	6
Kirkia acuminate Oliv. (white kirkia)	2
Lonchocarpus capassa (Klotzsch) Schrire (***Philenoptera violacea***) (apple-leaf)	6
Mundulea sericea (Willd.) A.Chev. (cork-bush)	2
Pappea capensis (Eckl. & Zeyh.) (jacket-plum)	50
Protorhus longifolia (Bernh.) Engl. (red beech)	1
Prunus africana (Hook.f.) Kalkm. (african almond)	6
Pyrostri hystrix (Bremek.) Bridson (porcupine-bush)	4

Rhigozum zambesiacum Baker (mopane rhigozum)	9
Rhus gueinzii Sond. (thorny karee)	3
Schotia brachypatela Sond. (weeping boer-bean)	9
Sclerocarya birrea (A.Rich) Hochst. **subsp. *caffra*** (Sond.) Kokwaro (marula)	1
Strychnos madagascariensis Poir. (black-monkey orange)	2
Suregada africana (Sond.) Kuntze (common canary-berry)	145
Terminalia sericea Burch. ex DC. (silver cluster-leaf)	5
Xanthocercis zambesiaca (Baker) Dumaz-le-Grand (nyala-tree)	1
Ximenia americana L. var. ***microphylla*** Welw. (blue sourplum)	2
X. caffra Sond. (sourplum)	2
Zanthoxylum capense (Thunb.) Harv. (small knobwood)	7
Ziziphus mucronata Willd. **subsp. *mucronata*** (buffalo-thorn)	7

Appendix 2: Total number and species of rodents sampled over six grids.

Rodent species	**N_i**
Mastomys coucha (Smith 1834) (multimammate mouse)	15
Rhabdomys pumilio (Thomas 1916) (striped mouse)	9
Aethomys chrysophilus (de Winton 1897) (red veld rat)	7
Tatera leucogaster (Peters 1852) (highveld gerbil)	11

CHAPTER 5
Discussion and recommendations

5.1. General discussion

5.1.1. Acacias in southern African savannas

Woody plants in southern African savannas are associated with both positive and negative aspects related to tree density and abundance (Smit 2004). Higher soil nutrient concentrations are often found under tree canopy cover, which increases plant nutrient concentrations and improves forage quality (Ludwig *et al.* 2004; Smit 2004). Woody plants also create habitats in savannas that support a diversity of species, while providing an important source of food for browser herbivore species (Smit 2004). However, the increase of woody plant density or cover in grasslands with formerly sparse tree occupation typically causes a dramatic decline in grass production (Scholes and Archer 1997). This can lead to intensified grazing pressure (Scholes and Archer 1997) and a decline in the grazing capacity of savanna areas (Smit *et al.* 1999). The thickening of existing woody species or their invasion into open grassland, which is commonly known as bush encroachment, is viewed as a serious problem in southern African savannas. Woody plant species from the generas *Acacia, Maytenus, Euclea* and *Grewia* are common invaders (Stuart-Hill and Tainton 1999).

As trees in savanna regions significantly influence their immediate surroundings, understanding their recruitment and regeneration processes is essential (Treydte *et al.* 2007). In savanna ecosystems *Acacia* trees are widespread (Goheen *et al.* 2004) and provide a valuable source of food to a range of herbivores (Coe and Coe 1987). *Acacias* are ecologically and economically important in savanna regions and as a result, interest in their regeneration strategy has increased (Midgley and Bond 2001). Seed production is an important regeneration mechanism in South African *Acacia* tree species (Munkert 2009). *Acacia* trees such as *A. karroo* and *A. nilotica* produce large quantities of seeds and may form large soil-stored seed banks, which can contribute to the progressive increase in the woody component of savannas (Walters and Milton 2003). For example, *A. nilotica* can produce a large number of seeds in one fruiting season (Tybirk 1989) and is prone to bush encroachment and thicket formation (Kriticos *et al.* 1999; Radford *et al.* 2001; Munkert 2009).

Seed bank ecology has become a subject of increasing interest to scientists, mainly because of the role seed banks play in conservation (Fenner 1995). Seed banks are regarded as natural 'storage facilities' (Fenner and Thompson 2005) and understanding seed bank dynamics may assist in increasing knowledge of seedling establishment (Garner and Witkowski 1997). The size, species richness and composition of seed banks can reflect the past, current and future state of ecosystems (Solomon *et al.* 2006).

An analysis of the trends in woody vegetation cover in the KNP between 1940 and 1998 showed that the density and cover of woody vegetation on granite substrates has increased, possibly due to a decrease in competition with grasses, which is a result of overgrazing (Eckhardt *et al.* 2000). However, in the absence of an interactive ecosystem modelling framework, and as a result of the wide range of factors which may drive these trends, it is difficult to identify the specific reasons for this observed increase in woody plant density and cover (Eckhardt *et al.* 2000). As different ecosystems require different research and management strategies, both types of strategies should be incorporated in order to generate an understanding of the management of the specific system under focus (Rogers 2003).

There is a large body of literature focusing on various aspects of the demography of African *Acacias*, as defined by Midgley and Bond (2001), namely pre- and post-dispersal seed predation (Southgate 1978; Coe and Coe 1987; Miller 1994; Miller 1996; Goheen *et al.* 2007; Zinn *et al.* 2007), seed dispersal (Garner and Witkowski 1997; Walters *et al.* 2005; Miller 1994), germination and seedling establishment (Smith and Shackleton 1988; Miller 1995; Wilson and Witkowski 1998; Argaw *et al.* 1999; Barnes 2001; Loth *et al.* 2005; Stave *et al.* 2006; Munkert 2009), saplings (Riginos and Young 2007), and adult trees (Blackmore *et al.* 1990; Midgley and Bond 2001; Cramer *et al.* 2007; Riginos *et al.* 2009). This study has focused on soil seed bank dynamics and touched on various other aspects of *Acacia* demography such as seed predation, seed dispersal and seed dormancy, viability and germination.

5.1.2. In situ soil seed banks

The main aim of this study was to investigate the density and distribution of *in situ* soil seed banks of four *Acacia* species that grow on granite substrate in the Skukuza land system of the KNP. Results from this study showed that *A. grandicornuta, A. nilotica and A. tortilis* form short-term persistent soil seed banks (seed that persist for 1-5 years), although seed density

varied between species as did seed viability of seed bank seeds. *A. senegal* seeds are transient and consequently do not form persistent soil seed banks. Seed bank density of *A. tortilis,* which had the densest seed banks of the four species, was almost twice as dense as that of *A. grandicornuta* and four times denser than that of *A. nilotica.* Viability of seed bank seeds of each species varied between 0% and 77%. In a study of soil seed banks in the Nylsvley Provincial Nature Reserve (NPNR) (Garner and Witkowski 1997) twice as many *A. tortilis* seeds and twenty-seven times more *A. nilotica* seeds were found than seed banks of the same species sampled from the KNP in this study. In this study, seeds sampled from pods collected from tree canopies were heavier and less spherical, with a larger volume, than seeds sampled from *in situ* soil seed banks for all species except *A. senegal,* where both types of seeds had similar characteristics. More spherically shaped seeds are generally found buried at greater depths in soil seed banks of savanna tree species (Garner and Witkowski 1997).

Bruchid beetles use *Acacia* seeds as a food source (Coe and Coe 1987), and this behaviour can affect seed fate. Almost ninety percent of bruchid beetle species have been found to feed predominantly on seeds in indehiscent pods (Johnson 1981). The results of this preliminary study of bruchid beetle infestation of seeds of four *Acacia* species showed that all four species (two indehiscent and two dehiscent species) suffered bruchid beetle predation, which ranged from 2.4 - 15.8% of 100 seeds per species.

Various seed, parent-tree and micro-site characteristics were analysed for their potential influences on seed bank density. The density of soil seed banks has been shown to increase with plant size for certain savanna woody plant species (Witkowski and Garner 2000). In this study seed bank density increased significantly with tree height for *A. senegal* and *A. tortilis* and tree canopy volume for *A. nilotica.* No other significant relationships were found between seed bank density and other associated tree characteristics such as stem diameter, bark thickness and tree canopy area for any of the species. Soil hardness and compaction may also affect the number of seeds stored in the soil (Garner and Witkowski 1997). This study showed that seed bank density generally decreased with depth of burial of seeds in the soil and distance from the centre of the tree canopy. Seed bank density was not related to herbaceous biomass, over-storey canopy shading or soil compaction for any of the species, except *A. senegal* for which seed bank density increased significantly with a decrease in soil compaction.

5.1.3. Seed burial trials

The ability of seeds to persist in the soil for extended periods of time was investigated, as was the response of buried seeds to natural field conditions as well as to simulated rainfall patterns in the laboratory. A smaller percentage of seeds remained intact and viable in the field seed burial trial compared with the greenhouse seed burial trial for all species. In the field the percentage of remaining, viable seeds was highest at micro-sites under tree canopy shading and buried below the surface of the soil for all species, as opposed to micro-sites in the open and on the soil surface. Seeds generally persist longer in the soil under canopy cover relative to those in the open (Garner and Witkowski 2000).

Relatively low percentages of *A. senegal* and *A. grandicornuta* seeds remained intact, while slightly higher percentages of *A. nilotica* and *A. tortilis* seeds remained intact. Of the remaining intact seeds more *A. tortilis* and *A. nilotica* seeds were viable, while a very low percentage of *A. grandicornuta* seeds and no *A. senegal* seeds were still viable. The number of germination events in the greenhouse burial trial was low for all species. Generally greater percentages of seeds remained intact and viable when watered with the average rainfall (523.5 mm annum^{-1} in 1970/1971), followed by the highest rainfall (1122.8 mm rainfall annum^{-1} in 1999/2000) and the lowest rainfall (237.4 mm annum^{-1} in 1999/1992). There was no association between rainfall treatments and the number of remaining intact, viable seeds for *A. nilotica*, while the association tended to be different for *A. tortilis.* There was a significant association for *A. grandicornuta,* where the number of remaining intact, viable seeds increased significantly with the average rainfall. No viable *A. senegal* seeds were found at the completion of both burial trials.

5.1.4. Predation of Acacia seeds

The role of large herbivores, termites and rodents as seed predators and their effect on seed fate was investigated. This study showed that large herbivores ingest different types of *Acacia* seeds and rodents select seeds of different woody plant species. The majority of seeds extracted from the dung of large herbivores were indehiscent species. Indehiscent seeds such as *A. tortilis* and *A. nilotica* are typically ungulate-dispersed (Tybirk *et al.* 1992). Only 10% of ingested *A. tortilis* seeds and no ingested *A. nilotica* seeds germinated after a six-week germination trial. It is possible that passage through the gut of a large herbivore (in these cases,

wildebeest) did not scarify the seed sufficiently to facilitate germination. Also greenhouse conditions during the germination trial may not have been conducive to *Acacia* seed germination. *Acacia* seeds often require specific environmental conditions to germinate (Wilson and Witkowski 1998; Loth *et al.* 2005) and may exhibit seed-coat imposed dormancy as a result of their hard seed coat (Coe and Coe 1987). More than half the ingested seeds of both *A. nilotica* and *A. tortilis* were found to be non-viable. Ingestion may be detrimental to seeds as germination of species such as *A. tortilis* and *A. nilotica* is often reduced after seeds have been eaten by animals (Tybirk *et al.* 1992; Shayo and Uden 1998).

Ingested *A. tortilis* seeds, as well as seeds sampled from *in situ* soil seed banks were lighter and more spherical than seeds removed from pods collected from the canopy of trees. Ingested *A. nilotica* seeds were similar in mass and sphericity to seeds from pods and were heavier than seeds sampled from seed banks. A possible reason for this finding is that smaller seeds survive ingestion by large herbivores, while larger seeds will be destroyed by herbivorous mastication (Calviño-Cancela *et al.* 2008).

The preliminary investigation into *Acacia* seed-termite association showed no relationship between the number of termitaria observed per grid and the number of *Acacia* trees growing on the mounds. Possibly termites do not select the seeds of the studied *Acacia* species as a food source and thereby do not disperse them to termitaria. However, seeds may be dispersed to mounds but may not germinate and establish successfully as seedlings in the conditions imposed by mounds. Seeds that are unable to germinate under a thick layer of soil and larger seeds are found less frequently in ant mounds (Dauber *et al.* 2006).

There was a significant difference in the percentage of seeds of woody plant species selected by rodents. Generally, larger seeds and seeds with high-fat content are harvested more frequently by seed predators (Xiao *et al.* 2006). In this study, a significantly larger percentage of seeds were removed from trays placed under vegetation cover as opposed to in the open. Rodents prefer foraging close to cover, indicating that the risk of predation in the open is a main concern (Hughes and Ward 1993).

5.2. Recommendations

5.2.1.Management

A better understanding of *in situ* soil seed bank dynamics and the internal (seed) and

external (parent-tree, micro-sites and other environmental factors such as seed predators) characteristics that influence these dynamics can assist in understanding the role that seed banks play in *Acacia* tree regeneration and recruitment. This understanding can assist decisions and strategies in the management of woody plants in the KNP. This investigation into soil seed banks in *Acacia* species in the KNP is only the first step in understanding the regeneration and recruitment process of these potentially invasive species.

Management of the KNP needs to be based on a strong scientific structure that permits rapid response and adaptability to an ever-changing system (Mabunda *et al.* 2003) and managers of national parks are challenged to incorporate concepts such as spatial heterogeneity and ecosystem flux into management policies (Rogers 2003). The management of large conservation areas such as the Kruger National Park is a complex issue, influenced by local and international stakeholders and having an effect on socio-political and economic factors (Mabunda *et al.* 2003). Thresholds of potential concern have been set up by KNP management, which represent hypotheses of the limits in acceptable change in the structure and function of an ecosystem. If these limits are reached an assessment of the causes in the ecosystem is activated and corrective action is initiated (Eckhardt *et al.* 2000; Rogers 2003). Rogers (2003) suggests managers need to identify the key agents of change in a system that can be manipulated with the tools at their disposal. This study has shown that different species have different seed bank densities, which is speculated to be related to variations in seed, pod and environmental characteristics. In this regard, several suggestions are made to KNP management:

1. Initiate the monitoring of change in the cover and density of known invader species such as *Acacia* species and *Dichrostachys cinerea* on granite substrates in the KNP over extended periods of time.
2. Monitor the impact of increased woody plant cover and density of those selected species on the quantity and quality of the grass layer on granite substrates in the KNP.
3. Monitor the effect of increased grazing pressure on the grass layer on granite substrates, as well as the feedback loop this pressure creates on the populations of grazing animals.
4. Adjust fire treatments and closure of artificial watering points where necessary to mitigate the negative effects of changes in woody plant density and cover (see

Eckhardt *et al.* 2000).

5.2.2. Future studies

The following suggestions are made for future studies that will allow a greater understanding of regeneration and recruitment in four *Acacia* species, and which can be used in conjunction with management strategies in successful management of the changes in woody plant density and cover on granite substrates in the KNP:

1. A study on the dynamics of resprouting and coppicing in *Acacia* tree species.
2. An analysis of the moisture content, as well as seed coat thickness of seeds in order to create a greater understanding of germination in *Acacia* species. Seeds with low water content may have seed coats impermeable to water, which has implications for germination as it is related to the loss of physical dormancy (Orozco-Almanza *et al.* 2003). *A. nilotica's* thick seed coat may account for low levels of germination in the species (Walters *et al.* 2004).
3. A further investigation into the processes of germination, dormancy, persistence and longevity in *Acacia* seeds using seed burial trials. Seeds requiring fluctuating temperatures or scarification as a means to break dormancy showed a greater potential for seed bank formation (Honda 2008).
4. An analysis of the effect of fire on *Acacia* germination and seed bank density. Radford *et al.* (2001) noted that fire plays a role in depleting soil seed banks and manipulating seed germination of *A. nilotica* due to scarification of the seed coat.
5. An in-depth analysis of the active selection of seeds and pods of *Acacia* trees by various animals such as rodents, ants, termites, birds and ungulates, and the effectiveness of dispersal of seeds by these animals to safe sites suitable for successful seedling establishment.
6. A study on the nutritional content of *Acacia* seeds and the effect of this on seed selection and predation by granivorous animals such as rodents and termites.

5.3.References

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